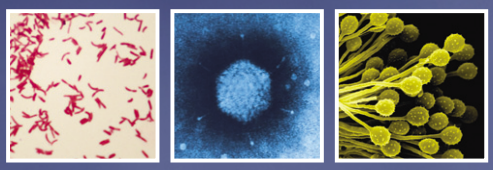
Proceedings

2014 한국미생물학회연합 국제학술대회

INTERNATIONAL MEETING OF THE FEDERATION OF KOREAN MICROBIOLOGICAL SOCIETIES



October 30 (Thu) ▶ 31 (Fri), 2014
KINTEX, Korea

New Horizons in Microbial Sciences

Organized by
The Federation of Korean Microbiological Societies (FKMS)

Hosted by
The Korean Society for Microbiology (KSMi)
The Korean Society for Microbiology and Biotechnology (KMB)
The Korean Society of Mycology (KSMy)
The Korean Society of Virology (KSV)
The Microbiological Society of Korea (MSK)

Sponsored by
Korean Federation of Science and Technology Societies
Korea National Microorganisms Research Resources Center
Korea Research Institute of Bioscience and Biotechnology
National Research Foundation of Korea
Gyeonggi Tourism Organization

한국미생물학회연합
www.fkms.kr

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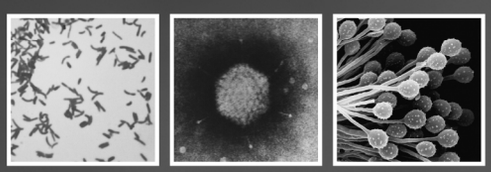

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Contents

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Tel: +82-2-887-3062 | Fax: +82-2-887-3063 | Email: fkms@fkms.kr | Homepage: www.fkms.kr

Timetable

October 30 (Thursday)

			Grand Ballroom A	Room 306 & 307	Room 305	Room 304	Room 303	Room 302
08:00-08:50	Registration							
08:50-09:00		Poster Session 1 & Exhibition	Opening Ceremony (Grand Ballroom A)					
09:00-11:00			S1 Outbreak Investigation and Control of Enteric Pathogens	S2 Pathogen Genomics	S3 New Challenge for Viral Vaccines	S4 Fungal Development and Pathogenesis	S5 Young Scientist Session I	S6 ACM 11 Symposium I
11:00-11:45			Plenary Lecture 1 (Grand Ballroom A)					
11:45-12:15			MSK GM (11:45~12:15)		KSV GM (11:45~12:15)	KSMy GM (11:45~12:15)		
			Poster Presentation 1 (11:45~13:00)					
12:15-13:00			Lunch					
13:00-13:45			Plenary Lecture 2 (Grand Ballroom A)					
13:45-15:45			SS1 Infectious Diseases and Industrialization I	S7 Metagenomics: from Resource to Biological Application	S8 Anti-Viral Drugs and Technology	S9 Mushroom Science and Industrial Application	2015 NRF, Division of Life Sciences (13:45~14:15)	S10 ACM 11 Symposium II
15:45-17:45			SS2 Infectious Diseases and Industrialization II	S11 Microbial Stress Response	S12 Host Defense against Viral Infections	S13 Fungal Taxonomy and Diversity		
17:45-18:30			Plenary Lecture 3 (Grand Ballroom A)					
18:30-21:00			Welcome Reception (Grand Ballroom B)					

October 31 (Friday)

			Grand Ballroom A	Room 306 & 307	Room 305	Room 304	Room 303	Room 302
08:00-09:00	Registration							
09:00-11:00		Poster Session 2 & Exhibition	S14 Systems and Synthetic Biology Approaches in Yeast (09:00~11:30)	S15 Recent Trends in Noroviral Research	S16 Re-emerging Infectious Diseases: Acute Febrile Illness	S17 Young Scientist Session II	S18 KNMRRC Session I (10:00~12:00)	S19 Young Scientist Session III
11:00-12:00					KSM GM (11:00~11:30)			
			Poster Presentation 2 (11:00~13:00)					
12:00-13:00			Lunch					
13:00-15:00			S20 Structural and Molecular Biology of Pathogenic Bacteria	S21 Recent Advances and Application in Microbial Genomics	S22 Molecular Basis of Bacterial Pathogenesis	S23 Young Scientist Session IV	S24 KNMRRC Session II	
15:00-15:45			Plenary Lecture 4 (Grand Ballroom A)					
15:45-17:45			S25 Research Trends in Biofilm and Biofouling	SS3 Infectious Diseases and Industrialization III	S26 Immunity to Infection	S27 Young Scientist Session V	S28 Young Scientist Session VI	
17:45-18:00			Closing Ceremony (Grand Ballroom A)					

* KSM: The Korean Society for Microbiology
KSV: The Korean Society of Virology
KSMy: The Korean Society of Mycology
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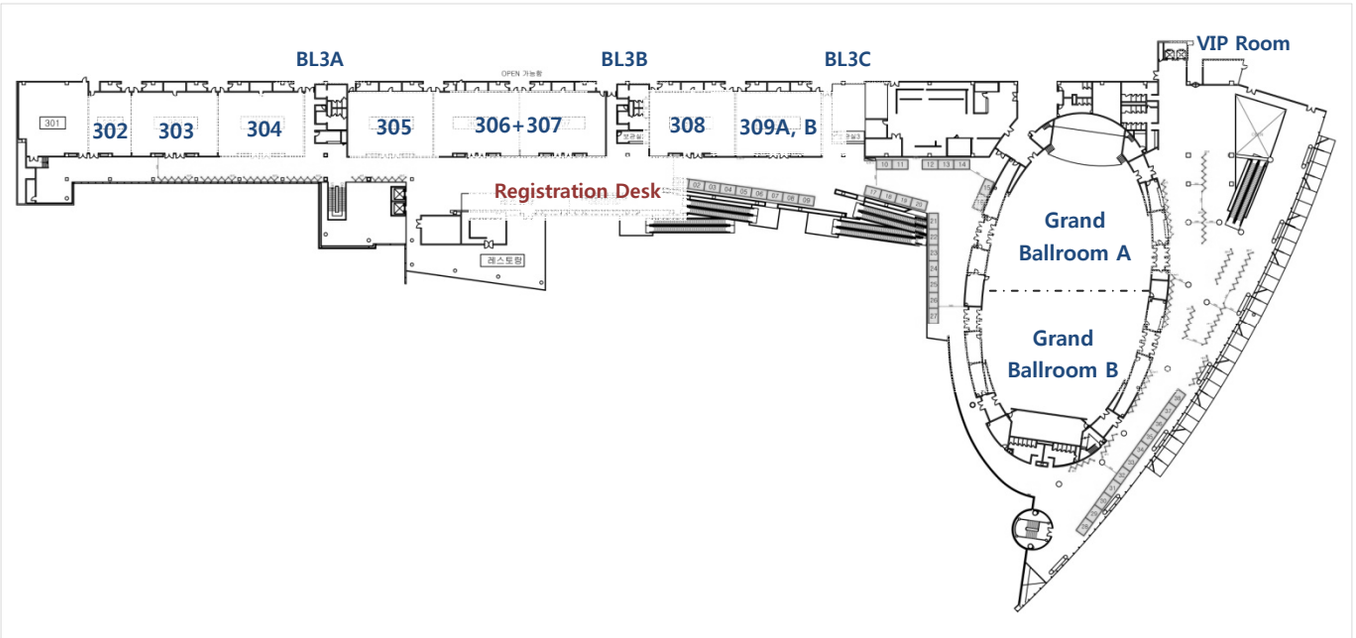
Floor Plan

3rd Floor



Room	302	303	304	305	306 + 307	Grand Ballroom A	Grand Ballroom B
30 (Thu)	Session(ACM)	Session (YS*)	Session(KSMy)	Session(KSV)	Session (KMB)	Session (MSK, FKMS), Opening Ceremony	Welcome Reception
31 (Fri)	Session (YS)	Session (YS)	Session (YS)	Session(KSM)	Session (KMB)	Session(MSK), Closing Ceremony	

* YS: Young Scientist

Room	308	309A	309B	BL3A	BL3B	BL3C	VIP Room
30 (Thu)	Meeting Room I	Meeting Room II	Honorary Member Room	Preview Room	Secretariat Office	VIP Room	Foreign VIP Room
31 (Fri)	Meeting Room I	Meeting Room II	Honorary Member Room	Preview Room	Secretariat Office	VIP Room	Foreign VIP Room

Program Schedule

■■■ Plenary Lectures

PL1 Plenary Lecture 1

October 30 (Thu.), Grand Ballroom A

Chair: Ok Bin Kim, Ewha Womans University

11:00-11:45

Fumarate and Succinate Metabolism in E. coli: Regulation by a Transporter/Sensor Complex

G. Unden, Johannes Gutenberg University Mainz, Germany

PL2 Plenary Lecture 2

October 30 (Thu.), Grand Ballroom A

Chair: Sang-Yeob Lee, NAAS

13:00-13:45

"Agro-environmental Microorganisms Inventory" - Application to Biocontrol and the Degradation of Plastic and Mycotoxin -

Motoo Koitabashi, National Institute for Agro-Environmental Sciences, Japan

PL3 Plenary Lecture 3

October 30 (Thu.), Grand Ballroom A

Chair: Yeonhee Lee, Seoul Women's University / KNRRC

17:45-18:30

The Role of BRCs as the Infrastructure of the Prokaryote Taxonomy

Ken-ichiro Suzuki, National Institute of Technology and Evaluation (NBRC), Japan

PL4 Plenary Lecture 4

October 31 (Fri.), Grand Ballroom A

Co-hosted by Infection Signaling Network Research Center, Chungnam National University and FKMS

Chair: Sang Sun Yoon, Yonsei University

15:00-15:45

Wall Teichoic Polymers in Staphylococcus Aureus Physiology and Host Interaction

Andreas Peschel, University of Tübingen, Germany

■■■ Special Symposium

SS1 Infectious Diseases and Industrialization I

October 30 (Thu.), Grand Ballroom A

Chair: Yoon-Won Kim, Hallym University

SS1-1 13:45-14:15

Regulatory Status for Drug Review and Approval of Anti-Infectives

So Hee Kim, Ministry of Food and Drug Safety

SS1-2 14:15-14:45

What Medical Inventions are Patentable- Ebola Patent Case

Min Son, Hanol Intellectual Property and Law

SS1-3 14:45-15:15

Development of Rapid Diagnostic Test Kit for Global Leptospirosis and its MFDS (KFDA) Approval

Young-Jin Kim, ImmuneMed

SS1-4 15:15-15:45

Ex vivo Expanded Allogeneic Natural Killer Cell Therapy for Cancer Patients

Yu-Kyeong Hwang, Cell therapy Research Center, Green Cross LanCell Corp.

SS2 Infectious Diseases and Industrialization II

October 30(Thu.), Grand Ballroom A

Co-hosted by Korea Research Institute of Bioscience and Biotechnology and FKMS

Chair: Yoon-Won Kim, Hallym University

SS2-1 15:45-16:15

IVD Approval Regulations of MFDS

Won Kyu Lee, National Institute of Food & Drug Safety Evaluation

SS2-2 16:15-16:45

LMO법률 및 시험 · 연구용 LMO 안전관리 제도

하주희, 한국생명공학연구원 LMO연구안전센터

SS2-3 16:45-17:15

유전자변형생물체(Living Modified Organism)연구 안전관리와 LMO법 제도

최경화, 한국생명공학연구원 LMO연구안전센터

SS2-4 17:15-17:45

Natural Killer Cell Immune function, as Assessed by the NKVue® Assay, Correlates with Clinical Cancer Stage

Jae Myun Lee, Yonsei University

SS3 Infectious Diseases and Industrialization III

October 31 (Fri.), Room 306 + 307

Co-hosted by Korea Research Institute of Bioscience and Biotechnology and FKMS

Chair: Woo Kon Lee, Gyeongsang National University

SS3-1 15:45-16:15

Development of DA-7218 (Tedizolid phosphate), a Next-Generation Oxazolidinone

Sunghak Choi, Dong-A ST

SS3-2 16:15-16:45

Triangular Cooperation of Industrialization of Diagnostic Kit for Acute Viral Infectious Diseases; A Lesson from Pandemic Influenza in 2009

Kisoon Kim, Korea National Institute of Health

SS3-3 16:45-17:05

Korea Chemical Bank: National Repository of Small Molecular Organic Compounds for New Drug Discovery

Hyeon-Kyu Lee, Korea Chemical Bank, Korea Research Institute of Chemical Technology

SS3-4 17:05-17:25

Acquisition, Management and Application of Bioproducts

Byoung-Chan Kim, Korea Research Institute of Bioscience and Biotechnology

SS3-5 17:25-17:45

Introduction of Biological Information Registration System for Biotechnology Research Outcomes

Haeyoung Jeong, Korea Research Institute of Bioscience & Biotechnology

■■■ Symposia

S1 Outbreak Investigation and Control of Enteric Pathogens

October 30 (Thu.), Grand Ballroom A

Chairs: Cheon-Kwon Yoo, Korea National Institute of Health

Kyu-Ho Lee, Sogang University

S1-1 09:00-09:30

Molecular Epidemiology and Characterization of Enteric Pathogens

Gyung Tae Chung, Korea National Institute of Health

S1-2 09:30-10:00

Genome-based Identification and Typing of Bacterial Pathogens

Jongsik Chun, Seoul National University

S1-3 10:00-10:30

Exogenous N-Acetylneuraminic Acid Promotes Fibronectin Adherence by Shigella flexneri 2457T

Jong Hyun Kim, Korea National Institute of Health

S1-4 10:30-11:00

Porcine Epidemic Diarrhea Virus: the New Era

Daesub Song, Korea Research Institute of Bioscience and Biotechnology

S2 Pathogen Genomics

October 30 (Thu.), Room 306 + 307

Chair: Heejoon Myung, Hankuk University of Foreign Studies

S2-1 09:00-09:30

Overcoming Bacterial Resistant Mutants Using Information Obtained from Genomic Analysis of Bacteriophages

Heejoon Myung, Hankuk University of Foreign Studies

S2-2 09:30-10:00

Regulation of Hepatitis C Virus Replication by Small Non-Coding RNAs

Jong-Won Oh, Yonsei University

S2-3 10:00-10:30

The Evolution of the Bacterial Antibiotic Resistance

Heenam Kim, Korea University

S2-4 10:30-11:00

Quorum Sensing Regulon and virulence of *Pseudomonas aeruginosa*

Joon-Hee Lee, Pusan National University

S3 New Challenge for Viral Vaccines

October 30(Thu.), Room 305

Chair: Yong-Soo Bae, Sungkyunkwan University

S3-1 09:00-09:30

T Cell Responses against Hepatitis C Virus and Vaccine Development

Eui-Cheol Shin, KAIST

S3-2 09:30-10:00

Universal Influenza Vaccine: Options and Hurdles

Baik Lin Seong, Yonsei University

S3-3 10:00-10:30

Towards a T-Cell Vaccine Based on Vaccinia Virus

Jin-Won Youn, Research Institute, Genexine, Inc.

S3-4 10:30-11:00

Non-invasive Nasal Adenovirus Vectored Vaccine for Broad Protection against Influenza – Implications for Development of Vaccines against Respiratory and Enteric Infectious Diseases

Huan Nguyen, International Vaccine Institute

S4 Fungal Development and Pathogenesis

October 30(Thu.), Room 304

Chair: Seong Hwan Kim, Dankook University

S4-1 09:00-09:30

Development of System-Wide Functional Analysis Platform for Pathogenicity Genes in *Magnaporthe oryzae*

Sook-Young Park, Sunchon National University

S4-2 09:30-10:00

Epigenetic Regulation of Fungal Development and Pathogenesis in the Rice Blast Fungus

Junhyun Jeon, Seoul National University

S4-3 10:00-10:30

Interaction between the Rice Pathogens, *Fusarium graminearum* and *Burkholderia glumae*

Jungkwan Lee, Dong-A University

S4-4 10:30-11:00

Genetic Control of Asexual Sporulation in *Fusarium graminearum*

Hokyoung Son, Seoul National University

S6 ACM 11 Symposium I

October 30 (Thu.), Room 302

Chair: Yeonhee Lee, Seoul Women's University / KNRRC

S6-1 **09:00-09:30**

Current Status and Future Prospects of ACM

Ken-ichiro Suzuki, National Institute of Technology and Evaluation (NBRC), Japan

S6-2 **09:30-10:00**

Patent Strategies for Biotechnology

Won-Hee Lee, WON International Patent & Law Firm

S6-3 **10:00-10:30**

Analysis of the Utilization of Microbial Resources Distributed from JCM

Moriya Ohkuma, Japan Collection of Microorganisms, Japan

S6-4 **10:30-11:00**

Application of Microbial Fermentation and Enzymatic Biotransformation in Cosmetic Industry

Byungyoung Kang, AMOREPACIFIC R&D Center

S7 Metagenomics: from Resource to Biological Application

October 30 (Thu.), Room 306 + 307

Chair: Jae Jun Song, Korea Research Institute of Bioscience and Biotechnology

S7-1 **13:45-14:15**

Discovery from Soil Microbial Diversity: Bioprospecting Soil Metagenomics

Seon-Woo Lee, Dong-A University

S7-2 **14:15-14:45**

Beyond "Who Are They?": Application of Metagenome to Plant

Choong-Min Ryu, Korea Research Institute of Bioscience and Biotechnology

S7-3 **14:45-15:15**

New Strategy for Ultra-high-throughput Screening (uHTS) of Novel Enzyme/Pathway by *in Vitro* Compartmentalization (IVC) Using Microbeads from Metagenomic Resources

Jong Hyun Choi, Korea Research Institute of Bioscience and Biotechnology

S7-4 **15:15-15:45**

Metagenomic Enzyme Discovery for the Use of Biocatalyst in Bio-based Chemical Industry

Jae Kwang Song, Korea Research Institute of Chemical Technology

S8 Anti-Viral Drugs and Technology

October 30 (Thu.), Room 305

Chair: Young Bong Kim, Konkuk University

S8-1 13:45-14:15

Cell-based High-throughput Screening of Chemical Libraries for Inhibitors of Influenza Virus

Meehyein Kim, Korea Research Institute of Chemical Technology

S8-2 14:15-14:45

Squirrel Poxvirus as a Novel Oncolytic Agent

Manbok Kim, Dankook University

S8-3 14:45-15:15

Structural Insight into the Extracellular Domain of Matrix Protein 2 of influenza A virus

Kyung Hyun Kim, Korea University

S8-4 15:15-15:45

차세대 염기서열 분석기법을 이용한 닭 허피스바이러스 전장유전체 분석

Sang-Won Lee, Konkuk University

S9 Mushroom Science and Industrial Application

October 30(Thu.), Room 304

Chair: Hyeon Su Ro, Gyeongsang National University

S9-1 13:45-14:15

Extraction and Application of Bulk Enzymes and Antimicrobial Substance from Spent Mushroom Substrates

Hee-Wan Kang, Hankyong National University

S9-2 14:15-14:45

Study on Species Diversity of Indigenous Mushrooms in Jeju

Pyung Yeol Ko, Jeju National University

S9-3 14:45-15:15

Distiller's Yeast Discovery for Industrial Application

Tae Wan Kim, Korea Food Research Institute

S9-4 15:15-15:45

Disinfection of *Fusarium*-infected Rice Seeds by Prochloraz and Gaseous Chlorine Dioxide

Young-ah Jeon, National Academy of Agricultural Science

S10 ACM 11 Symposium II (13:45 – 17:45)

October 30 (Thu.), Room 302

Chairs: Hiroko Kawasaki,
National Institute of Technology and Evaluation (NBRC), Japan

S10-1 Secretariat of the National Steering Committee for Biosafety
Oum Pisey, The Secretariat of the National Steering Committee for Biosafety

S10-2 China General Microbiological Culture Collection Center, IMCAS-BRC
Yu-Guang Zhou, Institution of Microbiology, Chinese Academy of Sciences, China

S10-3 Microbial Type Culture Collection and Gene Bank (MTCC)
D. Ananthapadmanaban, Microbial Type Culture Collection and Gene Bank, India

S10-4 Indonesian Culture Collection (InaCC)
Enny Sudarmonowati, Indonesian Institute of Sciences (LIPI), Indonesia

S10-5 Japan Collection of Microorganisms (JCM), RIKEN BioResource Center
Moriya Ohkuma, Japan Collection of Microorganisms (JCM), Japan

S10-6 KCTC, Korea Research Institute of Bioscience and Biotechnology
Doo-Sang Park, Korean Collection for Type Cultures (KCTC), Korea

S10-7 Malaysian Agricultural Research and Development Institute (MARDI)
Tosiah Sadi, Malaysian Agricultural Research and Development Institute (MARDI), Malaysia

S10-8 Korea National Research Resource Centers (KNRRC)
Kyungsook Ahn, Korea National Research Resource Centers (KNRRC), Korea

S10-9 Biological Resource Center, National Institute of Technology and Evaluation (NBRC)
Manabu Suto, Biological Resource Center, National Institute of Technology and Evaluation, Japan

S10-10 Korean Agricultural Culture Collection (KACC), National Academy of Agricultural Science (Korea)
Soon-Wo Kwon, Korean Collection for Type Cultures (KCTC), Korea

S10-11 Laboratory of Microbiology, Institute of Biology, MAS
Tsetseg Baljinova, Institute of Biology, MAS

S10-12 Microbial Culture Collection at the National Institute for Environmental Studies
Masanobu Kawachi, National Institute for Environmental Studies, Japan

S10-13 Philippine National Collection of Microorganisms (PNCM), National Institute of Molecular Biology and Biotechnology (BIOTECH)
Rosario G. Monsalud, The Philippine National Collection of Microorganisms (PNCM), Philippine

S10-14 Thailand Bioresource Research Center (TBRC) and the Mission to Encourage International Cooperation for Utilization of Microbial Resources in Asia
Lily Eurwilaichitr, Thailand Bioresource Research Center (TBRC), National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Thailand

S10-15 Updates of Activities of University of Santo Tomas Collection of Microbial Strains
Gina Rio Dedeles, The University of Santo Tomas Collection of Microbial Strains (USTCMS), Philippine

S10-16 TISTR Culture Collection, Thailand Institute of Scientific and Technological Research
Chatrudee Suwannchart, Thailand Institute of Scientific and Technological Research, Thailand

S10-17 Vietnam Type Culture Collection (VTCC), Institute of Microbiology and Biotechnology (IMBT), Vietnam National University, Hanoi, Vietnam (VNU)
Duong Van Hop, Vietnam Type Culture Collection (VTCC), Vietnam

S10-18 ACM Member Report
Gwo-Fang Yuan, Bioresource Collection and Research Center (BCRC), Food Industry R&D Institute, Taiwan

S11 Microbial Stress Response

October 30 (Thu.), Room 306 + 307

Chair: Won Hee Jung, Chung-Ang University

S11-1 **15:45-16:15**
Endeavors of Proteomic Studies in the Research of Microbial Stress Response with the Gap between Gene Expression and Function
Yong-Hak Kim, Catholic University of Deagu

S11-2 **16:15-16:45**
Cordycepin is A New Chemical Suppressor of EBV Replication
Hyojeung Kang, Kyungpook National University

S11-3 **16:45-17:15**
Role and Application of Two-Component Systems (TCS) of Radiation-Resistant Bacterium, *Deinococcus radiodurans*
Sangyong Lim, Korea Atomic Energy Research Institute

S11-4 **17:15-17:45**
Non-replicative Helicases, UvrD and DinG, Regulate the Replication of Theta (θ)-replicative Plasmids in *Escherichia coli*
Jihwan Hwang, Pusan National University

S12 Host Defense against Viral Infections

October 30 (Thu.), Room 305

Chair: Joong-bok Lee, Konkuk University

S12-1 15:45-16:15

Upregulation of PD- on Regulatory T Cells Potentiates Their Suppressive Function during Chronic Viral Infection

Sang-Jun Ha, Yonsei University

S12-2 16:15-16:45

Development of a Novel Therapeutic DNA Vaccine against Human Papillomavirus-Associated Pre-Malignant Lesions; from Bench to Bedside

Hyun-Tak Jin, Genexine

S12-3 16:45-17:15

Cytokine-mediated Suppression of Hepatitis B Virus

Kyun-Hwan Kim, Konkuk University

S12-4 17:15-17:45

Deregulation of Immune Response Genes in Epstein-Barr Virus Associated Gastric Cancer Reflects Favorable Prognosis

Myung-Soo Kang, Sungkyunkwan University

S13 Fungal Taxonomy and Diversity

October 30 (Fri), Room 304

Chair: Kap-Hoon Han, Woosuk University

S13-1 15:45-16:15

Are Cryptic Species Real?

Pedro W. Crous, CBS Fungal Biodiversity Centre, Netherland

S13-2 16:15-16:45

The Origin of *Meju* Fungi - Fungal Diversity of Soybean, Rice Straw and Air for *Meju* Fermentation

Dae-Ho Kim, Kangwon National University

S13-3 16:45-17:15

Morphological and Genetic Characteristics of *Colletotrichum gloeosporioides* Isolated from Newly Emerging Static-Symptom Anthracnose in Apple

Yongho Jeon, Andong National University

S13-4 17:15-17:45

Ecological Characteristics and Unique Diagnostic Techniques of Apple Blotch Disease Caused by *Marssonina coronaria* in Korea

Chang-Gi Back, Kyungpook National University

S14 Systems and Synthetic Biology Approaches in Yeast

October 31 (Fri.), Grand Ballroom A

Chair: Hyunah Kang, Chung-Ang University

S14-1 09:00-09:30

Nitric Oxide-mediated Antioxidative Mechanism in Saccharomyces Cerevisiae and its Application to Baker's Yeast

Hiroshi Takagi, Nara Institute of Science and Technology, Japan

S14-2 09:30-10:00

Systematic Genome Deletion of Fission Yeast and its Applications

Kwang-Lae Hoe, Chungnam National University

S14-3 10:00-10:30

Regulatory Proteolysis by N-Terminal Acetylation and the N-End Rule Pathway

Cheol-Sang Hwang, Pohang University of Science and Technology

S14-4 10:30-11:00

Synthetic Glyco-engineering of Yeast for Production of Therapeutic Enzymes

Doo-Byoung Oh, Korea Research Institute of Bioscience and Biotechnology

S14-5 11:00-11:30

Metabolic Engineering of *Saccharomyces cerevisiae* for Production of Lactic Acid

Ji-Yoon Song, Samsung Advanced Institute of Technology

S15 Recent Trends in Noroviral Research

October 31(Fri.), Room 306 + 307

Chair: Yong-Soo Bae, Sungkyunkwan University

S15-1 09:00-09:30

Insight into VPg-mediated RNA Synthesis in Norovirus

Kyung Hyun Kim, Korea University

S15-2 09:30-10:00

Application of Omics Technology for Food Material Development

Joseph Kwon, Korea Basic Science Institute

S15-3 10:00-10:30

Prevention of Norovirus Associated Foodborne Outbreak: Diagnostics and Viral Control

GwangPyo Ko, Seoul National University

S15-4 10:30-11:00

An Plasmid Based Human Norovirus Reverse Genetics System

Kazuhiko Katayama, National Institute of Infectious Diseases, Japan

S16 Re-emerging Infectious Diseases: Acute Febrile Illness

October 31(Fri.), Room 305

Chair: Myung-Sik Choi, Seoul National University

S16-1 09:00-09:30

Epidemiology of Scrub Typhus: Current Issues on Environmental Factors

Nam-Hyuk Cho, Seoul National University

S16-2 09:30-10:00

Richettsial Deseases in Korea

Won-Jong Jang, Konkuk University

S16-3 10:00-10:30

Re-emerging Infections in Sri Lanka-the Challenge

Ranjan Premaratna, Univ. of Kelaniya, Sri Lanka

S16-4 10:30-11:00

Clinical Validation of Scrub Typhus Diagnosis Kit in Asian Countries

Sungman Park, Hallym University

S18 KNMRRC Session I

October 31(Fri.), Room 303

Organized by Korea National Microorganisms Research Resources Center

Chair: Yong Hwan Lee, Seoul National University

S18-1 10:00-10:30

Korea Bank for Pathogenic Viruses

Song Ki-Joon, Korea University

S18-2 10:30-11:00

Campylobacter in Korea: Their Relateness

Eunju Shin, Seoul Women's University

S18-3 11:00-11:30

Development of Transformation System of Lichen-Forming Fungus, *Umbilicaria muehlenbergii*

Jae-Seoun Hur, Sunchon National University

S18-4 11:30-12:00

Helicobacter pylori Korean Type Culture Collection

Hyung-Lyun Kang, Gyeongsang National University

S20 Structural and Molecular Biology of Pathogenic Bacteria

October 31(Fri.), Grand Ballroom A

Chair: You-Hee Cho, CHA University

S20-1 13:00-13:30

Structure of the Tripartite Multidrug Efflux Pump AcrAB-TolC Shows an Intermeshing Cogwheel Interaction between AcrA and TolC

Nam-Chul Ha, Seoul National University

S20-2 13:30-14:00

Reciprocal Regulation of the Autophosphorylation of Enzyme I^{Ntr} by Glutamine and α-Ketoglutarate in *Escherichia coli*

Chang-Ro Lee, Myongji University

S20-3 14:00-14:30

FeoC Regulation of Fe(II) Uptake in *Salmonella enterica*

Dongwoo Shin, Sungkyunkwan University

S20-4 14:30-15:00

Stringent Control of EPEC Pathogenesis

Jang Won Yoon, Kangwon National University

S21 Recent Advances and Application in Microbial Genomics

October 31(Fri.), Room 306 + 307

Chair: Seung-Hwan Park, Korea Research Institute of Bioscience and Biotechnology

S21-1 13:00-13:30

Small Yet Big: Interpretation of Microbial Big Data

Changhoon Kim, Macrogen

S21-2 13:30-14:00

Harnessing the Power of Adaptive Laboratory Evolution and Genome-Scale Sciences for Strain Engineering

Daehee Lee, Korea Research Institute of Bioscience and Biotechnology

S21-3 14:00-14:30

Genome-wide Analysis of the Extremophilic Bacterium *Fervidobacterium islandicum* AW- Revealed the Degradation Mechanism of Feather Keratin

Dong-Woo Lee, Kyungpook National University

S21-4 14:30-15:00

Comparative Genomic Analysis of Complete Genome Sequence of 4 Microbes, Isolated from the Real Food-Borne Outbreak in South Korea

Heebal Kim, Seoul National University

S22 Molecular Basis of Bacterial Pathogenesis

October 31(Fri.), Room 305

Chair: Hyun E. Choy, Chonnam National University

S22-1 13:00-13:30

An Anti-virulence Strategy to Combat *Pseudomonas aeruginosa* Infection

Sang Sun Yoon, Yonsei University

S22-2 13:30-14:00

Host Partners of an RTX Toxin in *Vibrio vulnificus* Cytotoxicity

Young Ran Kim, Chonnam National University

S22-3 14:00-14:30

Virulence Factors of Acinetobacter baumannii as Promising New Drug Target

Chul Hee Choi, Chungnam National University

S22-4 14:30-15:00

Differential Modulation of Cytokine Production in Gram-negative and Gram-positive Bacterial Infection by ATF3, a Stress Inducible Eukaryotic Gene

Dong-Kwon Rhee, Sungkyunkwan University

S24 KNMRRC Session II

October 31(Fri.), Room 303

Organized by Korea National Microorganisms Research Resources Center

Chair: Heejoon Myung, Hankuk University of Foreign Studies

S24-1 13:00-13:30

Importance of Isolating Bacterial Viruses for the Interpretation of Metagenomes

Jang-Cheon Cho, Inha University

S24-2 13:30-14:00

The Applications of Genome Information to Understand Biology

Ik-Young Choi, Seoul National University

S24-3 14:00-14:30

Myung Kyum Kim, Seoul Womens University

S24-4 14:30-15:00

Detection of Coliforms in Drinking Water Using Skin Patches: A Rapid, Reliable Method that Does Not Require an External Energy Source

Gyu-Cheol Lee, Water Quality Research Center, K-water

S25 Research Trends in Biofilm and Biofouling

October 31(Fri.), Grand Ballroom A

Chair: Woojun Park, Korea University

S25-1 15:45-16:15

Studying Mechanical Properties of Bacterial Appendages Using Atomic Force Microscopy and Traction Force Microscopy

Sungsu Park, Sungkyunkwan University

S25-2 16:15-16:45

The Cocci-shaped *Escherichia coli*: Biofilm Formation and Antibiotics Resistance

Younghoon Kim, Chonbuk National University

S25-3 16:45-17:15

Inhibition of Biofilm Formation using Ginger Chemicals: 6-gingerol and Raffinose

Hee-Deung Park, Korea University

S25-4 17:15-17:45

Diverse Quorum Quenching Bacteria Isolated from Wastewater Treatment Plant, and Their Application for Controlling Biofilm Formation

Jung-Kee Lee, Paichai University

S26 Immunity to Infection

October 31 (Fri.), Room 305

Chair: In-Hong Choi, Yonsei University

S26-1 15:45-16:15

Preconditioning of High Mobility Group Box Protein (HMGB) Attenuates TLR4-mediated Inflammatory Response

Jeon-Soo Shin, Yonsei University

S26-2 16:15-16:45

Helicobacter pylori-Mediated Inflammation in Host Cells: Mechanism for VEGF and IL-b Production

Jong-Hwan Park, Chonnam National University

S26-3 16:45-17:15

Role of Streptococcal Phage $Lysin_{sm}$ in the Pathogenesis of Infective Endocarditis

Ho Seong Seo, Korea Atomic Energy Research Institute

S26-4 17:15-17:45

A GPCR9 Agonist Alleviates Systemic Inflammation by Expansion of Immune Regulatory Cells

Seung-Yong Seong, Seoul National University

2015 NRF, Division of Life Sciences

October 30 (Thu.), Room 303

Organized by National Research Foundation of Korea

13:45- 14:15

Microbiological Societies and NRF of Korea, 2015

Joon Kim, Korea University

■■■ Young Scientist Sessions

S5 Young Scientist Session I

October 30(Thu.), Room 303

Chair: Sang Sun Yoon, Yonsei University

S5-1 09:00-09:15

Impact of Obesity on Influenza Infection

Joo Young Kim, Ewha Womans University College of Pharmacy

S5-2 09:15-09:30

Association between Respiratory Virus Specific IgE Detection in Sputa and Asthma Exacerbation

Yong Won Lee, Catholic Kwandong University

S5-3 09:30-09:45

Antigenic and Phylogenetic Dynamics of Influenza A(H3N2) Viruses in Korea in 2011-2012

Jin Il Kim, Korea University

S5-4 09:45-10:00

Deglycosylation at Influenza A Neuraminidase Stalk Confers Enhanced Pathogenicity in Mice

Sehee Park, Korea University

S5-5 10:00-10:15

A Single Amino Acid Mutation in the PA Improves Viral Yield of Influenza A Candidate Vaccine Virus

Ilseob Lee, Korea University

S5-6 10:15-10:30

Control of Salmonella Infection by Modulating Hepcidin

Jae-Ho Jeong, Chonnam National University Medical School

S5-7 10:30-10:45

A Critical Role of Defective Viral Genomes Arising *In Vivo* for the Triggering of Innate Antiviral Immunity

Won-keun Kim, Korea University

S17 Young Scientist Session II

October 31(Fri.), Room 304

Chair: Jung-Shin Lee, Kangwon National University

S17-1 09:00-09:15

Quorum Sensing for Biofilm Formation and Oil Degradation in *Acinetobacter oleivorans* DR

Jisun Kim, Korea University

S17-2 09:15-09:30

Genotyping of *Agaricus bisporus* Strains by PCR Fingerprints

Kyong-Jin Min, Hankyong National University

S17-3 09:30-09:45

Analysis of Mating System in Lentinula edodes and Development of Mating Type-Specific Markers

Byung-Suk Ha, Gyeongsang National University

S17-4 09:45-10:00

Invesigation of Functional Roles of a Protein Kinase in a Fungal Plant Pathogen, *Magnaporthe oryzae*

Jong-Hwan Shin, Kangwon National University

S17-5 10:00-10:15

Heterologous Expression of Der Homologues in *Escherichia coli der* Mutant and Their Functional Complementation

Eunsil Choi, Pusan National University

S17-6 10:15-10:30

A Small Nucleotide Regulator (p)ppGpp Directs Metabolic Fate of Glucose in *Vibrio Cholerae*

Young Taek Oh, Yonsei University

S17-7 10:30-10:45

Isolation, Identification and Physiological Functionality of Yeasts from Wild Flowers in Islands and Mountains of Korea

Se-Hee Hyun, Paichai University

S19 Young Scientist Session III

October 31(Fri.), Room 302

Chair: So-Youn Woo, Ewha Womans University

S19-1 09:00-09:15

FVB Gulo$^{-/-}$ Congenic Mice as An Animal Model for *H. pylori* Infection

Jong-Hun Ha, Gyeongsang National University

S19-2 09:15-09:30

Mouse Susceptibility to *Vibrio Cholerae* Infection is Influenced by Altered Composition of Gut Microbiota

Mi Young Yoon, Yonsei University

S19-3 09:30-09:45

Mycobacterium Avium MAV2054 Protein Induces Macrophage Apoptosis through Targeting to Mitochondria and Enhances Intracellular Survival of the Bacteria

Kang-In Lee, Chungnam National University

S19-4 09:45-10:00

Comparison of Genotyping of *Helicobacter pylori* CagA in Gastric Disease

Dong-Hae Lee, Gyeongsang National University

S19-5 10:00-10:15

Clinical Evaluation of Immunemed *Leptospira* Rapid Kit

Jin-Woo Lee, ImmuneMed

S19-6 10:15-10:30

Production of Reference Standard for Determining the Cut-off of the Scrub Typhus Rapid Kit

Min-Woo Kim, Hallym University

S19-7 10:30-10:45

Genipin as a Novel Chemical Activator of the Gammaherpesvirus Lytic Cycle

Myoungki Son, Kyungpook National University

S23 Young Scientist Session IV

October 31(Fri.), Room 304

Chair: Hee-Wan Kang, Hankyong National University

S23-1 13:00-13:15

Ecophysiological and Genomic Characterization of Methylotrophic Bacteria belonging to the LD28 Clade from Lake Soyang

Mihye Im, Inha University

S23-2 13:15-13:30

Loss of Microbial Diversity Resulted in Enhanced Diesel-Bioremediation at a Cost of Trade-off in Ecological Functions

Jaejoon Jung, Korea University

S23-3 13:30-13:45

Physiology and Genomic Characteristics of Strain IMCC3023, a Marine Actinobacterium Isolated from Arctic Seawater, Encoding Actinorhodopsin Gene

Taeyang Kwon, Inha University

S23-4 13:45-14:00

Diversity of fungi from Dokdo Island Soil, Korea and Their Antimicrobial and Hydrolytic Enzyme Activity

Hye Won Lee, Chonnam National University

S23-5 14:00-14:15

Intraspecific Functional Variation of Arbuscular Mycorrhizal Fungi Originated from Single Population on Plant Growth

Eun-Hwa Lee, Korea National University of Education

S23-6 **14:15-14:30**

Isolation and Characterization of Fungal Diversity from Crop Field Soils of Nigeria

Dil Raj Yadav, Kangwon National University

S23-7 **14:30-14:45**

Analysis of Fungal Communities on Ulleungdo and Dokdo Islands

Yoon-Jong Nam, Kyungpook National University

S27 Young Scientist Session V

October 31 (Fri.), Room 304

Chair: Nam-Chul Ha, Seoul National University

S27-1 **15:45-16:00**

Heterologous Expression of a New Manganese-Dependent Peroxidase Gene from *Peniophora incarnata* KUC8836 *in Saccharomyces cerevisiae*

Hwanhwi Lee, Korea University

S27-2 **16:00-16:15**

Cold Stress Improves the Ability of *Lactobacillus plantarum* L67 to Survive Freezing

Sooyeon Song, Chonnam National University

S27-3 **16:15-16:30**

The Acid Stress Response in *Lactobacillus rhamnosus* LGG

Miseon Bang, Chonnam National University

S27-4 **16:30-16:45**

Changes of Bacterial Communities in Myeolchi-jeot, Fermented Anchovy, during Fermentation

Se Hee Lee, Chung-Ang University

S27-5 **16:45-17:00**

HPr of the *Vibrio vulnificus* PTS Confers Resistance to H_2O_2 Stress by Stimulating Pyruvate Kinase A Activity

Hey-Min Kim, Seoul National University

S27-6 **17:00-17:15**

proP P promoter Derived Transcript is Posttranscriptionally regulated by RNase Ⅲ activity in *Escherichia coli*

Boram Lim, Chung-Ang University

S27-7 **17:15-17:30**

Functional Characterization of *Cryptococcus neoformans KRE2/MNT* and *OCH* Gene Family encoding Novel Mannosyltransferases involved in *O*-linked Glycans Biosynthesis

Dong-Jik Lee, Chung-Ang University

S27-8 **17:30-17:45**

Isolation and Characterization of Extended-spectrum-β-lactamase-producing Non-typhoidal *Salmonella* in Retail Chicken Meat in South Korea

Dasom Choi, Konkuk University

S28 Young Scientist Session VI

October 31 (Fri.), Room 303

Chair: Gyu-Cheol Lee, Water Quality Research Center, K-water

S28-1 **15:45-16:00**

MicroRNA-25a Inhibits Autophagy Activation and Antimicrobial Responses during Mycobacterial Infection

Jin Kyung Kim, Chungnam National University

S28-2 **16:00-16:15**

Evaluation of an Autotransporter protein of *Orientia tsutsugamushi* as a Vaccine Antigen for Scrub Typhus

Na Young Ha, Seoul National University

S28-3 **16:15-16:30**

Regulation of Metabolic Signaling of T cell by a Herpesviral Protein

Kim Yuri, Seoul National University

S28-4 **16:30-16:45**

Human Endogenous Retrovirus Envelope-Coated, Baculovirus-Based, VLP forming DNA Vaccine for Influenza pdmHN

Yongdae Gwon, Konkuk University

S28-5 **16:45-17:00**

Oral Administration of Multivalent White Spot Syndrome Virus DNA Vaccine Fused *Salmonella Typhimurium* Flagellin 2 in *Macrobrachium Nipponense*

Hansam Cho, Konkuk University

S28-6 **17:00-17:15**

Structure of the Tripartite Multidrug Efflux Pump AcrAB-TolC Shows an Intermeshing Cogwheel Interaction between AcrA and TolC

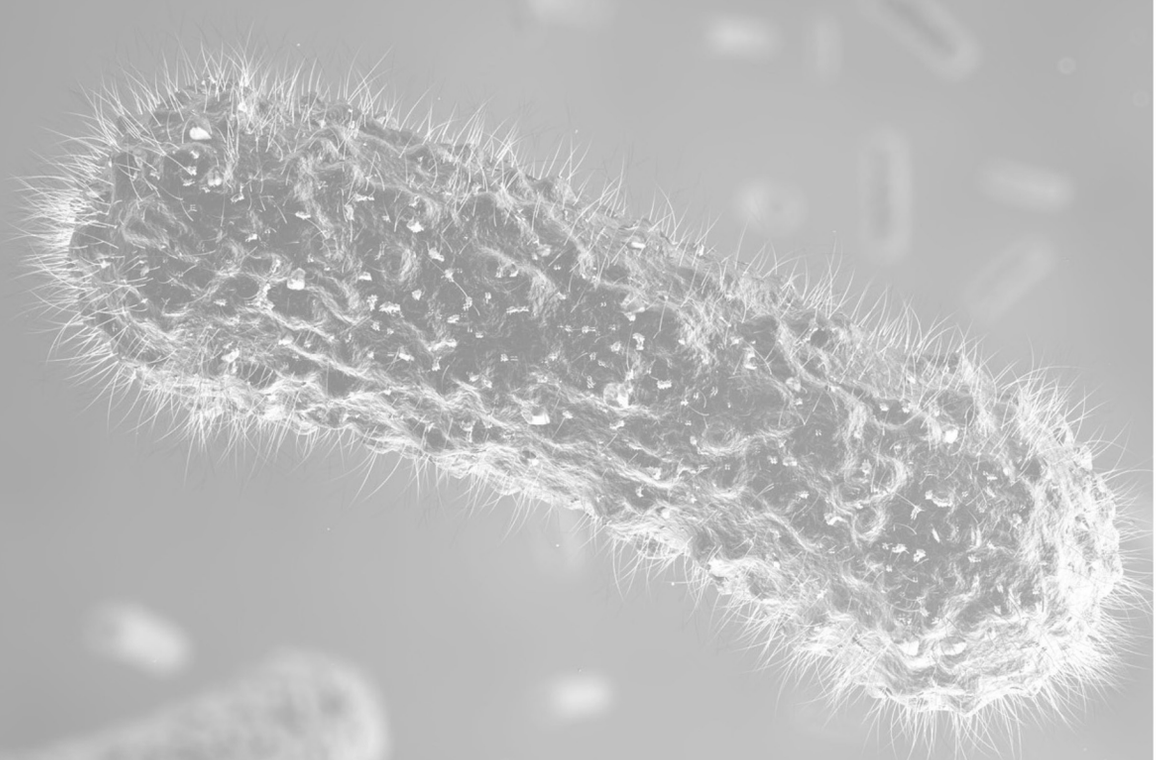
Jin-Sik Kim, Seoul National University

S28-7 **17:15-17:30**

Effects of Various Culture Media on the Expression of Shiga Toxins in Enterohemorrhagic *Escherichia coli* O157:H7

Hyung Tae Lee, National University

Plenary Lectures



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INTERNATIONAL MEETING OF
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PL1

Fumarate and Succinate Metabolism in *E. coli*: Regulation by a Transporter/Sensor Complex

G. Unden

Johannes Gutenberg University Mainz, Germany

C_4-dicarboxylates like fumarate, succinate, or L-malate represent for many bacteria important substrates or products of energy metabolism that are closely connected to central metabolic pathways. Their pathways for aerobic and anaerobic degradation differ during aerobic and anaerobic metabolism, resulting in oxidation to CO_2 under the former and fumarate reduction to succinate under the latter conditions. Under both conditions different transporters are used due to the need for C_4-dicarboxylate uptake in aerobic and antiport under anaerobic growth conditions.

In *E. coli* the C_4-dicarboxylates are sensed by the membrane integral C_4-dicarboxylate sensor kinase DcuS. DcuS responds to externally supplied C_4-dicarboxylates and becomes autophosphorylated. The phosphoryl group is transferred to the response regulator DcuR that activates the expression of the genes encoding the transporters (DctA for the C_4-dicarboxylate uptake during aerobic growth, and DcuB forC_4-dicarboxylate/succinate antiport, fumarase FumC and fumarate reductase FRD under anaerobic conditions).

The function of the sensor kinase DcuS depends on the transporters DctA and DcuB as co-regulators, respectively. DcuS forms with the transporters a DcuS/DctA or DcuS/DcuB complex with a new mode of signal perception and processing. Signal perception is effected only by the extracytoplasmic sensor domain of DcuS, a PAS domain. The transporter are bifunctional proteins with regulatory and transport functions which can be separated by mutation. The transporters confer C_4-dicarboxylate responsiveness to the sensor kinase but have no sensory function on their own. Transport activity or flux sensing is not involved in signal perception. Signal transduction across the membrane occurs by a piston type movement of one transmembrane helix of DcuS, and by the cytoplasmic PAS domain the signal is transferred in an unknown mode to the kinase domain.

Keywords: DctA transporter, DcuB antiporter, DcuS sensor kinase, DctA/DcuS sensor complex, bifunctional transporter, PAS domain, signal perception, signal transduction

PL2

"Agro-environmental Microorganisms Inventory" – Application to Biocontrol and the Degradation of Plastic and Mycotoxin –

Motoo Koitabashi

National Institute for Agro-Environmental Sciences, Japan

The plant surface, also known as the phyllosphere, is one of the most common habitats of terrestrial microorganisms. On the plant surface, microorganisms encounter various environmental stresses, such as rain, desiccation, ultraviolet radiation, and exposure to chemicals. This implies that these microorganisms have many different functions compared with microorganisms in other environments. Then, we have named the microbial inventory, which we constructed, '*microForce*®'. The microbial inventory '*microForce*®' contains various phyllosphere microorganisms databases. We can use utilization and development for practical.

[1] Biocontrol of plant disease

Microorganisms isolated from wheat leaf surfaces were screened for inhibition of wheat powdery mildew. A new screening method, in which wheat leaves were inoculated with *Blumeria graminis* f. sp. *tritici* and incubated with the cultured microorganisms under non-contact conditions, was developed in our study. Among these strains, a fungus designated as Kyu-W63 had an especially strong inhibitory effect. Kyu-W63 had a strong aromatic odor when being cultured. Nuclear magnetic resonance analysis revealed that Kyu-W63 produced two types of volatile substances, 5-pentyl-2-furaldehyde and 5-(4-pentenyl)-2-furaldehyde. The antifungal activity of 5-(4-pentenyl)-2-furaldehyde is first confirmed in our study. Strain Kyu-W63 completely agreed with *Irpex lacteus* in the rDNA internal transcribed spacer (ITS) sequences, and strain Kyu-W63 was inferred to be *I. lacteus*. Biocontrol of parsley powdery mildew was examined using a filamentous fungus, Kyu-W63, that produces antifungal volatiles, for 3 years under greenhouse conditions. Kyu-W63 treatment significantly inhibited disease severity compared to control plots. In addition, Kyu-W63 suppressed the other harmful fungi such as *Penicillium* sp., *Aspergillus* sp. which are the pathogens of plant or the allergens of human existing in environment.

[2] Biodegradable plastic degradation

To improve the biodegradation of biodegradable plastic (BP) mulch films, 1227 fungal strains were isolated from plant surface and evaluated for BP-degrading ability. Among them, B47-9 a strain isolated from the leaf surface of barley showed the strongest ability to degrade poly-(butylene succinate-co-butylene adipate) (PBSA) and poly-(butylene succinate) (PBS) films. The strain grew on the surface of soil-mounted BP films, produced breaks along the direction of hyphal growth indicated that it secreted a BP-degrading enzyme, and has directly contributing to accelerating the degradation of film. The deduced amino acid sequence suggested that this enzyme belongs to the cutinase family. Treatment with the culture filtrate decomposed 91.2 wt%, 23.7 wt%, and 14.6 wt% of PBSA, PBS, and commercially available BP polymer blended mulch film, respectively, on unsterilized soil within 6 days. The PCR-DGGE analysis of the transition of soil microbial community during

film degradation revealed that the process was accompanied with drastic changes in the population of soil fungi and *Acantamoeba* spp., as well as the growth of inoculated strain B47-9.

[3] Mycotoxin degradation

Deoxynivalenol (DON) is a hazardous and globally prevalent mycotoxin in cereals. It commonly accumulates in the grain of wheat, barley and other small grain cereals affected by Fusarium head blight (caused by several *Fusarium* species). The concept of reducing DON in naturally contaminated grain of wheat or barley using a DON-degrading bacterium is promising but has not been accomplished. We isolated a novel DON-utilising actinomycete, *Marmoricola* sp. strain MIM116, from wheat heads through a novel isolation procedure including an in situ plant enrichment step. The inoculation of MIM116 cell suspension plus 0.01% Tween 80 into 1,000 harvested kernels of wheat and barley resulted in a DON decrease from approximately 3 mg kg(-1) to less than 1 mg kg(-1) of dry kernels, even when cells had only basal levels of DON-degrading activity.

References

[1] Koitabashi M. (2005) Journal of General Plant Pathology 71:180-184.
[2] Koitabashi M. et al. (2012) AMB Express 2:40. Suzuki K. et al. (2014) Appl. Microbiol. Biotechnol. 98:4457-4465.
[3] Ito M. et al. (2012) Appl. Microbiol. Biotechnol. 96:1059-1070.

Keywords: plant surface, phylloplane fungi, biocontrol, biodegradable plastic, mulch film, mycotoxin, deoxynivalenold

PL3

The Role of BRCs as the Infrastructure of the Prokaryote Taxonomy

Ken-ichiro Suzuki

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Culture collections are the important infrastructure for microbiology and contributing to the ex situ conservation of microbial diversity. Culture collections are providing microbial strains for the study of microbial taxonomy as well as for the references of industrial standardized tests and the screening sources to explore new bioactive compounds or new functions.

Recent progress of microbiology and demands of new genetic resources require culture collections to have high level techniques and knowledge for cultivation, preservation and quality management. In addition, different from the plants and animals, both of which assign dead specimen as the taxonomic type of species, the type of the prokaryotes must be a living culture in principle.

In 1980, as the starting point of the new taxonomic system of the prokaryotes, Approved lists of bacterial names (1) were published in accordance with the Bacteriological Code, which contained only 1,792 species of 290 genera of bacteria and archaea as the correct names. Since then, proposals of new taxa have been actively published in International Journal of Systematic and Evolutionary Microbiology (IJSEM) including publication outside of IJSEM followed by the validly published in IJSEM later, the number of names of the prokaryotes accounted for 2,393 genera and 12,391 species as of the end of August, 2013 (2). In these 9 years, progresses of isolation and cultivation techniques as well as the taxonomic characterization have accelerated the publication so that more than 500 species have been published every year. All of the type strains of these species have been essentially preserved in some culture collections in the world for further utilization, especially for taxonomic comparison.

In 1999, International Committee on Systematic Bacteriology (ICSB) decided to request authors to deposit type strains in at least two culture collections in two different countries to make it publically accessible (3). One of the reasons of this system is to avoid possible loss of type strains by their death or unexpected accident in preservation by mutual back-up system. The other reason is to secure the international accessibility of the type strains.

Since the soverign rights of states over their biological resources have been recognized on the bases of the Convention on Biological Diversity (CBD) (4), international transfer of biological materials has become challenging issue. In academia, biological resources had been freely shared among scientists for their studies by their spontaneous exchange. It is true that this custom supported the progress of science by sharing materials commonly as shown in the case of *Escherichia coli* strain K-12. Principles of the CBD do not intend to prohibit but rather promote the research contributing to the conservation and sustainable use of biological diversity. In addition, the Nagoya Protocol entered into force on 12 October of this year. The roles of biological resource centers (BRC) including the function of culture collection have become more important in legitimate access and utilization in compliance with the relevant laws and regulations internationally and domestically. The CBD also promotes and encourages research particularly in corporation with developing countries. Taxonomy is one of the

most suitable sciences for conservation and sustainable use of biological resources. Therefore, BRCs are expected to be national centers for the depository of the taxonomic type strains of the prokaryotes as components of the international network of BRCs.

References

[1] Skerman, V.B.D., McGowan, V. & Sneath, P.H.A. (1980). Approved lists of bacterial names. Int. J. Syst. Bacteriol. 30, 225-420.
[2] Euzby, J. List of prokaryotic names with standing nomenclature. http://www.bacterio.net/
[3] International Committee on Systematic Bacteriology (2000). Minutes of the meetings, IXth International (IUMS) Congress of Bacteriology and Applied Microbiology. Int. J. Syst. Bacteriol. 50, 2245-2247.
[4] Convention on Biological Diversity. http://www.cbd.int/

Key words:culture collection, biological resource center, Convention on Biological Diversity, Bacteriological Code, type strain

PL4

Wall Teichoic Polymers in *Staphylococcus aureus* Physiology and Host Interaction

Andreas Peschel

Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Germany

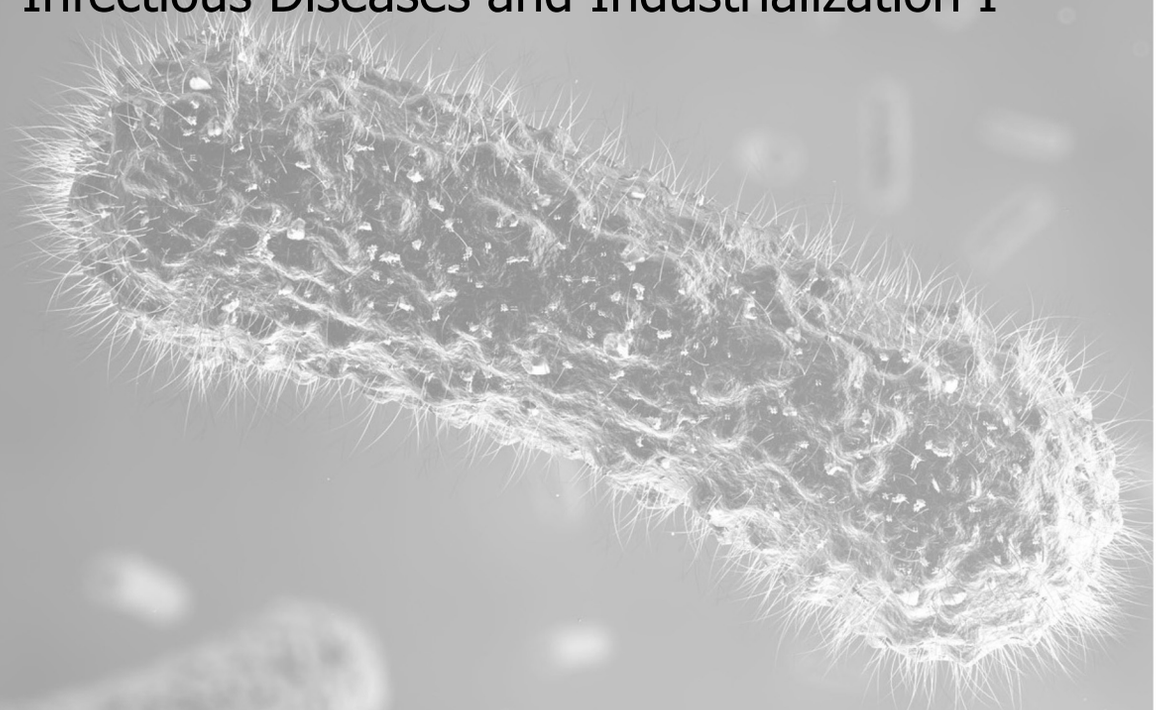
The thick peptidoglycan layers of Gram-positive bacteria are connected to polyanionic glycopolymers called wall teichoic acids (WTA). Pathogens such as *Staphylococcus aureus* produce WTA with diverse, usually strain-specific structure. Extensive studies on *S. aureus* WTA mutants revealed important functions of WTA in cell division, growth, morphogenesis, resistance to antimicrobials, and interaction with host or phages. While most of the *S. aureus* WTA-biosynthetic genes have been identified it remained unclear for long how and why *S. aureus* glycosylates WTA with α- or β-linked N-acetylglucosamine (GlcNAc). Only recently the discovery of two WTA glycosyltransferases, TarM and TarS, yielded fundamental insights into the roles of *S. aureus* WTA glycosylation. Mutants lacking WTA GlcNAc are resistant towards most of the *S. aureus* phages and, surprisingly, TarS-mediated WTA β-O-GlcNAc modification is essential for β-lactam resistance in methicillin-resistant *S. aureus*. Notably, *S. aureus* WTA GlcNAc residues are major antigens and activate the complement system contributing to opsonophagocytosis. Moreover, WTA structure was found to direct the routes of horizontal gene transfer even across long phylogenetic distances via WTA-binding phages.

Keywords: bacterial cell envelope, microbe-host interaction, glycopolymers, innate and adaptive immunity, antibiotic resistance, bacterial pathogens

Co-hosted by Infection Signaling Network Research Center, Chungnam National University and FKMS

Special Symposium [1]

Infectious Diseases and Industrialization I



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INTERNATIONAL MEETING OF

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SS1-1

Regulatory Status for Drug Review and Approval of Anti-Infectives

So Hee Kim

Oncology & Antimicrobial Products Division, Ministry of Food and Drug Safety

One of the missions of Ministry of Food and Drug Safety (MFDS) is to ensure that drugs marketed are safe and effective. Drug evaluation department has responsibility for evaluation of quality, safety and efficacy both prescription and nonprescription drugs before they can be sold.

For Drug development process, after obtaining promising data from laboratory studies, drug developers take the next step and submitted an Investigational New Drug (IND) application to MFDS. Once the IND application is in effect, the drug sponsor could begin their clinical trials. Clinical trials are experiments that use human subjects to see whether a drug is effective, and what side effects it may cause. Drug sponsor analyzed the clinical trials data and concluded that enough evidence existed on the drug's safety and effectiveness to meet MFDS's requirements for marketing approval. The sponsor submitted a New Drug Application (NDA) with full information on manufacturing specifications, stability and bioavailablility data, method of analysis of each of the dosage forms the sponsor intends to market, packaging and labeling, and the results of any additional toxicological studies not already submitted in the Investigational New Drug application.

Infectious diseases are still one of humanity’s greatest problems. For public health there is an urgent medical need to discover and develop novel classes of anti-infectives. The MFDS established several scientific guidance on clinical evaluation of anti-infectives to facilitate drug development. In this presentation, I’d like to show overview of drug evaluation and approval process with some related statistics and also introduce clinical guidelines for anti-infectives.

Keywords : drug review, approval, anti-infectives

SS1-2

What Medical Inventions are Patentable-Ebola Patent Case

Min SON, Ph.D., Patent Attorney

Hanol Intellectual Property and Law

Ebola may be one of the most dangerous infectious diseases in the world. A potential drug for this deadly disease, ZMapp, was developed by Mapp Biopharmaceuticals, a small private company based in San Diego. The technique of ZMapp is covered by a US patent application. ZMapp has received worldwide attention due to the patent application covering this potential drug.

The first patent application for ZMapp was filed back in 2011 in the United States. It received the first Office Action recently. Because of recent changes in the patent system in the United States, especially in terms of patentable subject matters, people expressed concern as to whether the antibody claimed in an Ebola patent could be granted under the new guidelines issued after the *Myriad Genetics* case.

Medical inventions are usually marketed globally. They also heavily rely on patent protection for the success of the product in the market. Patent protection is crucial for commercialization of medical inventions because of the high risk and long-term investment to make one marketable product. Planning a global patent strategy is, therefore, one of the most important things for pharmaceutical companies to do for successful commercialization of a drug, but is more complex than is usually thought.

One of the reasons is the diversity of patent systems between countries. Each country has a different patent system, even though they share many basic concepts. Biotechnology is the area where the global patent system shows least harmonization, especially in terms of patentable subject matters and substantial patent examination. The other reason may be rapid changes in countries economic, social, and legal environment. It is important to understand these diversities and recent changes in patent systems to prepare a good patent strategy for the global market.

Medical inventions could be categorized into several important subject matters:

(i) Diagnostics, (ii) Medical treatment, (iii) Drugs (Second medical use), (iv) Genes & Proteins, (v) Stem cells, (vi) Animals, and (vii) personalized medicines as an emerging market. Recently, the patent law and practices of the United States has changed dramatically. For example, in 2013, the U.S. Supreme Court ruled in the *Myriad* case that a human genome cannot be patented. The impact of this decision on bio-medical business is hardly comprehendible, and there is still much debate about how far this decision should be applied to biological materials. In another decision called the *Mayo* case, the Supreme Court held that the correlation between naturally-produced metabolites and their therapeutic efficacy and toxicity to be an unpatentable “natural law”. However, many European and other countries see these subject matters differently.

What is important here, is that, companies who make diagnostics or develop any therapeutics need to be aware of these changes, and plan their patent strategies accordingly. This presentation will summarize how these important patentable subject matters are treated differently in various countries.

Keywords: ebola, patentable subject matter, patentability, biotechnology, patent commercialization strategy, global patent strategy

SS1-3

Development of Rapid Diagnostic Test Kit for Global Leptospirosis and Its MFDS (KFDA) Approval

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Leptospirosis is classified as one of 3rd class legal infectious diseases in Korea and is caused by bacteria, called *Leptospira interrogans*. It is transmitted by both wild and domestic animals. The most common animal that spreads the disease is rodent. It is often transmitted by animal urine or water containing animal urine through contact with breaks in the skin, or with mucosa of the eyes, mouth or nose. In the developing world, the disease commonly occurs in farmers and poor people who live in cities.

Symptoms can range from none or mild symptom such as headache, muscles pain, and fever to severe with bleeding from the lungs or meningitis. These symptoms are common among other acute febrile illnesses, which is made doctors to have difficulties to differentiate this leptospirosis from others. Without early treatment, the disease would be severe and often go to death. Therefore, early and accurate diagnosis is very important for the treatment of patient. Until now, the gold standard diagnostic method is MAT (microscopic agglutination test). To perform this MAT, the endemic serovars of *Leptospira interrogans* should always be culturing for diagnosis, and each serovar is mixed with patient serum by specialist to diagnose under dark field microscopy. In interpreting the result, it tremendously occur that untrained personnel misinterprets the result as false positive or false negative.

Overcoming these difficulties ImmuneMed Inc. has developed a rapid and accurate diagnostic kit for leptospirosis. This kit is developed based on genus specific antigen using lateral flow immunochromatographic assay and it takes only 15 minutes to interpret the result. This study is to validate the performance of the rapid diagnostic kit for Leptospirosis in terms of domestic and international clinical evaluation. In Korea, the sensitivity and specificity are 96.4% and 98.4% respectively. From international clinical evaluations done by Bulgaria, Argentina and Malaysia, each sensitivity and specificity are 100% and 100%, 81% and 95.4% and 87.7% and 62%.

According to WHO report, it is known that over 200 serovars of *Leptospira* are widely distributed worldwide. For a high sensitivity against majority of serovars, the genus specific antigen must be necessary. Because of ImmuneMed Leptospira Rapid Kit is excellent, the antigen, surface polysaccharide of *Leptospira* which is used in this kit, is interpretated as genus specific antigen.

Now, we are proceeding the manufacturing and sales approval of this kit as in vitro diagnostic medicine from MFDS(KFDA).

Keywords: leptospirosis, rapid diagnostic test kit, MFDS, approval

SS1-4

Ex vivo Expanded Allogeneic Natural Killer Cell Therapy for Cancer Patients

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NK cells from healthy donors under good manufacturing practice (GMP) conditions. From healthy donor's peripheral blood mononuclear cells (PBMCs), the NK cells were expanded for 14 days, resulting in a highly pure population of CD3−CD16+CD56+ NK cells which is desired for allogeneic purpose. Compared with freshly isolated NK cells, these expanded NK cells showed robust cytokine production and potent cytolytic activity against various cancer cell lines. Of note, expanded NK cells selectively killed cancer cells without demonstrating cytotoxicity against allogeneic non-tumor cells in coculture assays. The anti-tumor activity of expanded human NK cells was examined in SCID mice injected with human lymphoma cells. Ex vivo-expanded, allogeneic natural killer (NK) cells are infused into 18-advanced and refractory cancer patients with various solid tumor and lymphoma. No adverse effects are reported after, and changes of immune cell population and cytokine/chemokine levels are showed by NK cell infusion. Therefore, allogeneic NK cell treatment without T cell contamination is feasible for cancer patients, and is expected good clinical outcome if it is used with adequate preconditioning regimen.

Special Symposium [2]

Infectious Diseases and Industrialization II

Co-hosted by
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2014 한국미생물학회연합 국제학술대회
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SS2-1

IVD Approval Regulations of MFDS

Won Kyu Lee

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The in vitro diagnostics(IVD) sector will be the largest medtech segment in 2018 due to the change of desease management paradigm from treatment to prevention by early diagnosis, according to a recent UN report. IVD products are those reagents, instruments, and systems intended for use in diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, or prevent disease or its sequelae. Ministry of Food and Drug Safety (MFDS) classifies IVD products into Class I~IV according to the level of regulatory control that is necessary to a assure safety and effectiveness. For Class I devices list Pre-Market Notification application including basic device informations to regional Food and Drug Administration, and for Class II~IV devices submit general technical files or safety & effectiveness technical files to MFDS for approval, respectively.

SS2-2

LMO 법률 및 시험 · 연구용 LMO 안전관리 제도

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「유전자변형생물체의 국가간 이동 등에 관한 법률」(이하 "LMO법")에 따라, 유전자변형생물체를 개발하거나, 이를 이용하여 실험을 하는 시설(이하 "연구시설")은 안전관리등급별로 신고 또는 허가 받아야 한다.

1등급 및 2등급 연구시설은 미래창조과학부에 신고하고, 3등급 및 4등급 연구시설은 환경위해성과 인체위해성을 구분하여 환경위해성 3, 4등급 연구시설은 미래부에, 인체위해성 3, 4등급 연구시설은 보건복지부의 허가를 받아야 한다.

신고 또는 허가받은 연구시설은 그 종류 및 안전관리등급에 따른 안전기준(LMO 통합고시 별표 9-1~4)을 준수해야 하며, 수출입 등 관리·운영에 관한 기록을 작성하여 보관하여야 한다.

또한, 시험·연구용으로 사용하기 위해 유전자변형생물체를 수입하려는 자는 사전에 미래부에 신고해야 하며, 수출하려는 경우에는 사전에 미래부에 통보해야 한다.

시험·연구용 LMO를 이용하여 포장시험 등 환경방출 실험을 하려는 경우에는 미래부에 개발·실험 승인을 신청하고, 미래부 전문가심사위원회의 심의를 거쳐 승인받은 후에 실험을 실시할 수 있다.

LMO법에 따른 안전관리제도의 미 이행 시에는 벌칙 및 과태료가 부과될 수 있으므로 해당 연구기관 및 연구자는 이를 숙지해야 하며, 관련 세부 내용은 "시험연구용 LMO 정보시스템(http://biosafety.msip.go.kr)" 및 "한국생명공학연구원 LMO연구안전센터"에서 안내 받을 수 있다.

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Keywords: 유전자변형생물체, 시험·연구용 LMO, 안전관리, 연구시설, 안전관리등급

SS2-3

유전자변형생물체(Living Modified Organism)연구 안전관리와 LMO법 제도

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인위적인 유전자재조합 기술에 의하여 만들어진 유전자변형생물체(Living Modified Organisms, LMO)는 질병, 기아, 환경오염 등 인류가 직면하고 있는 각종 문제점을 해결하기 위하여 전 세계적으로 연구개발이 활발하게 이루어지고 있다. LMO의 연구개발 및 이용, 수입 및 수출이 증가하면 할수록 LMO개발부터 상업화에 이르기까지 전 과정에 대한 철저한 안전관리에 대한 요구와 관심도 높아지고 있다. 이에 따라 국제적으로 LMO가 인체 및 환경에 잠재적으로 미칠 수 있는 부정적인 영향을 사전에 예방하기 위하여 바이오안전성의정서(Biosafety Protocol)가 채택·발효되었고 국내에서도 의정서 이행을 위하여 제정한 「유전자변형생물체의국가간이동등에 관한법률」(이하 "LMO법"이라 한다)이 2008년 1월부터 발효되었다. 동 법률에 따라 연구시설에서 이용되는 LMO는 시험·연구용으로 분류하고 미래창조과학부에서 안전관리를 담당하고 있다. 미래창조과학부와 한국생명공학연구원 LMO연구안전센터는 시험·연구용 LMO의 수출입 신고, 각 부처 소관 연구기관을 제외한 전국의 모든 대학, 병원, 기업연구소의 1,2등급 LMO연구시설에 대한 신고와 안전점검, 지속적인 사후모니터링을 수행하고 있다. 본문에서는 유전자 재조합과 형질전환, 형질특성 평가 등을 수행하는 연구시설을 운영하는 기관과 LMO연구자들이 이행해야 하는 LMO법 제도 사항을 소개하고자 한다.

SS2-4

Natural Killer Cell Immune Function, as Assessed by the NKVue® Assay, Correlates with Clinical Cancer Stage

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Natural killer (NK) cells are lymphocytes of the innate immune system and have the ability to kill tumor cells and virus-infected cells without prior sensitization. Malignant tumors and viruses have developed, however, strategies to suppress NK cells to escape from their responses. Thus, the evaluation of NK cell activity (NKA) could be invaluable to estimate the status and the outcome of cancers, viral infections, and immune-mediated diseases. Established methods that measure NKA, such as ^{51}Cr release assay and CD107a degranulation assay, may be used to determine NK cell function, but they are complicated and time-consuming because they require isolation of peripheral blood mononuclear cells (PBMC) or NK cells. In some cases these assays require hazardous material such as radioactive isotopes. To overcome these difficulties, we developed a simple assay that uses whole blood instead of PBMC or isolated NK cells. This novel assay is suitable for high-throughput screening and the monitoring of diseases, because it employs serum of ex vivo stimulated whole blood to detect interferon (IFN)-γ secreted from NK cells as an indicator of NKA. After the stimulation of NK cells, the determination of IFNγ concentration in serum samples by enzyme-linked immunosorbent assay provided a swift, uncomplicated, and high-throughput assay of NKA ex vivo. The NKA results variety of cancer patients was showed significantly lower NKA compared with healthy subjects. Therefore, the NKA could be utilized as a supportive and add-on diagnostic marker for cancer.

Keywords: NK cell, NKVue Kit, prostate cancer, gastric cancer

Special Symposium [3]

Infectious Diseases and Industrialization III

Co-hosted by
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2014 한국미생물학회연합 국제학술대회
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SS3-1

Development of DA-7218 (Tedizolid phosphate), a Next-Generation Oxazolidinone

Sunghak Choi

Dong-A ST

The oxazolidinones represent a novel chemical class of synthetic antimicrobial agents. Tedizolid phosphate (DA-7218) is a second generation oxazolidinone prodrug antibiotic that is rapidly converted in vivo by phosphatases to the microbiologically active moiety Tedizolid (DA-7157). Tedizolid is a protein synthesis inhibitor that interacts with the 23S ribosomal ribonucleic acid (rRNA) of the bacterial ribosome, thereby preventing the initiation of translation by inhibiting formation of the initiation complex. Due to its unique molecular structure and target site-binding properties, Tedizolid has a potent antibacterial activity against Gram-positive pathogens such as methicillin-resistant *Staphylococccus aureus*, penicillin-resistant *Streptococcus pneumoniae* and vancomycin-resistant enterococci. It is also active against pathogens that have developed resistance to linezolid, a first generation oxazolidinone antibiotic. With a long half-life, high oral bioavailability and solubility and high potency, Tedizolid phosphate will provide clinicians with flexible dosing and a possible shorter course of therapy thereby enabling the early transition of hospitalized patients from IV to oral treatment and subsequent early discharge from the hospital. Tedizolid phosphate had successfully completed Phase I, Phase II and Phase III clinical trials. Sivextro (Tedizolid phosphate) has been approved by the FDA on June 20, 2014 for the treatment of acute bacterial skin and skin structure infections (ABSSSI) caused by Gram-positive bacteria. In this presentation, overall progress of the development of Tedizolid phosphate will be discussed.

Keywords: tedizolid, DA-7218, oxazolidinone, ABSSSI, gram-positive bacteria

SS3-2

Triangular Cooperation of Industrialization of Diagnostic Kit for Acute Viral Infectious Diseases; a Lesson from Pandemic Influenza in 2009

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Majority of infectious diseases caused by viral infection is often characterized by acute progress of clinical symptoms within several days of pathogen invasion. The early detection of causative agent plays a key factor for intervention of disease progression of patients and helpful for clinicians to decide treatment strategy and even disease control practices. Beside sporadic case of infectious disease, regional or nation-wide spreads should be accompanied with large quantity production of diagnostic kits when outbreak of the disease is occurred. In this reason, standardized and continuous supply of diagnostic reagents with reasonable specificity and sensitivity is required through industrial level of production to control diseases or outbreaks effectively. To encompass contentment for both sides of necessities from accuracy and availability of the diagnostic strategy, public health sector and industrial affiliation should work together. Here we would like to share valuable experience to expedite development of rapid antigen test (RAT) kit for prompt response of pandemic influenza which happened in 2009. Precise and crucial information of newly emerged influenza virus (pdm09) was consolidated and development of diagnostic strategy was established with accessibility and sustainability of follow-up steps. Once the viral characteristic was clarified, then industrial counterpart, which had enough experience of mass production of corresponding diagnostic agents took over next process. Prototype kit was developed and sent back to public health sector again to evaluate efficacy where sufficient field specimens were available. Fast track investigation of permission was done by the licensing authority in consideration of pandemic situation. In conclusion, at least three factors should be allowed for successful industrialization of infectious disease diagnosis. Sophisticated understanding of pathogen should be examined with priority. Sustainability of target material production is well-appointed. Finally and most importantly, cooperative network between public health research and industrial sector should be maintained to value the product.

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Keywords : industrialization, diagnostic kit, acute viral infection, pandemic influenza

SS3-3

Korea Chemical Bank: National Repository of Small Molecular Organic Compounds for New Drug Discovery

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Screening a large number of small molecular weight-chemicals obtained from synthetic or natural sources has become a mainstay in the chemical biology and new drug discovery researches. Success in high throughput screening (HTS) for biological activities is mainly depending on the quality of the chemical library to be screened, such as diversity, physicochemical properties, and the number of the compounds. Consequently, an expansion of compound collection with structurally diverse compounds and a proper management of the chemical library to support HTS has become a critical task in the drug discovery process. Korea Chemical Bank (KCB) has been officially designated as the chemical library management institution by Korea Government and serving as the national repository of small molecular weight-chemicals for supporting the chemical biology and drug discovery researches.

The chemical library of KCB is composed of, currently, more than 260,000 biologically-relevant stock compounds collected from domestic and abroad, and these compounds have been provided to researchers in the field of drug discovery, chemical biology, and chemical genomics.

KCB has also been managing millions of bioassay data of the provided-compounds in the form of cheminformatics database. The combined bioassay data and chemical structure information are being analyzed and under processing to establish an integrated chemical structure-bioassay data information system in KCB.

The current status of KCB chemical library utilizations, managing system of KCB including compound and biological activity information management systems developed by KCB will be introduced.

Keywords : HTS, Chemical Library, Drug Discovery, Chemical Biology, Chemical Genomics

SS3-4

Acquisition, Management and Application of Bioproducts

Byoung-Chan Kim and Doo-Sang Park

Korean Collection for Type Cultures (KCTC), Biological Resource Center (BRC), Korea Research Institute of Bioscience and Biotechnology (KRIBB)

Research products refer to eight types of products procured through the National R&D Project. In order to build a basis to vitalize the utilization of research products through systematic management and establishment of their use, the Ministry of Science, ICT and Future Planning (MSIP) enacted the 'Regulation on National R&D Project Management (Executive Order) and the enforcement rule for the regulation. According to the regulation, research products on biological resources (Bioproducts) are to be deposited in and registered to the institution specialized in research products, appointed by the Central Administrative Agency; the MSIP appointed and notified (2013.9.05) that biological resources be under the jurisdiction of the KRIBB as the specialized institution. Hence, the KCTC/BRC of the KRIBB currently carries out tasks concerning the deposition, management and utilization of research outcomes in various resources including microorganisms, animals, plants and genomes through relevant procedures. As a result, more than 1,100,000 biological resources have been deposited and more than 5,000 bioproducts have been distributed. The national systematic managements of biological resources for preservation and distribution will stimulate their scientific and industrial application.

Keywords: bioproducts, management, preservation, distribution

SS3-5

Introduction of Biological Information Registration System for Biotechnology Research Outcomes (http://biodata.kr/)

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Korea Research Institute of Bioscience & Biotechnology (KRIBB)

Large amount of biological information, deposited by the research communities and freely available as a public database such as GenBank and web-based subsidiary services, has been considered to be an invaluable resource for the scientific research and industrial applications. Nowadays, it is a new challenge and opportunity to unveil biological phenomena with big data derived from Next-Generation Sequencing (NGS) technology. It is also important to integrate and manage those big data including sequencing data and associated metadata for public access, enhancing biological research resources. KOBIC is responsible for the management and distribution of research outcomes such as biological information (genomes, transcriptomes, proteomes, etc.) conforming to the Regulation on Management of National Research and Development Projects. While previous version of registration system for biological big data were presented as a simple web-based platform with several fields to deal with only small amount of data files, we recently developed a new version of registration system to handle large dataset based on the high-throughput file transfer application (KoDS, KOBIC Data Transfer System), user authentication, and retrieval of project information from the NTIS (National Science & Technology Information Service). These registration systems are composed of database management system and massive data storage systems. NGS-derived primary data or further analyzed data is allocated into one of six categories of newly designed biological information classification schema (simple sequence, genome, transcriptome, proteome, molecular marker, and other type). Then, these data will be transferred using the RAPIDANT-based, dedicated user application that can be run regardless of the operating system. Finally, users will submit metadata, and relevant project information retrieved from NTIS in the registration system by using keywords of project or principle investigator of project. After verification of uploaded files and their associated information, registration numbers will be officially issued for each submission. To maximize the utilization of deposited data, an integrated biological information service system (a part of KOBIC "Omics Portal") and a customizable data analysis system for large scale data are now under construction.

Keywords: research outcome, biological information, KoDS, Next-generation sequencing

Symposium [S1]

Outbreak Investigation and Control of Enteric Pathogens

2014 한국미생물학회연합 국제학술대회
INTERNATIONAL MEETING OF
THE FEDERATION OF KOREAN MICROBIOLOGICAL SOCIETIES

S1-1

Molecular Epidemiology and Characterization of Enteric Pathogens

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Nowadays, industrialized and globalized food marketing systems threaten our health and increase the probability of rapid and large outbreak of foodborne diseases.

In May 2013, foodborne disease outbreak by enteropathogenic *E. coli* O157:H45 has occurred. This strain has eaeA gene and the intimin type was γ. 10 strains isolated from specimens were identified to be tbe same pulsotype strains by PFGE analysis.

From November 2013 to December 2014, mass outbreaks of shigellosis were reported in Incheon, Gyeonggi-do, and Busan. Since there were a total of 483 cases, this event was the largest outbreak in the past decade.

From December 2013 to February 2014, clusters of gastroenteritis were occurred in Taiwanese and Hongkong tourists. It was attributed to food poisoning caused by norovirus contamination in underground water used for cooking at a restaurant.

In February 2014, an outbreak was reported with the symptom of diarrhea, nausea and abdominal pain at military facility. Astrovirus was detected in all patients (10/10, 100%), asymptomatic control soldiers (9/17, 52.9%), and asymptomatic food handler in outside restaurant (1/5, 20%). In addition, astrovirus also detected in the cooking water, especially groundwater of military service and food and cooking implements in restaurant. Astrovirus generally induced mild diarrhea in infant but this case was verified to induce in adult.

Rapid subtyping of foodborne pathogens are essential for outbreak detection. Current genotyping methods such PFGE, MLVA, SNPs and MLST yield only some of the characteristics desired for an optimal subtyping method. Technologies used for pathogen subtyping continue to advance and become accessible to more public health laboratories. United States CDC requested $40 million for advanced molecular infection and response to infectious disease outbreaks. Furthermore the *Listeria monocytogenes* real-time surveillance proof-of-concept study has successfully demonstrated the utility of whole genome sequencing (WGS) technology for the identification of Listeria clusters.

Conclusively, new perspective of preventive measures for infection prevention and control would be necessary in the future. Whole genome sequencing may eventually be a practical technique to regularly differentiate organisms. By sequencing the entire genome, we will be able to characterize and subtype foodborne pathogens in a single efficient workflow in real-time therby making laboratory surveillance more efficient and enhancing outbreak investigation.

S1-2

Genome-based Identification and Typing of Bacterial Pathogens

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Thanks to the next generation sequencing, high-throughput sequencing is readily available to routine diagnostic laboratories. There is a clear tendency towards genome-based taxonomy and classification over other technologies, such as biochemical and chemetaxonomic methods. Genome sequence data not only provide accurate identification at the species or subspecies level but also enable typing of isolates involved in large scale outbreaks. However, if genome-based technologies are widely used in routine laboratories, high quality database and sophisticated bioinformatics tools are prerequisites. In the presentation, current status of bacterial genomics and related bioinformatics tools will be reviewed and future perspectives will be given in the light of the potential use of genomics in clinical diagnostic laboratories.

S1-3

Exogenous *N*-Acetylneuraminic Acid Promotes Fibronectin Adherence by *Shigella flexneri* 2457T

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Shigella flexneri, the causative agent of shigellosis, is a Gram-negative bacterial pathogen that initiates infection by invading cells of the colonic epithelium. The mechanism of adherence of the pathogen to the extracellular matrix is poorly characterized. In this study, we investigated the binding of *S. flexneri* strain 2457T to basolateral fibronectin (Fn) on colonic epithelial cells. We also examined the role of exogenous sialic acid in the adherence of *S. flexneri* 2457T. The bacteria bound to Fn immobilized on microtiter plates in a concentration-dependent manner ($P<0.005$). Soluble Fn inhibited the binding of *S. flexneri* to immobilized Fn by 80% ($P<0.05$). Various extracellular matrix components had no effect on the adherence to Fn. However, pretreatment with mucin caused pronounced inhibition. Additionally, pretreatment with exogenous sialic acid resulted in a 2.5-fold increase in binding to immobilized Fn ($P<0.001$). The addition of exogenous sialic acid (*Lactobacillus casei* supernatant pretreated with mucin) or a sialic acid preparation (200 mM) led to a 4-fold increase in the adherence of *S. flexneri* to Fn ($P<0.0006$). It has been reported that *S. flexneri* adheres to a host molecule prior to invading the cell. Here, we provide strong evidence that *S. flexneri* binding to Fn is promoted by host-derived sialic acid. These findings suggest novel therapeutic strategies for *S. flexneri* infections.

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Keyword: *N*-acetylneuraminic acid, fibronectin, *S. flexneri*, mucin

S1-4

Porcine Epidemic Diarrhea Virus: the New Era

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The porcine epidemic diarrhoea virus (PEDV), a member of the Coronavirus family, causes acute diarrhoea and dehydration in pigs. Although it was first identified in Europe, it has become increasingly problematic in many Asian countries, including Korea, China, Japan, the Philippines, and Thailand. The economic impacts of the PEDV are substantial, given that it results in significant morbidity and mortality in neonatal piglets and is associated with increased costs related to vaccination and disinfection. Recently, progress has been made in understanding the molecular epidemiology of PEDV, thereby leading to the development of new vaccines. In the current review, we first describe the molecular and genetic characteristics of the PEDV. Then we discuss its molecular epidemiology and diagnosis, what vaccines are available, and how PEDV can be treated.

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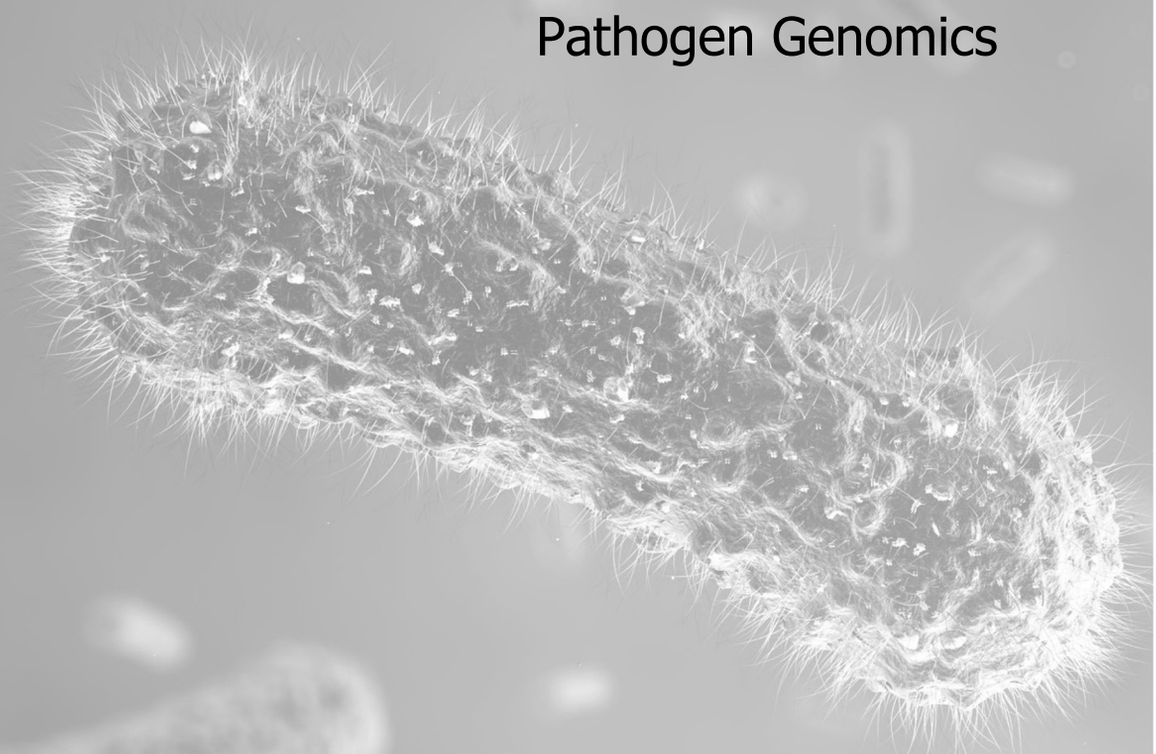
Kewwords: PEDV (porcine epidemic diarrhea virus), epidemiology, diagnosis, vaccine

Symposium [S2]

Pathogen Genomics



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S2-1

Overcoming Bacterial Resistant Mutants Using Information Obtained from Genomic Analysis of Bacteriophages

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Phage therapy is a legitimate alternative to antibiotics in the current era of antibiotic-resistant pathogens. Phages are currently used as food additives for human and feed additives for animals. Many human clinical trials of phage therapy for medical use have been conducted recently.

One problem with phage therapy is the appearance of phage-resistant bacterial strains. For prolonged use of phages, this problem needs to be overcome. Usually, a cocktail of phages is applied for therapeutic use. We present a novel strategy for overcoming phage-resistant bacteria.

In this study, we isolated a bacteriophage T7-resistant mutant strain of *Escherichia coli* and then proceeded to characterize it. The mutant bacterial colonies appeared to be mucoid. Microarray analysis revealed that genes related to colanic acid production were upregulated in the mutant. Colanic acid production actually increased in the mutant bacteria when they were biochemically measured, and protective capsule formation was observed under an electron microscope. We found a point mutation in the *lon* gene promoter in S3, the mutant bacteria. Overproduction of colanic acid was observed in phage-resistant mutant bacteria after infection with other bacteriophages, T4 and lambda. Colanic acid overproduction was also observed in clinically isolated strains of *E. coli* upon phage infection. The overproduction of colanic acid resulted in the inhibition of bacteriophage adsorption to the host. Biofilm formation also increased after 48 hours of incubation by the emergence of the mutant bacteria.

Whole genome analysis of bacteriophage PBECO4 revealed a putative open reading frame encoding a colonic acid-degrading enzyme activity. The bacteriophage PBECO4 was shown to infect the colanic acid-overproducing mutant strains of *E. coli*. We confirmed that gene product of ORF 547 of PBECO4 harbored colanic acid degrading enzymatic (CAE) activity. The mixed infection of T7 and PBECO4 or its purified enzyme (CAE) to the T7-resistant bacteria led to the successful infection of T7. Biofilm formation decreased with the mixed infection, too. This represents a novel strategy for overcoming phage-resistant mutant bacteria where phage cocktails different from those exploiting solely receptor differences are used.

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S2-2

Regulation of Hepatitis C Virus Replication by Small Non-coding RNAs

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Hepatitis C virus Hepatitis C virus (HCV) is a positive-sense single stranded RNA virus causing chronic hepatitis and liver cirrhosis, the latter often leading to hepatocellular carcinoma. Like other viruses, HCV infection, replication, and propagation steps are dependent on host cells and are thus subjected to regulation by multiple virus-host interaction events mainly through the interplay between viral and cellular proteins [1, 2]. In addition to the cellular proteins interacting with various HCV proteins, small non-coding RNAs including microRNAs (miRNAs), which have diverse physiological functions such as gene expression regulation, oncogenesis, and development, are potential cellular factors that may also modulate the HCV life cycle. Several miRNAs have been found to have a role in antiviral host defense responses or sometimes virus-promoting activity as in the case of HCV. Interestingly, HCV genome abundance is positively regulated by miR-122, a liver-specific miRNA, which appears as the most highly expressed miRNA in adult liver [3]. miR-122 has previously been found to increase the accumulation and translation of HCV RNA in infected cells through its direct interaction with the 5' untranslated region of HCV genome [4]. Besides miRNAs, recent deep-sequencing analysis of small RNAs from various cell lines revealed the existence of diverse types of small non-coding RNAs derived from tRNAs. Unlike cellular and viral miRNAs or virus-originated siRNAs, a role of tRNA fragments, if any, in viral life cycle has not yet been disclosed. HCV core protein is the viral nucleocapsid protein that binds and packages the viral RNA genome [5]. Besides its function as a viral structural protein, the core protein is implicated in chronic HCV infection-associated liver diseases by induction of reactive oxygen species and modulation of apoptosis [6]. In this presentation, I will present our current data describing the role of HCV core protein in regulation of miR-122 abundance in HCV-infected cells and the novel functions of tRNA-derived small RNA fragments in HCV replication.

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Keywords: hepatitis C virus, RNA replication, miR-122, small non-coding RNA, HCV core

S2-3

The Evolution of the Bacterial Antibiotic Resistance

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The continuous evolution of β-lactamases resulting in bacterial resistance to β-lactam antibiotics is a major concern in public health, and yet the underlying molecular basis or the pattern of such evolution is largely unknown. We investigated the mechanics of the substrate spectrum expansion of the class A β-lactamase using PenA of *Burkholderia thailandensis* as a model. We found twelve positions with single amino acid substitutions (altogether twenty-nine different substitutions), co-localized at the active-site pocket area. Simulation studies suggested that all substitutions caused a congruent effect, expanding the space in a conserved structure called the omega loop, which in turn increased flexibility at the active site. These mapped substitutions represent a comprehensive set of general mechanical paths to substrate spectrum expansion in class A β-lactamases that all share a functional evolutionary mechanism using common conserved residues. We also describe five deletion mutations, each also conferring extended substrate spectrum. Single-amino-acid deletions, E168del, T171del, I173del, and P174del, and a two-amino-acid deletion, R165_T167delinsP, occurred in the omega loop, increasing the flexibility of the binding cavity. This rare collection of mutations have significance allowing exploration of the diverse evolutionary trajectories of β-lactamases, and as potential future isolates in clinical settings, conferring high-level ceftazidime resistance compared with amino-acid-substitution mutations. Lastly, we describe various duplications (*de novo* TRs) that occurred in the coding region of a β-lactamase gene, where the omega loop is encoded. These duplications that occurred under selection using ceftazidime conferred substrate spectrum extension to include the antibiotic. Under selective pressure with one of the original substrates (amoxicillin), a high level of reversion occurred in the mutant β-lactamase genes completing a cycle back to the original substrate spectrum. The *de novo* TRs coupled with reversion makes a genetic toggling mechanism enabling reversible switching between the two phases of the substrate spectrum of β-lactamases. This toggle exemplifies the effective adaptation of *de novo* TRs for enhanced bacterial survival. We found pairs of direct repeats that mediated the DNA duplication (TR formation). In addition, we found different duos of sequences that mediated the DNA duplication. These novel elements—that we named, SCSs (same-strand complementary sequences)—were also found associated with β-lactamase TR mutations from clinical isolates. Both direct repeats and SCSs had a high correlation with TRs in diverse bacterial genomes throughout the major phylogenetic lineages, suggesting that they comprise a fundamental mechanism shaping the bacterial evolution.

S2-4

"Quorum Sensing Regulon and Virulence of *Pseudomonas aeruginosa*"

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Pseudomonas aeruginosa is a major opportunistic human pathogen that can cause cystic fibrosis, microbial keratitis, and burn wound infections. In *P. aeruginosa*, quorum sensing (QS) plays an essential role in pathogenesis and the QS response controls many virulence factors. Transcriptome studies suggest that over 300 genes (6 ~ 10% of the genome) are under QS control in *P. aeruginosa*. In particular, the Las and Rhl systems are known to regulate the expression of multiple extracellular virulence factors including elastase, alkaline protease, pyocyanin, and hydrogen cyanide. Unlike Las and Rhl systems, third QS regulator, QscR constitutes a different system in which there is no associated synthase gene. While QscR appears to repress a subset of Las and Rhl regulons, more comprehensive analysis of the QscR regulon identified 424 genes under the control of QscR. Of these, some genes were induced but majority was repressed. Based on this transcriptome-based information, we addressed how the QS system incapacitated the innate immune responses of insect, using mealworm *Tenebrio molitor* as a host model. We found that a QS-regulated exoprotease of *P. aeruginosa*, Protease IV functions as a key virulence effector modulating the insect innate immunity by degrading the components in Toll signaling. Protease IV converted zymogens of spätzle processing enzyme (SPE) and SPE-activating enzyme (SAE) into inactive forms, which blocks the activation of spätzle, a ligand of Toll receptor. This dampens the antimicrobial peptide (AMP) production by Toll signaling. Independently of the Toll pathway, the melanization response, another innate immunity was still generated, since Protease IV directly converted *Tenebrio* prophenoloxidase into active phenoloxidase. Protease IV also worked as an important factor in the virulence to brine shrimp and nematode. These results suggest that Protease IV provides *P. aeruginosa* with a sophisticated way to escape the immune attack of host by blocking the production of AMPs.

Symposium [S3]

New Challenge for Viral Vaccines

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S3-1

T Cell Responses against Hepatitis C Virus and Vaccine Development

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Hepatitis C virus (HCV) infection often develops into chronic hepatitis which progresses to liver cirrhosis and hepatocellular carcinoma. After acute HCV infection, 20-40% of patients recover spontaneously, but 60-80% of patients ultimately suffer from chronic HCV infection. HCV escapes from host immune responses by diverse mechanisms, thus is able to persist in the host. In innate immune responses, HCV circumvents the antiviral action of type I interferons (IFNs) by inhibiting both the induction and the signaling of type I IFNs. In adaptive immune responses, HCV evades from neutralizing antibodies by mutations of envelop proteins. HCV also circumvents virus-specific T cell responses during chronic infection. Once chronic HCV infection is established, HCV-specific T cells are exhausted and become dysfunctional. During chronic HCV infection, HCV-specific T cells overexpress T-cell inhibitory receptors such as PD-1, CTLA-4 and Tim-3 which are responsible for T-cell dysfunction. Interestingly, blocking these inhibitory receptors restores the function of HCV-specific T cells and leads them to proliferate and exert effector functions such as cytokine production and cytotoxicity. Recently, the blockade of the inhibitory receptors is considered as a novel strategy for the treatment of chronic HCV infection. In the present lecture, recent update of immunology of HCV infection will be reviewed, including the course of immune responses during acute infection, the mechanisms of immune evasion, and the development of immune-based therapies.

Keywords: hepatitis C virus, T cell, exhaustion, PD-1, vaccine

S3-2

Universal Influenza Vaccine: Options and Hurdles

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Since the discovery of antibodies specific to a highly conserved stalk region of the influenza virus hemagglutinin (HA), eliciting such antibodies has been considered the key to developing a universal influenza vaccine that confers broad-spectrum protection against various influenza subtypes. To achieve this goal, a prime/boost immunization strategy has been heralded to redirect host immune responses from the variable globular head domain to the conserved stalk domain of HA. While this approach has been successful in eliciting cross-reactive antibodies against the HA stalk domain, protective efficacy remains relatively poor due to the low immunogenicity of the domain, and the cross-reactivity was only within the same group, rather than among different groups. Additionally, concerns are raised on the possibility of vaccine-associated enhancement of viral infection and whether multiple boost immunization protocols would be considered practical from a clinical standpoint. Live attenuated vaccine hitherto remains unexplored, but is expected to serve as an alternative approach, considering its superior cross-reactivity. The present presentation will summarize recent advancements in the HA stalk-based universal influenza vaccines, discusses the pros and cons of these approaches with respect to the potentially beneficial and harmful effects of neutralizing and non-neutralizing antibodies, and suggests future guidelines towards the design of a truly protective universal influenza vaccine.

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Keywords: influenza virus, universal vaccine, hemagglutinin, cross-protection, antibody

S3-3

Towards a T-Cell Vaccine Based on Vaccinia Virus

Jin-Won Youn[1,5], Ji-Sun Kwon[1], Jungsoon Yoon[1], Yeon-Jung Kim[1], Kyuho Kang[1,6], Sunje Woo[2], Dea-Im Jung[2], Man Ki Song[2], Eun-Ha Kim[3], Hyeok-il Kwon[3], Young Ki Choi[3], Jihye Kim[4], Jeewon Lee[4], Yeup Yoon[1,7], and Eui-Cheol Shin[4]

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Antibody-inducing conventional vaccines have been successful in conquering diverse infectious diseases until 1980s, however hard-to-prevent pathogens, such as malaria, tuberculosis, Dengue, CMV, herpes, etc., still remain unconquered. The emergence of HIV and HCV clearly revealed the limitation of conventional vaccine, and necessitated introduction of a next generation vaccine, capable of inducing T cell immunity. Despite many approaches to T-cell based vaccine, the translation of the results from preclinical efficacy study has often been controversial.

For the last decade, we have focused on T cell vaccine which is critical for protection against highly variable viruses. We achieved complete protection against chronic hepatitis C virus progression in chimpanzees, and then heterosubtypic protection against diverse influenza viruses. The latter may lead to universal protection against any unpredictable influenza pandemic, called 'Universal Influenza Vaccine'. The promising results using prototype T cell vaccines encourage the feasibility of T cell vaccine in acute lytic as well as chronic nonlytic viruses. The proposed T cell vaccine platform will provide versatile protection against hard-to-conquer infectious diseases, and will shed lights on therapeutic vaccine strategy against hard-to-treat diseases including chronic infection and cancer.

S3-4

Non-invasive Nasal Adenovirus Vectored Vaccine for Broad Protection against Influenza – Implications for Development of Vaccines against Respiratory and Enteric Infectious Diseases

Huan Nguyen

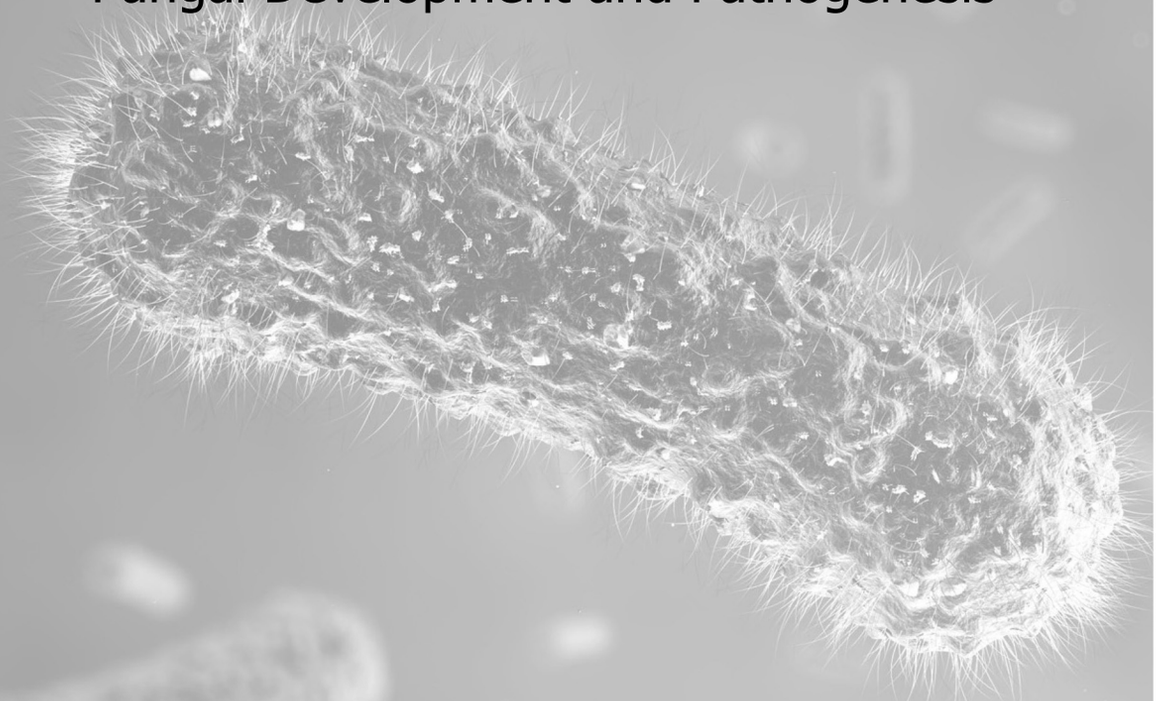
International Vaccine Institute

Influenza vaccines aimed at inducing antibody (Ab) responses against viral surface hemagglutinin (HA) and neuraminidase (NA) provide sterile immunity to infection with the same subtypes. Vaccines targeting viral conserved determinants shared by the influenza A viruses (IAV) offer heterosubtypic immunity (HSI), a broad protection against different subtypes. We hypothesized that vaccines targeting multiple influenza virus antigens would provide broader protection against infection with different subtypes. We report that single intranasal (i.n.) immunization with a recombinant adenovirus (rAd) vector encoding both HA of H5 virus and M2e (rAdH5/M2e) induced significant HA and M2e specific Ab responses along with protection against heterosubtypic challenge in mice. The protection is superior as compared to that induced by rAd vector encoding either HA (rAdH5), or M2e (rAdM2e). While protection against homotypic H5 virus is primarily mediated by virus neutralizing (VN) antibodies (Abs), the cross-protection is associated with Abs directed to conserved stalk HA and M2e that seem to have additive effect. Consistently, adoptive transfer of antisera induced by rAdH5/M2e provided the best protection against heterosubtypic challenge as compared to that provided by antisera derived from mice immunized with rAdH5 or rAdM2e. The findings support the development of rAd vectored vaccines encoding both H5 and M2e as universal vaccines against different IAV subtypes. In addition, the nasal vectored vaccine induced also significant level of specific Abs in intestinal secretion suggesting that, the nasal vectored vaccine could be developed to control enteric infectious diseases.

Keywords: non-invasive, vaccine, adenovirus vector, influenza, broad protection, respiratory, enteric, infectious diseases

Symposium [S4]

Fungal Development and Pathogenesis



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S4-1

Development of System-Wide Functional Analysis Platform for Pathogenicity Genes in *Magnaporthe oryzae*

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Null mutants generated by targeted gene replacement are frequently used to reveal function of the genes in fungi. However, targeted gene deletions may be difficult to obtain or it may not be applicable, such as in the case of redundant or lethal genes. Constitutive expression system could be an alternative to avoid these difficulties and to provide new platform in fungal functional genomics research. Here we developed a novel platform for functional analysis genes in *Magnaporthe oryzae* by constitutive expression under a strong promoter. Employing a binary vector (pGOF1), carrying *EF1β* promoter, we generated a total of 4,432 transformants by *Agrobacterium tumefaciens*-mediated transformation. We have analyzed a subset of 54 transformants that have the vector inserted in the promoter region of individual genes, at distances ranging from 44 to 1,479 bp. These transformants showed increased transcript levels of the genes that are found immediately adjacent to the vector, compared to those of wild type. Ten transformants showed higher levels of expression relative to the wild type not only in mycelial stage but also during infection-related development. Two transformants that T-DNA was inserted in the promotor regions of putative lethal genes, *MoRPT4* and *MoDBP5*, showed decreased conidiation and pathogenicity, respectively. We also characterized two transformants that T-DNA was inserted in functionally redundant genes encoding alpha-glucosidase and alpha-mannosidase. These transformants also showed decreased mycelial growth and pathogenicity, implying successful application of this platform in functional analysis of the genes. Our data also demonstrated that comparative phenotypic analysis under over-expression and suppression of gene expression could prove a highly efficient system for functional analysis of the genes. Our over-expressed transformants library would be a valuable resource for functional characterization of the redundant or lethal genes in *M. oryzae* and this system may be applicable in other fungi.

Keywords: the rice blast fungus, functional analysis, *Agrobacterium tumefaciens*-mediated transformation, over-expression, system development

S4-2

Epigenetic Regulation of Fungal Development and Pathogenesis in the Rice Blast Fungus

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Fungal pathogens have huge impact on health and economic wellbeing of human by causing life-threatening mycoses in immune-compromised patients or by destroying crop plants. A key determinant of fungal pathogenesis is their ability to undergo developmental change in response to host or environmental factors. Genetic pathways that regulate such morphological transitions and adaptation are therefore extensively studied during the last few decades. Given that epigenetic as well as genetic components play pivotal roles in development of plants and mammals, contribution of microbial epigenetic counterparts to this morphogenetic process is intriguing yet nearly unappreciated question to date. To bridge this gap in our knowledge, we set out to investigate histone modifications among epigenetic mechanisms that possibly regulate fungal adaptation and processes involved in pathogenesis of a model plant pathogenic fungus, *Magnaporthe oryzae*. *M. oryzae* is a causal agent of rice blast disease, which destroys 10 to 30% of the rice crop annually. Since the rice is the staple food for more than half of human population, the disease is a major threat to global food security. In addition to the socioeconomic impact of the disease it causes, the fungus is genetically tractable and can undergo well-defined morphological transitions including asexual spore production and appressorium (a specialized infection structure) formation *in vitro*, making it a model to study fungal development and pathogenicity. For functional and comparative analysis of histone modifications, a web-based database (dbHiMo) was constructed to archive and analyze histone modifying enzymes from eukaryotic species whose genome sequences are available. Histone modifying enzymes were identified applying a search pipeline built upon profile hidden Markov model (HMM) to proteomes. The database incorporates 22,169 histone-modifying enzymes identified from 342 species including 214 fungal, 33 plants, and 77 metazoan species. The dbHiMo provides users with web-based personalized data browsing and analysis tools, supporting comparative and evolutionary genomics. Based on the database entries, functional analysis of genes encoding histone acetyltransferases and histone demethylases is under way. Here I provide examples of such analyses that show how histone acetylation and methylation is implicated in regulating important aspects of fungal pathogenesis. Current analysis of histone modifying enzymes will be followed by ChIP-Seq and RNA-seq experiments to pinpoint the genes that are controlled by particular histone modifications. We anticipate that our work will provide not only the significant advances in our understanding of epigenetic mechanisms operating in microbial eukaryotes but also basis to expand our perspective on regulation of development in fungal pathogens.

S4-3

Interaction between the Rice Pathogens, *Fusarium graminearum* and *Burkholderia glumae*

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Species belonging to the genus *Fusarium* are widely distributed and cause diseases in many plants. Isolation of fungal strains from air or cereals is necessary for disease forecasting, disease diagnosis, and population genetics [1]. Previously we showed that *Fusarium* species are resistant to toxoflavin produced by the bacterial rice pathogen *Burkholderia glumae* while other fungal genera are sensitive to the toxin, resulting in the development of a selective medium for *Fusarium* species using toxoflavin [2]. In this study, we have tried to elucidate the resistant mechanism of *F. graminearum* against toxoflavin and interaction between the two pathogens in nature.

To test whether *B. glumae* affects the development of *F. graminearum*, the wild-type *F. graminearum* strains were incubated with either the bacterial strain or supernatant of the bacterial culture. Both conditions increased the conidial production five times more than when the fungus was incubated alone. While co-incubation resulted in dramatic increase of conidial production, conidia germination delayed by either the bacterial strain or supernatant. These results suggest that certain factors produced by *B. glumae* induce conidial production and delay conidial germination in *F. graminearum*.

To identify genes related to toxoflavin resistance in *F. graminearum*, we screened the transcriptional factor mutant library previously generated in *F. graminearum* [3] and identified one mutant that is sensitive to toxoflavin. We analyzed transcriptomes of the wild-type strain and the mutant strain under either absence or presence of toxoflavin through RNAseq. Expression level of total genes of 13,820 was measured by reads per kilobase per million mapped reads (RPKM). Under the criteria with more than two-fold changes, 1,440 genes were up-regulated and 1,267 genes were down-regulated in wild-type strain than mutant strain in response to toxoflavin treatment. A comparison of gene expression profiling between the wild type and mutant through gene ontology analysis showed that genes related to metabolic process and oxidation-reduction process were highly enriched in the mutant strain. The data analyses will focus on elucidating the resistance mechanism of *F. graminearum* against toxoflavin and the interaction between the two pathogens in rice. Further evolutionary history will be traced through figuring out the gene function in populations and in other filamentous fungi.

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Keywords: *Burkholderia glumae*, conidiation, *Fusarium graminearum*, interaction, toxoflavin

S4-4

Genetic Control of Asexual Sporulation in *Fusarium graminearum*

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Fusarium graminearum (teleomorph *Gibberella zeae*) is an important plant pathogen that causes head blight of major cereal crops such as wheat, barley, and rice, as well as causing ear and stalk rot on maize worldwide. Plant diseases caused by this fungus lead to severe yield losses and accumulation of harmful mycotoxins in infected cereals [1].

Fungi utilize spore production as a mean to rapidly avoid unfavorable environmental conditions and to amplify their population. Spores are produced sexually and asexually and their production is precisely controlled. Upstream developmental activators consist of *fluffy* genes have been known to orchestrate early induction of condiogenesis in a model filamentous fungus *Aspergillus nidulans*. To understand the molecular mechanisms underlying conidiogenesis in *F. graminearum*, we characterized functions of the *F. graminearum fluffy* gene homologs [2]. We found that FlbD is conserved regulatory function for conidiogenesis in both *A. nidulans* and *F. graminearum* among five *fluffy* gene homologs. *flbD* deletion abolished conidia and perithecia production, suggesting that *FlbD* have global roles in hyphal differentiation processes in *F. graminearum* .

We further identified and functionally characterized the ortholog of AbaA, which is involved in differentiation from vegetative hyphae to conidia and known to be absent in *F. graminearum* [3]. Deletion of *abaA* did not affect vegetative growth, sexual development, or virulence, but conidium production was completely abolished and thin hyphae grew from abnormally shaped phialides in *abaA* deletion mutants. Overexpression of *abaA* resulted in pleiotropic defects such as impaired sexual and asexual development, retarded conidium germination, and reduced trichothecene production. AbaA localized to the nuclei of phialides and terminal cells of mature conidia. Successful interspecies complementation using *A. nidulans* AbaA and the conserved AbaA-WetA pathway demonstrated that the molecular mechanisms responsible for AbaA activity are conserved in *F. graminearum* as they are in A. nidulans.

F. graminearum ortholog of *Aspergillus nidulans wetA* has been shown to be involved in conidiogenesis and conidium maturation [4]. Deletion of *F. graminearum wetA* did not alter mycelial growth, sexual development, or virulence, but the *wetA* deletion mutants produced longer conidia with fewer septa, and the conidia were sensitive to acute stresses, such as oxidative stress and heat stress. Furthermore, the survival rate of aged conidia from the *F. graminearum wetA* deletion mutants was reduced. The *wetA* deletion resulted in vigorous generation of single-celled conidia through autophagy-dependent microcycle conidiation, indicating that WetA functions to maintain conidia dormancy by suppressing microcycle conidiation in *F. graminearum.*

In *A. nidulans*, FlbB physically interacts with FlbD and FlbE, and the resulting FlbB/FlbE and FlbB/FlbD complexes induce the expression of *flbD* and *brlA*, respectively. BrlA is an activator of the AbaA-WetA pathway. AbaA and WetA are required for phialide formation and conidia maturation, respectively [5]. In *F. graminearum*, the AbaA-WetA pathway is similar to that of *A. nidulans*, except a *brlA* ortholog does not exist. Amongst the *fluffy* genes, only *fgflbD* has a conserved role for regulation of the AbaA-WetA pathway.

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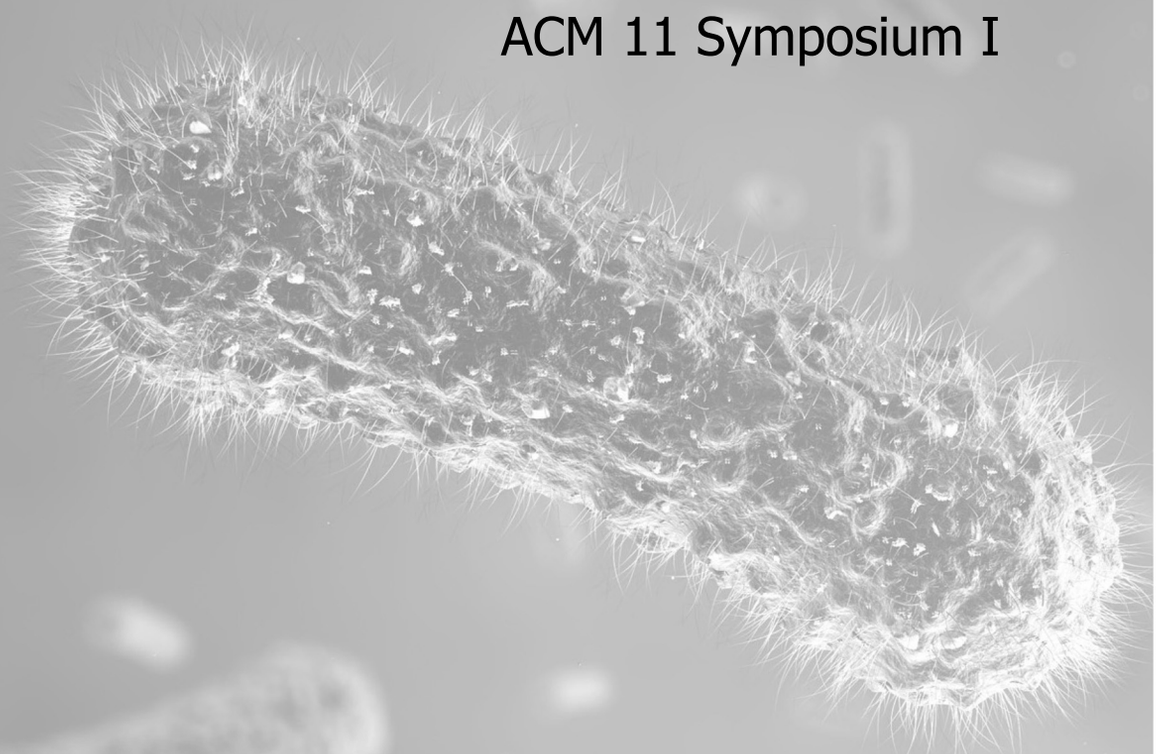
Keywords: conidiogenesis, *fluffy* gene, *Fusarium graminearum*, wetA, abaA

Symposium [S6]

ACM 11 Symposium I



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INTERNATIONAL MEETING OF

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S6-1

Current Status and Future Prospects of ACM

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ACM is the Asian Consortium on Conservation and Sustainable Utilization of Microbial Resources, which was established in 2004 by the microbiologists from twelve Asian countries. The object of ACM is to promote utilization of microbial resources for research and development in life sciences and biotechnology. Convention on Biological Diversity (CBD) was enforced in 1993 and sovereign right on genetic resources is given to the countries of origin. Since then, the scientists are paying attention when they use genetic resources of foreign countries especially for commercial use. In such background, the roles of biological resource centers (BRC) have become important for management of the biological resources for (international) transfer and the utilization bridging the depositors and the users of microbial materials.

ACM has meeting annually consisted of the member report, general assembly, and the taskforce report. Three taskforces (TF) of ACM are (1) Asian BRC Network (ABRCN) TF to develop the common database and to assist the development of individual database for member collections, (2) Human Resource Development (HRD) TF to improve the technique and knowledge of the members by organizing training courses and workshops, and (3) Management of Material Transfer (MMT) TF for standardization and establishment of a common platform for international transfer of biological materials for research. The report and discussion in these TFs are very useful for the members to lead the construction of infrastructure in their countries to use microbial resources.

Microbial resources of BRCs are used especially for (1) standardized tests such as specified in the Pharmacopoeia, industrial standard, etc., (2) type strain depository for proposal of new species of the prokaryotes as in nomenclatural rule, and (3) sources for exploitation of new microorganisms to discover new bioactive compounds and enzymes for biotechnology and industry. Commonly sharing of microbial strains among the community is necessary for these purposes to use through BRCs of each country.

Large biodiversity of Asian countries is expected to be the supplier of such materials with potential. In addition, more than half of the new taxa of the prokaryotes are from Asia, especially from China, Korea and Japan and followed by India and Thailand. These facts suggest that international collaboration in Asia will surely produce good achievement in life sciences and biotechnology.

2014 is the memorial year for the Nagoya Protocol (Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity) to be enforced in October. Ten years' effort and trial of ACM have become more realistic. This meeting will be a new starting point of the next stage of ACM to support Asian microbiology community in promoting the correct access and use of microbial resources.

http://www.acm-mrc.asia/

KeyWords: Convention on Biological Diversity, Nagoya Protocol, biological resource center, Asian countries

S6-2

Patent Strategies for Biotechnology

Won-Hee LEE, Ph.D

WON International Patent & Law Firm

Biological resources (natural products) are mankind's valuable property, and benefits arising from sustainable use should be shared by developers and resource providers.

Sharing of benefits through an access to biological resources should be supported by development of biotechnology industry utilizing biological resources, and to accomplish it, database of biological resources should be constructed, research utilizing biological resources should be conducted and patent rights on research results should be obtained, benefits should be arisen by commercializing those patent rights, and benefits should be shared fairly and equitably.

Biotechnology industry is a knowledge-based industry, and patent roles as a major factor in this field.

Specifying objects of patent for biological resources (animals, plants and microorganisms), requirements for obtaining patents, strategies for obtaining strong patents to be commercialized in the market, and reflecting Nagoya Protocol from the early stage of research will be discussed.

S6-3

Analysis of the Utilization of Microbial Resources Distributed from JCM

Moriya Ohkuma

Japan Collection of Microorganisms (JCM), RIKEN BioResource Center

The Japan Collection of Microorganisms (JCM) in RIKEN BRC has been collecting, preserving, and distributing microbial cultures of diverse bacteria, archaea, and fungi since established in 1981. An important mission of JCM is to contribute to scientific communities by responding the demands of the users and to enhance researches particularly in health and environmental science as well as general microbiology.

JCM has been trying to improve its functions as a microbial resource center such as quality control of the microbial strains and satisfactory services for the users under the quality management system accredited by ISO 9001:2008. JCM has been trying to characterize the strains such as genome sequencing and to enrich the strain information, which is continuously updated and opened to the public through our on-line catalog database. Deposition of a type strain and its availability must be ensured when researchers describe novel microbial taxon. Some journals ask the authors to deposit the materials used in the publications to a public bioresource center. Indeed, the number of depositions to JCM increased dramatically in these years. As the results, JCM now successfully houses a great microbial diversity characterized well, authentic, and useful for researches.

JCM annually received near 800 strain depositions, >70% of which came from abroad. JCM annually distributed >3,400 strains and approximately one-forth of them went to abroad. In addition, publications using or describing JCM strains are very important and effective to measure our contributions. Last year, JCM strains appeared in 540 original scientific papers including those published in highly impacted journals, and used near 100 patent applications. Particularly, more than half of the original papers were published by researchers in Asia excluding Japan, reflecting a large number of depositions from and distributions to Asian countries. These numeric data are good measures of the contribution of JCM to scientific communities in the world, particularly in Asia.

Keywords: bioresource, microbial diversity, scientific community, deposition, distribution

S6-4

Application of Microbial Fermentation and Enzymatic Biotransformation in Cosmetic Industry

Kyeong hwan Hwang, Joon Ho Park, Jun seong Park and Byungyoung KANG

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Microbial fermentation and enzymatic reactions have become very important in industry due to their valuable properties, i.e., rapid and efficient action at low concentrations under mild pH values and temperatures, high substrate specificity, low toxicity, and ease of termination of activity. Microbial enzymes are economical on a large scale due to inexpensive media and short fermentation cycles. Different microbes produce somewhat different enzymes that catalyze the same reaction. This offers flexibility with respect to operating conditions in the reactor. For all those reasons microbial enzymes are of great importance in the development of industrial bioprocesses. Current applications are focused on many different industries including pulp and paper, leather, detergents and textiles, pharmaceuticals, chemical, food and beverages, bio-fuels, animal feed and personal care, among others.

Use of Enzyme in cosmetics. There have been attempts to use enzymes as active ingredients in cosmetics, especially for skin exfoliation products since various proteases have been studied extensively. Among those proteases, enzymes with keratinolytic activities hold potential as natural exfoliation agent since keratin is a major constituent in dead skin cells [1]. To explore the keratinolytic enzyme from microorganism in green tea, total of 4 strains were isolated from green tea. Among these isolates, AP sulloc 331261 of microorganism with keratinolytic activities were screened using keratinase assay and identified. The strains named AP sulloc 331261 belonged to the genus *Lactobacillus planetarium*. The enzymes extracted from *Lactobacillus planetarium* AP sulloc 331261 were found to have stronger keratinolytic activity compared to Papain which is a popular protease used in exfoliation products in cosmetic industry. Despite its strong activity towards keratin degradation, the bare enzyme cannot be applied to cosmetic products due to stability issues. In order to minimize the activity loss of the enzymes, the enzymes were first adsorbed to alkyl acrylate cross-polymer in 1,3-butyleneglycole environment. After the adsorption, the enzymes were dispersed in a pH 5.0 buffer solution which is the optimum pH for the enzymes. The stabilized enzyme maintained its activity more than a month in 40°C while non-stabilized enzymes lost most of its activity within a day. The stabilized enzymes were dispersed in silicone producing a W/S formula for cosmetic purposes. A clinical trial was done using this formula and the result showed significant decrease in stratum corneum on human skin

Use of enzymatic biotransformation in cosmetic. Ginseng has been used as a traditional medicine in Asian countries, to strengthen immunity, supply nutrition, and decrease fatigue. These beneficial functions are attributed mainly to the ginsenosides in ginseng. The minor ginsenosides (F2, Rg3, Rh1, Rh2, and compound K) are more pharmaceutically active than the major ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1), which comprise more than 80% of the total ginsenosides. Therefore, many studies have focused on the production of minor ginsenosides by hydrolyzing the sugar moieties of major ginsenosides. The ginsenoside compound K has attracted attention in recent years because of its pharmaceutical activities, including anti-tumor, anti-inflammatory, anti-allergic, and hepatoprotective effects [2-4]. Ginsenoside compound K was produced

from ginseng root extract using a recombinant β-glycosidase from microbes. Under optimum conditions, ginsenosides Rb1, Rb2, Rc, and major protopanaxadiol ginsenosides in ginseng root extract were completely converted to compound K after 18hr *via* biotransformation pathway Rb1, Rb2 or Rc → Rd → F2 → compound K.

Use of microbial fermentations in cosmetic. Sake is a Japanese alcoholic beverage produced from rice and water by fermentation, but is little known for its effect on melanogenesis. To identify the effect of sake extract on melanin synthesis, a melanin assay was performed in melan-A murine melanocytes. Sake extract treatment significantly inhibited melanin production in a dose-dependent manner, and tyrosinase, the rate-limiting enzyme of melanogenesis, decreased significantly at the protein level. Further investigations were performed with multiple assay systems; a sake extract reduced melanin production in melan-A/SP-1 murine cell co-culture, and also in MelanoDerm, a skin equivalent model of human keratinocytes-melanocytes. Finally, subjects were treated with a formula containing the sake extract. Topical application of the sake extract product improved skin lightness (L*) significantly within 7 days. We identified sake extract as a new anti-melanogenic ingredient through in vitro and in vivo experiments. These results suggest that a sake extract can be used to improve skin hyperpigmentation. And similar study of wild flower ferment extracts using *Lactobcillus sakei* species which identified as symbiotic resident microbes, showed anti inflammatory efficacy on topical application promising for cosmetic products.

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Keywords: biotransformation, enzyme, microbial, fermentation, cosmetic

Symposium [S7]

Metagenomics: from Resource to Biological Application

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S7-1

Discovery from Soil Microbial Diversity: Bioprospecting Soil Metagenomics

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Majority of soil microorganisms are not culturable by a standard cultivation method. Thus, one may expect that tapping the unculturable microbial resources would provide the unique opportunity to obtain novel microbial resources. Metagenome, a total microbial genome directly obtained from microbial habitat, could be a rich source for bioprospecting purpose. Soil metagenome are cloned in a surrogate host bacterium to constitute metagenome library and can be also used for selecting novel genetic resources from the majority of unculturable bacteria. Either expression-dependent or sequence homology based screening of the constructed library can be performed to obtain novel resources. Based on the expression-dependent screening of various soil metagenomic libraries, we have obtained novel enzymes and novel gene clusters for secondary metabolite production. The functional screening also allowed us to re-annotate several hypothetical proteins in genome DB into novel enzymes. We obtained a number of enzymes involved in lipid metabolism through functional screening of metagenomic libraries, including lipase/esterase, wax ester synthase (WES), 3-OH palmitate methyl ester (3-OH PAME) hydrolase. In addition, we also isolated and identified genes and gene clusters for antimicrobial activities and resistance to antibioitcs. In this presentation, I would like to present an interesting enzymes WES from soil metagenome, which was recently characterized from my lab. The overall identity of WES from metagenome was 22-30% to known WES from bacteria and plant. The expression of *wes* in *Escherichia coli*, tobacco and Arabidopsis plant produced wax esters with various chain length. Transgenic Arabidopsis plants expressing *wes* were tolerant to drought stress and showed the slower transpiration than wild type. This result suggested that introduction of metagenome-derived genes into plants will give unique opportunity for enhanced plant function and agricultural productivity.

Keywords: bioprospecting metagenomics, soil metagenome, wax ester synthase

S7-2

Beyond "Who Are They?": Application of Metagenome to Plant

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Biological control, as defined by Cook and Baker, was proposed half a century ago. Fifty years later, biological control of plant diseases is still recognized as a unique alternative to chemical control. The modes of action of biological control are known as antagonism, competition, hyperparasitism, and induced systemic resistance (ISR). Due to its ability to induce resistance against a broad spectrum of pathogens and its long-lasting effect, plant growth-promoting rhizobacteria (PGPR)-elicited ISR can be an attractive tool for biological control. Here, we provide new genetic resources to elicit ISR. A metagenome library is an important and indispensable resource for identifying novel biomolecules by a culture-independent technique to obtain DNA from diverse microbiota. However, little information are available to exploit agricultural field because scientists mostly focused on community analysis (so called "who are they?"). We employed the soil metagenome to search for new genetic resources for prompting ISR. Our metagenome pools were screened for ISR capacity against *Erwinia carotovora* by drench-application of each pool *in vitro*. Drench application of the selected clones' metabolites reduced symptom development caused by *Xanthomonas axonopodis* and *Cucumber mosaic virus* in pepper plants under field conditions. Our results broaden our knowledge of genetic resources to be utilized as BCAs.

Keywords: biological control, functional metagenome, induced systemic resistance, pepper

S7-3

New Strategy for Ultra-high-throughput Screening (uHTS) of Novel Enzyme/Pathway by *In Vitro* Compartmentalization (IVC) Using Microbeads from Metagenomic Resources

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It is very difficult to screen for novel enzymes/pathways using traditional methods owing to limitations in resources and screening methods. This study is the first to demonstrate direct screening for novel enzymes/pathways using an ultra-HTS (uHTS) system by *in vitro* compartmentalization (IVC) using microbeads from metagenomic resources. The goal of IVC is to divide every reaction between many microscopic compartments. This strategy would be useful in fields of biochemistry where large populations of variants are handled.

Recently, a screening protocol for robotic HTS system have been enabled us to perform activity verifications on more than 10^4 clones per day and identify various enzymes with activity. Recently, we developed new screening system on more than 10^7 clones per hour by single cell level. In the present study, we can generate very easily microbeads without microfluidic system and show a rapid, simple, and efficient method for screening enzyme/pathway including hydrolase using an uHTS system by using fluorescence-activated cell sorting (FACS) analysis.

The generation of water-in-oil-in-water (W/O/W) droplets in homogeneous size distribution was very difficult without microfluidic devices, and then the fabrication speed using such a device was at least 4 orders of magnitude slower than that of homogenizing method. In this study, we develop a rapid and simple method by which the agarose-based microbeads containing single cell could be generated in homogeneous size distribution without microfluidic devices. Such as W/O/W emulsion, we confirmed that the agarose-based microbeads could efficiently form micro-reactors which encapsulate single, enzyme expressing cells and fluorogenic substrate to analyze and screen by FACS.

This IVC method using microbeads can detect and screen various enzyme/pathway in the single cell level without diffusion of fluorescent substrate in IVC using W/O/W emulsion method. This new uHTS system is a model system for ultra-speedy, sensitive, multiplex screening of enzyme/pathway from various genetic libraries and holds promise for providing new enzymes/pathways for bioindustrial applications.

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Key words: ultra-high-throughput screening (uHTS), *in vitro* compartmentalization (IVC), microbeads, metagenome, functional enzyme

S7-4

Metagenomic Enzyme Discovery for the Use of Biocatalyst in Bio-based Chemical Industry

Jae Kwang Song, Min-A Kwon, Eun Young Yu, Miae Lee, Ji Young Lee, Hee Taek Kim, Ji-Eun Choi, Hye Young Na, Hye Jin Lim, Joon Young Oh

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A metagenome, the total genetic material recovered from environmental samples, includes the genomes from as-yet uncultured or uncultivable microorganisms. By enabling us to harness the natural genetic diversity present in the environment, a metagenomic DNA library plays an important role in revealing novel sequences, genes and biological pathways [1]. On the basis of the functional and sequence-based search of the collective microbial genomes in a given habitat, metagenomics has been used to discover novel and potentially important enzymes for industrial applications [2,3]. Many metagenomic enzymes often displayed novel substrate/product ranges and are also often highly stable under extreme conditions.

We obtained industrially promising enzymes such as lipases, esterases, isomerase, alkane hydroxylases and long-chain-alcohol O-fatty-acyltransferase from metagenome, and identified their ability to catalyze the desired chemical reactions. As a case in point, a simple and efficient high-throughput screening (HTS) system to identify novel isomerization activity for aromatic compounds from soil metagenomic libraries. The fosmid DNA conferring the positive activity was isolated and then the 35-kb metagenomic insert DNA was sequenced. The gene encoding the new isomerase was identified out of about 30 putative enzyme-encoding genes, which was confirmed through the subcloning experiment followed by activity assay. Then we attempted to develop a novel biotransformaion reaction for organic synthesis. This enzyme was overexpressed in *E. coli* and tested for a biotransformation reaction in various reaction conditions for an optimization. Moreover, the combination of reduction and isomeration was designed for an easy use of this reaction, and the whole cell having dual activity was prepared. Using the whole cell biocatalyst, multi-step reaction was successfully carried out to give rise to good yields. We will discuss the whole process from finding a novel enzyme to developing a new biotransformation.

The biological technology such as biocatalyst and biocatalysis will be increasingly important for the chemical industry to be environmentally, socially, and economically sustainable. So far, exploration of metagenomic libraries has been effective in identifying a novel enzyme with desired properties and potential applications as biocatalysts in industries including the production of fine chemicals. Another example was several genes encoding esterases and lipases with distinguished features such as cold-adapted activity [4], and alkaliphilic activity [5]. Therefore, discovery of new biocatalysts from metagenome, accompanied with the other parts of biocatalyst development, should continue to be part of a “more green chemistry” movement.

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Keywords: metagenome, isomerase, lipase, biocatalyst, high-throughput screening

Symposium [S8]

Anti-Viral Drugs and Technology

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S8-1

Cell-based High-throughput Screening of Chemical Libraries for Inhibitors of Influenza Virus

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Influenza virus is an enveloped virus with eight segmented negative sense RNA genomes and belongs to the family *Orthomyxoviridae*. There are three different types of influenza virus, A, B and C. Only types A and B infect humans causing respiratory diseases [1]. Particularly, influenza A virus is further classified into subtypes based on the antigenic properties of surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). There are two approved antivirals against influenza A virus infection, viral NA inhibitors (oseltamivir and zanamivir) and ion channel M2 inhibitors (amantadine and rimantadine) [2,3]. The M2 inhibitors, which are intrinsically ineffective to influenza B virus, are not recommended anymore even against influenza A virus because of the rapid emergence of drug-resistant viruses. Not only influenza A virus but also influenza B virus with more reduced susceptibility or increased resistance to the NA inhibitors started to be isolated from humans as well as *in vitro* cell cultures [4]. It suggests that the potential for infection of drug-resistant influenza viral isolates could increase in the future and thus there is an urgent need for development of a new class of antivirals targeting alternative influenza viral proteins or cellular factors essential for virus replication.

In our laboratory, a chemical library of approximately 20,000 small organic compounds was screened to discover hit compounds with activity against two human influenza A viruses (H1N1 and H3N2) and one influenza B virus in a cell culture-based system. In this presentation, chemical structures and antiviral modes of action of three representative antiviral compounds will be presented. The first one is (-)-Epigallocatechin-3-gallate (EGCG), one of the major flavonoid components of green tea [5]. It is known to have a broad antiviral activity against several enveloped viruses, including the influenza virus. In our study, it was observed that EGCG blocked an early step of the influenza viral life cycle, but did not affect viral adsorption to target cells or viral RNA replication. It inhibited hemifusion events between virus particles and cellular membrane by impairing the viral membrane integrity, resulting in the loss of cell penetration ability of influenza virus. Although EGCG also suppressed viral and non-viral neuraminidase (NA) activity in an enzyme-based assay system, it was marginal. Thus, it can be suggested that the anti-influenza viral efficacy of EGCG is exhibited due to damage in the physical property of viral envelope accompanied with partial inhibition of the NA surface glycoprotein. The second one is a spiro compound [6]. An efficient and novel two step synthetic procedure to prepare various substituted 3*H*,3'*H*-spiro[benzofuran-2,1'-isobenzofuran]-3,3'-diones, was established from very simple and easily available starting materials. The prepared compounds were tested against influenza virus type A, such as A/Taiwan/1/86 (H1N1), A/Hong Kong/8/68 (H3N2), and type B, such as B/Panama/45/90, B/Taiwan/2/62, B/Lee/40, B/Brisbane/60/2008. Among 31 compounds tested, some of them showed good activity (selective index values >10) against the various type B viruses. The most active compound **3b** showed activity in 3.0 to 16.1 μM range with a selectivity index value between 30 to 166 against these type B viruses. The third hit compound selected is (***Z***)-1-((5-fluoro-1*H*-indol-3-yl)methylene)-6-methyl-4-thioxo-4,5- dihydrofuro[3,4-***c***]pyridin-3(1***H***)-one (**15a**) with half-maximal effective concentrations of 17.4–21.1 μM against influenza A/H1N1, A/H3N2 and B viruses

without any cellular toxicity at 900 μM [7]. To investigate the structure-activity relationships, two dozens of the hit analogs were synthesized. The anti-influenza viral compounds efficiently suppressed not only viral protein level of the infected cells but also production of viral progeny in the culture supernatants in a dose-dependent manner. Based on a mode-of-action study, they did not affect virus entry or RNA replication. Instead, they suppressed viral neuraminidase activity. This study is the first to demonstrate that dihydrofuropyridinones could serve as lead compounds for the discovery of alternative influenza virus inhibitors.

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S8-2

Squirrel Poxvirus as a Novel Oncolytic Agent

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SQPV (Squirrel poxvirus) was identified in 1936 by Kilham et al.[1] SQPV natural host tropism is highly restricted to gray squirrels or red squirrels [2]. Due to a SQPV host range restriction, SQPV doesn't infect and cause pathogenesis in other species such as sheep, wood mice or bank voles and there is no known human infection/pathogenesis caused by SQPV in nature [3]. We found that non-human pathogenic SQPV can be used as a novel oncolytic viral agent in order to treat human cancers, such as lymphoma, leukemia, and various solid malignancies. SQPV didn't affect normal non-squirrel tissues. Our data showed that SQPV infected various types of human cancers such as brain, ovarian, liver cancers as well as human hematopoietic malignancies such as lymphoma and leukemia. Suppression of human tumor growth was observed following SQPV treatment in vivo. Therefore, SQPV can be used for a viral oncolytic agent with a high safety profile specifically targeting human cancers while sparing normal human tissues. In the future clinical setting, SQPV viral safety can be derived from a highly restricted poxviral tropism on non-squirrel hosts such as human or companion animal.

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Keywords: oncolytic virus, Poxvirus, Leporipoxvirus, Squirrelpoxvirus, cancer therapy

S8-3

Structural Insight into the Extracellular Domain of Matrix Protein 2 of Influenza A Virus

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Influenza viruses continuously undergo antigenic changes with gradual accumulation of mutations and there is an urgent need for novel vaccine and antiviral development. We determined the structures of the HA proteins from A/Korea/01/2009 (KR01) and A/Thailand/CU44/2006 (CU44). The crystal structure of KR01 HA revealed a V-shaped head-to-head arrangement, and the complex structure of KR01 HA and Fab0757 derived from a broadly neutralizing H1-specific monoclonal antibody also exhibited a head-to-head arrangement of HA. Both native and Fab complex structures reveal different spatial orientation of HA1 relative to HA2. The extracellular domain of influenza A virus matrix protein 2 (M2e) is highly conserved among human and nonhuman influenza A viruses. The crystal structures of soluble, leucine-zippered, tetrameric M2e (M2e-tGCN4) and a complex of M2e peptide and monoclonal antibody-65 (mAb-65) directed against M2e were determined. In the complex structure, M2e adopts a U-shaped conformation that is facilitated by the engagement of crucial residues within the short peptide epitope. The structures of viral proteins and their complexes with protective antibodies may guide the design of a universal vaccine with a stabilized conformation.

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Keywords: Influenza virus, protein structure, structure-based vaccine design, hemagglutinin, M2e

S8-4

차세대 염기서열 분석기법을 이용한 닭 허피스바이러스 전장유전체 분석

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Development of next generation sequencing (NGS) technology allowed to determine complete genome sequences from animals or microorganisms possessing large size of genomic DNA. In Korea NGS has been used intensively for complete genome sequencing of human, animals, plants and bacteria. However complete genome sequencing of virus was performed rarely using NGS before. Here I would like to introduce complete genome sequencing method for viral genome using NGS. In this study, I determined complete genomic sequences of several strains of Infectious laryngotracheitis virus (ILTV; gallid herpesvirus 1) using NGS. ILTV is a double stranded DNA virus that causes acute upper respiratory disease in chickens. One to five micrograms of purified ILTV genomic DNA was used to prepare fragment libraries that were sequenced in parallel using the SOLiD system (Applied Biosystems) with a flow cell divided into 8 segments. Each segment also contained a similarly generated library of an unrelated bacterial genome of around one megabase pairs. The resulting reads were unambiguously mapped to each viral and bacterial genome. *De novo* assembly of the viral reads produced contigs that were aligned to the complete concatenated genomic ILTV sequence described above with the exception of the terminal repeat region which were excluded from the analysis. Geneious software (Biomatters Ltd) was then used to manually curate these alignments and, with reference to the original reads, produce consensus sequences for each strain. Genome sizes of each strain were found to vary, however all genomes had a G + C content of 47.5% and contained 79 predicted ORFs. In the full genomic alignment of the strains, the lowest DNA sequence identity was 99.2%. These results suggested that ILTV has a highly conserved genome. When the genome sequence of two closely related ILTV vaccine strains (SA2 and A20 ILTV) were compared, the two viruses were found to share 99.99% DNA sequence identity with 24 single nucleotide polymorphisms (SNPs) detected between them. Ongoing work in our laboratories is focused on using this sequence data to develop improved diagnostic tools and to better understand the epidemiology and pathogenesis of disease due to infection with ILTV.

Symposium [S9]

Mushroom Science and Industrial Application

2014 한국미생물학회연합 국제학술대회

INTERNATIONAL MEETING OF

THE FEDERATION OF KOREAN MICROBIOLOGICAL SOCIETIES

S9-1

Extraction and Application of Bulk Enzymes and Antimicrobial Substance from Spent Mushroom Substrates

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Pleurotus ostreatus, *P. eryngii*, and *Flammulina velutipes* are major edible mushrooms that account for over 89% of total mushroom production in Korea. Recently, *Agrocybe cylindracea*, *Hypsizygus marmoreus,* and *Hericium erinaceu* are increasingly being cultivated in mushroom farms. In Korea , the production of edible mushrooms was estimated to be 614,224 ton in 2013. Generally, about 5 kg of mushroom substrate is needed to produce 1 kg of mushroom, and consequently about 25 million tons of spent mushroom substrate (SMS) is produced each year in Korea. Because this massive amount of SMC is unsuitable for reuse in mushroom production, it is either used as garden fertilizer or deposited in landfills, which pollutes the environment. It is reasonably assumed that SMS includes different secondary metabolites and extracellular enzymes produced from mycelia on substrate. Three major groups of enzymes such as cellulases, xylanases, and lignin degrading enzymes are involved in breaking down mushroom substrates. Cellulase and xylanase have been used as the industrial enzymes involving the saccharification of biomass to produce biofuel. In addition, lignin degrading enzymes such as laccases have been used to decolorize the industrial synthetic dyes and remove environmental pollutions such as phenolic compounds. Basidiomycetes produce a large number of biologically active compounds that show antibacterial, antifungal, antiviral, cytotoxic or hallucinogenic activities. However, most previous researches have focused on therapeutics and less on the control of plant diseases. SMS can be considered as an easily available source of active compounds to protect plants from fungal and bacterial infections, helping alleviate the waste disposal problem in the mushroom industry and creating an environmentally friendly method to reduce plant pathogens. We describe extraction of lignocellulytic enzymes and antimicrobial substance from SMSs of different edible mushrooms and their potential applications.

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Keyword: antimicrobial activity, lignocellulytic enzymes, spent mushroom substrate
* Aknowlegement: this research is supported by research grant (Agenda project No. PJ009969) from Rural Department Administration, Suwon, Korea.

S9-2

Study on Species Diversity of Indigenous Mushrooms in Jeju

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The importance of utilizing biological resources has become magnified and it has been a big issue to share the benefit among nations as Nagoya Protocol began in 2010. This study was conducted to research the diversity and distribution of wild mushrooms, and to survey the traditional mushroom knowledge of the people in Jejudo which is a volcanic island having a distinctive climate and forest environment.

The research sites were Dongbaekdongsan, Keuneonggot, Hallasan National Park, Muryeongarioreum, Saryeonisupgil and other important area where mushrooms are growing spontainously in Jejudo. A total of 511 species comprising 2 phylums, 8 classes, 20 orders and 74 genera were identified from 1600 specimens collected from 2006 to 2012. In previous studies, a total of 561 species comprising 69 families and 99 genera were investigated. As a result, a total of 755 species comprising 23 orders, 87 families and 263 genera were documented in Jejudo.

In this study, 137 species were newly identified as unrecorded species in Jejudo and 9 species, Amanita gemmata, Tricholoma aurantiipes, Panellus violaceofulvus, Leucopaxillus septentrionalis, Bondarzewia montana, Psilocybe argentipes, Boedijnopeziza insititia, Sarcoscypha occidentalis for. occidentalis and Morchella patula var. semilibera were the first record for Korea. Also, 7 species, Amanita gemmata, Tricoloma aurantiipes, Panellus violaceofulvus, Leucopaxillus septentrionalis, Boedijnopeziza insititia, Sarcoscypha occidentalis for. occidentalis and Morchella patula var. semilibera were known as only growing in Jejudo.

The traditional knowledge was collected from visiting and questionnaire survey in 50 villages in Jejudo. A total of 23 mushrooms were found in which 12 species were used for food, 2 species were poisonous, 6 species were medicinal, 2 species were used for folk religion and 3 species were used for play purposes.

Macrolepiota procera was the most commonly used as an edible mushroom and *Chlorophyllum neomastoidea* was the most well known poisonous mushroom. Also, 267 cases of traditional knowledge about using mushrooms as a food and medicine were collected.

This study has significance for supplementing previous studies about distribution of wild mushrooms in Jejudo and documenting unrecorded species in Korea. Also, it is valuable by providing important data of traditional knowledge for using mushrooms since old times.

Keywords: biodiversity, Hallasan, Jeju Island, Oreum, wild mushroom

S9-3

Distiller's Yeast Discovery for Industrial Application

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There are many yeast strains have been discovered for industrial usage in global scale. In the point of view for the alcoholic fermentation performance and producing alcoholic beverage products, recently many countries have known about the importance of microorganisms as a valuable resource. Discovered with well performed yeasts have potential industrial application in diverse ways such as foods, beverages, cosmetics, pharmaceutical functions, and so on.

In Korea, the yeast research has not been sufficiently performed especially for distilled spirits industry. As a result, not so little manufacturers use exotic yeasts from overseas even included the expensive royalties. Besides of those, to produce distilled spirits, many manufacturers do not use specialized yeast for distilled spirits.

Distiller's characterized yeasts such as whisky, brandy, vodka, Japanese shochu and awamori, are all well-known industrialized. For decades, the distillers, except us, have selected, developed, and practised yeasts in accordance with distilled spirits characters.

This study is about selection and industrial application of yeasts for the Korean pot distilled spirits. Finally 7 yeast strains were selected among over 1,000 yeasts from the traditional Nuruks, through the essential related tests based on brewing and distilling science. The selected yeasts show the appropriate characteristics of distilled spirits. The result of this study could help our distilled spirits industry be activated and stand independent from the exotic microbes.

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Keywords: distiller's yeast, distillery yeast, distilled spirits, spirits, yeast, yeast strains

S9-4

Disinfection of *Fusarium*-infected Rice Seeds by Prochloraz and Gaseous Chlorine Dioxide

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Three species of *Fusarium*, *F. fujikuroi*, *F. verticillioides* and *F. proliferatum*, are known to be associated with bakanae disease of rice [1, 2]. *F. fujikuroi* infects rice flowers and survive in endosperm and embryo of the seeds. Infected seed is an important source of primary inoculum of pathogens [3]. Seeds of rice (*Oryza sativa* cv. Boramchan) collected from bakanae-infected field were found to be 96% infected with *Fusarium* sp., 52% with *F. fujikuroi*, 42% with *F. verticillioides*, and 12% with *F. proliferatum* as determined by incubation method and species-specific PCR assays. *F. fujikuroi* was detected at lemma/palea, endosperm and embryo whereas *F. verticillioides* and *F. proliferatum* were recovered only from lemma/palea by means of component plating test.

Seed disinfection methods have been developed to control bakanae disease and prochloraz has been most widely used for rice seeds. Two chemicals formulated with prochloraz (PC 1) and prochloraz + hexaconazole (PC 2) that inhibit biosynthesis of ergosterol strongly reduced the incidence of *Fusarium* spp. on selective media to 4.7% and 2.0%, respectively. Disease symptoms of rice seedlings in nursery soil were alleviated by chemical treatment; seedlings with elongated leaves or wide angle between leaf and stem were strikingly reduced from 15.6 to 3.2% (PC 1) and 0 (PC 2), stem rots were reduced from 56.9 to 26.2% (PC 1) and 32.1% (PC 2), and normal seedling increased from 0.4 to 13.3% (PC 2).

Prochloraz has some disadvantages and risks such as the occurrence of tolerant pathogens [4] and effects on the sterol synthesis in animals and humans [5]. For these reasons, it is necessary to develop new disinfection method that do not induce fungal tolerance and are safe to humans and animals. Chlorine dioxide (ClO_2), that is less toxic, produces no harmful byproducts, and has high oxidizing power, has been reported to be effective at disinfection of several phytopathogenic fungi including *Colletotrichum* spp. and *Alternaria* spp. [6]. Gaseous ClO_2 applied to rice seeds at a concentration of 20 ppm strongly suppressed mycelial growth of *Fusarium fujikuroi, F. verticillioides* and *F. proliferatum*. The incidence of *Fusarium* spp. in dry seed with 8.7% seed moisture content (SMC) tended to decrease as the concentration of ClO_2 increased from 20 to 40 ppm. Applying 40 ppm ClO_2 at 90% relative humidity, incidence was reduced to 5.3% and resulted in significant reduction of disease symptoms on MS media. In nursery soil, stem rot was reduced from 56.9 to 15.4% and the number of normal seedlings increased from 0.4 to 25.5%. With water-soaked seeds (33.1% SMC) holding moisture in the endosperm and embryo, the effectiveness of disinfection using ClO_2 increased, even when treated with only 20 ppm for four hours. This suggests that moisture was a key element for action of ClO_2. Removal of the palea and lemma from seeds significantly decreased the incidence of *Fusarium* spp. to 3.0%. Seed germination appeared to decrease slightly by water-soaking at 30°C because of increased SMC and by physical damage of embryos from hulling. These results indicate that the use of gaseous ClO_2 was effective as a means to disinfect rice seeds infected with *Fusarium* spp. and that moisture around the pathogens in the seed was an important factor for the action of ClO_2. Further investigations should be conducted to ascertain the best conditions for complete disinfection of *Fusarium* spp. that infect deep site of rice seeds.

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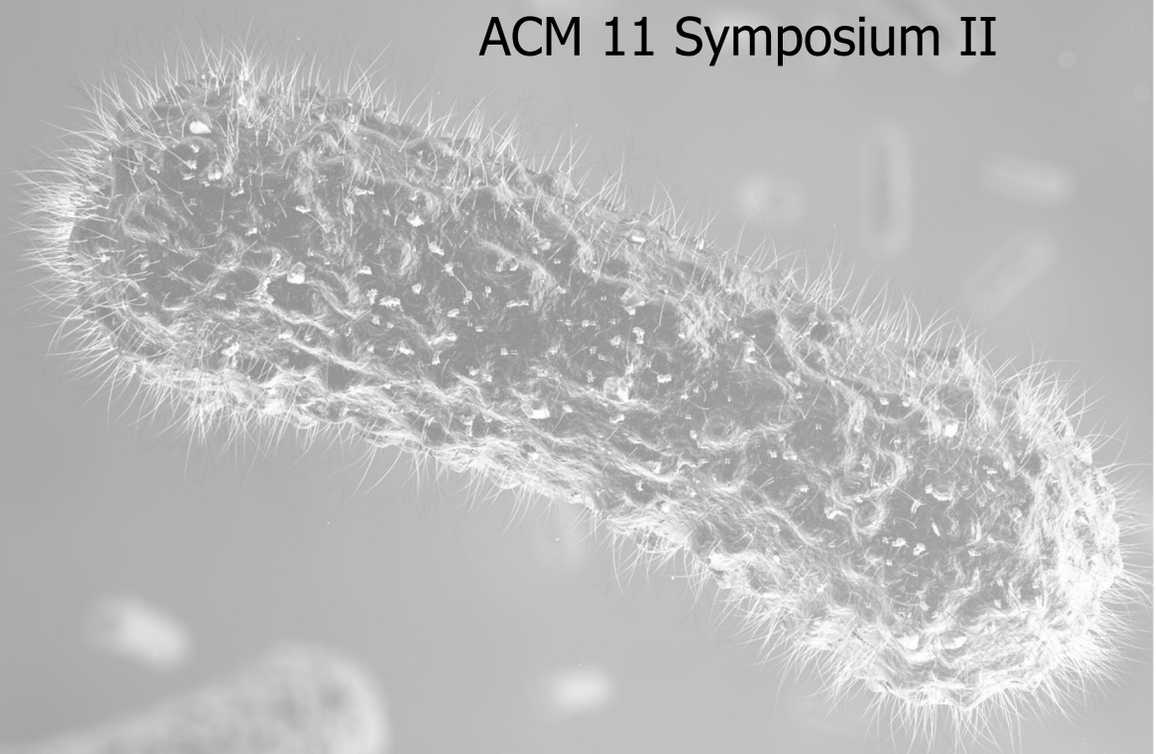
Keywords: chlorine dioxide, disinfection, *Fusarium* spp., prochloraz, rice seed

Symposium [S10]

ACM 11 Symposium II



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INTERNATIONAL MEETING OF

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S10-1

Secretariat of the National Steering Committee for Biosafety

Oum Pisey

http://www.bch.gov.kh

The Secretariat of the National Steering Committee for Biosafety (NSCB) is in charge of detecting living modified organisms, conducting risk assessment, risk management, monitoring and presenting advice to the government. NSCB has been conducting testing to identify genetically modified crops through applying strip test and real time PCR-Machine. Training workshops on LMOs Detection have been organized throughout Cambodia to train 400 custom officers, border inspectors, environmental officers, and agricultural agents to be able to familiar with Law on Biosafety, detecting and monitoring of LMOs. Several crops have been tested such as Cotton, Maize, Papaya, Rice and Soybean. NSCB has recently conducted a country-wide inventory on agro-industry farm to be able to monitor possible illegal commercial plantation of genetically modified crops and propose some response measures in case of risk exposure.

S10-2

China General Microbiological Culture Collection Center, IMCAS-BRC

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The Institute of Microbiology of the Chinese Academy of Sciences (IMCAS) was founded on December 3, 1958, through the merger of the Institute of Applied Mycology and the Beijing Laboratories of Microbiology, both of which were affiliated to the Chinese Academy of Sciences (CAS). After over 50 years of development, it has become the nation's largest comprehensive research institution of microbiological science. Since 2008, the Institute has endeavored to reorganize research and development activities into an innovative value chain with a biological resource center, a scientific research system and a technology transfer and transformation center as three interconnected units, and to carry out basic, strategic and prospective research in the areas of microbial resources, microbial biotechnology and pathogenic microbiology and immunology to meet national needs in industrial upgrading, agricultural development, human health, environmental protection, etc.

China General Microbiological Culture Collection Center (CGMCC), a department of Institute of Microbiology CAS, is a non-profit organization established in 1979 as the central culture collection in the cooperative network of China Committee for Culture Collection of Microorganisms (CCCCM). The CGMCC has started to carry out its tasks as a depository organization of China designated by Patent Bureau of China since 1985 and as an International Depository Authority (IDA) under the regulations of the Budapest Treaty since 1995.

Biological Resources Center, Institute of Microbiology Chinese Academy of Sciences (IMCAS-BRC) has been set up by integrating the China General Culture Collection Center (CGMCC), the Information Center and the newly developed high throughput evaluation platform of microbial metabolites. The mission of IMCAS-BRC is to serve as an integrated public infrastructure of culture collection, research and utilization of microorganisms, in order to sustain biotechnology innovation. In addition to preservation and management of microbial strains, in recent years, IMCAS-BRC is engaged in the function evaluation of the preserved microbes, especially the functional genes to promote utilization of microbial resources.

Recently, three projects on microbial investigation and resource collection from some special environment in china, supported by MOST, have been co-organized by IMCAS-BRC. There are hundreds of researchers from the 18 institutes and universities take part in the projects. The major purpose of these projects is to collect, preserve and provide valuable microbial resources for bioscience and biotechnology.

S10-3

Microbial Type Culture Collection and Gene Bank (MTCC)

D. Ananthapadmanaban, S. Mayilraj, Korpole Suresh, P. Anil Kumar, S. Krishnamurthi, Amit Arora and G.S.Prasad

http://mtcc.imtech.res.in

Introduction of the Institute: Microbial Type Culture Collection and Gene Bank (MTCC) located in CSIR-Institute of Microbial Technology (CSIR-IMTECH) is the premier culture collection of the country providing quality services and helping serving the scientific community of the country for more than two decades. MTCC is an International Depositary Authority (IDA) under Budapest Treaty on 4 October, 2002, by the World Intellectual Property Organization (WIPO), Geneva, Switzerland, thus becoming the first IDA in India. It is a National Biodiversity Authority (NBA) of India, Designated National Repository (DNR) for microbial cultures. MTCC has mandate to accept deposits of actinomycetes, bacteria, fungi, plasmids and yeasts for patent purposes. Besides accepting cultures under general deposit, it accepts two kinds of confidential deposits viz., 1. Safe deposit : Confidential deposit service for those valuable cultures for which patent protection has not been sought, if requested, the culture can be transformed to a patent strain deposit at a later date 2. Deposits under Budapest Treaty: Valid for filing patents in all the PCT countries.

Current Status of Culture Collections: MTCC has around 25,000 microbial cultures (actinomycetes, bacteria, fungi, yeasts and plasmids) in its collections. Of which 12,000 are available for public distribution, and information about these cultures is available at the website http://mtcc.imtech.res.in/catalogue.php. This collection includes type strains of several taxa, strains used for teaching purposes, genetic stock, cultures used for various quality control tests etc. More than 80% of MTCC general collection is of Indian origin from various ecological niches of India. MTCC has been in the forefront in offering quality services related to microbiology to several thousands of researchers of the country. The customer database of MTCC (around 9,500 customers) ranges from a college in a remote location to top-most biotech/ pharmaceutical company of the country. MTCC has supplied more than thirty five thousand (35,000) microbial cultures, and has characterized more than 2,500 microorganisms for other researchers during the last five years. Recently CSIR-IMTECH and Indian Phamacopoeia Commission (IPC) have assigned the responsibility of providing certified reference microbial cultures to Indian pharma stakeholders.

Microbial Diversity, Taxonomy and Genomics research: Besides the service component, MTCC scientists are actively involved in research activities related to microbial diversity, microbial taxonomy, environmental biotechnology etc. Over the last decade, MTCC scientists have described 70 novel microbial genera/species from different ecological niches of India. We have initiated whole-genome sequencing of taxonomically and biotechnologically important microbial strains of Indian origin available at the MTCC in order to explore biodiversity and evolution. Genomics data will form the underlying basis for fine-grained taxonomic classification. In collaboration with other researchers in our organization we have started two complementary projects “The Genome Annotation Collaborative (TGAC)” and “Augmenting Classical Taxonomy & Genomics (ACTG)”.

S10-4

Indonesian Culture Collection (InaCC)

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http://inacc.biologi.lipi.go.id/

Indonesian Culture Collection (InaCC) was established by mergering two big culture collections in Indonesian Institute of Sciences (LIPI), i.e. LIPI Microbial Culture Collection (LIPIMC) and Biotechnology Culture Collection (BTCC). Starting from 2007, InaCC has been developed with various modern facilities which are crucial to support research and depository activities. A new building of InaCC was inaugurated by Vice President of Indonesia on September 11, 2014 located in Cibinong Science Center and Botanical Garden, West Java. Certification of ISO 9001 on quality management has been received in the begining of 2014 and thus InaCC will be able to give optimal services as national depository of microorganisms. InaCC has been trying to become Competence National Authority (CNA) which is essential for Indonesia as a country who has ratified Nagoya Protocol in October 2013 (UU No. 11 Year 2013). The collection from two previous culture collections have exceeded 6000 isolates of filamentous fungi, yeast, actinomycetes, bacteria, archaea, microalgae, and bacteriophage, and currently those are on the process of standardized deposition in InaCC, with the additional at least 2000 microbial strains deposition by 2016 under the project of Science and Technology Research Partnership for Sustainable Development (SATREPS). Most research activities were foccused on taxonomic studies of indigenous microbial resources from several sources including soil, plant litter, fermented foods, plant roots, animal supporting with molecular and bioinformatic analyses. Those microbial resources are potentially used in drug discovery, biorefinery and bioenergy processes, food and feed applications, as well as soil quality and agricultural improvements. In the future InaCC will play roles as centers for microbial preservation, microbial access, patented microbial preservation, research on microbial exploration and high-throughput screening, training on microorganisms handling, and public awareness on microbial roles and bioprospects.

S10-5

Japan Collection of Microorganisms (JCM), RIKEN BioResource Center

Moriya Ohkuma

http://jcm.brc.riken.jp

JCM has been collecting, preserving, and distributing cultured microbial strains, since established in 1981, as one of the leading culture collections in the world. JCM aims to contribute to scientific communities by maintaining and serving useful microbial resources for general microbial studies and various researches. After joining to RIKEN BioResource Center (RIKEN-BRC) at 2004, we have particularly focused on microbial resources for health and environmental sciences. JCM has participated in the National BioResource Project (NBRP) supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan as the core facility for "General Microbes" since 2007. JCM has been acquired the certification of ISO9001:2008 for its quality management system to maintain and improve the quality of its service.

JCM holds 22,455 microbial strains as of Sep. 2014; 15,049 strains of bacteria, 679 strains of archaea, and 6,727 strains of fungi including yeasts. Among them, >14,700 strains are open to public. JCM annually received a deposition of approximately 800 strains including many type strains. On receiving the depositions, we quickly and extensively check their growth, purity, and authenticity, to find 12.4% of them had any problems in 2013. We asked the depositor to resend the authentic strains. More than 3,400 strains are annually distributed to domestic and overseas researchers. For convenience of users, genomic DNA samples are served in cooperation with RIKEN-BRC DNA Bank, and we have started the distribution of active culture upon request. As the consequence, JCM strains appeared in 540 original scientific papers and >85 patent applications published in 2013.

JCM also has research activities to exploit new microbial resources, to describe novel microbial taxa, and to develop the methods for investigating and handling extremophiles, yet-uncultured microorganisms and microbial communities. In collaborations with researchers outside JCM, we published 38 original scientific papers including descriptions of 24 novel prokaryotic species in 2013. We also determined draft genome sequence of more than 300 JCM strains of prokaryotes under the NBRP genome information upgrading program, and the genome information has been opened through public databases and our web site. This year we are determining draft genomes of 100 eukaryotic JCM strains under the same program. These research activities enable to enrich our holdings and to add values to JCM strains. Strain information is exhibited through our continuously updated online database.

S10-6

KCTC, Korea Research Institute of Bioscience and Biotechnology

Doo-Sang Park

http://kctc.kribb.re.kr

Korean Collection for Type Cultures (KCTC) is located at the main campus of Korea Research Institute of Bioscience and Biotechnology (KRIBB) in Daejeon. It was established in 1985 by Korean government as an infrastructure to support biotechnological research in Korea. KCTC joined the World Federation of Culture Collections in 1985. It has also gained the status of an International Depositary Authority (IDA) from the World Intellectual Property Organization in June 1990.

Total number of holding of biological resources in KCTC is 24130 including 5013 patent strains, 4120 Gram-negative bacteria, 4093 molds, 2473 yeasts, 1968 Gram-positive bacteria, 1686 actinobacteria, 1367 anaerobic bacteria, 188 archaea, and 173 extremophiles. KCTC also has 1,477 microalgae, 781 plant cell lines and 158 animal cell lines (as of September 2014). Total distribution to research institutes or universities was 7,099 strains in 2013. In 2013, KCTC members published 67 scientific papers which comprised of the description of 39 microbial taxa including 7 novel genera. Researches performed by KCTC are mainly on microbial taxonomy, but it also covers microbial ecology, enzyme characterization derived from bacteria, and bioinformatics.

KCTC provides regular education programs for domestic users and young researchers abroad. There are 3 workshops a year where grad students and technicians at private companies participate one day workshop of lectures and experiments to learn basic skills of growing and preserving microorganisms or cell lines. KCTC also holds 9 workshops for analyzing cellular fatty acids using MIDI system for bacterial taxonomy and 2 workshops for molecular phylogenetic analysis. The 4^{th} international training course on microbial taxonomy was held for 6 weeks in spring 2014 with four participants from ACM countries.

S10-7

Malaysian Agricultural Research and Development Institute (MARDI)

Tosiah Sadi

http://www.mardi.gov.my

Introduction of the Institute

Malaysian Agricultural Research and Development Institute (MARDI) is a statutory body which has been mandated to conduct research in agriculture, food and agro-based industries. MARDI research endeavors for almost 45 years had generated many new crop (varieties and clones) and animal breeds together with their management practices. However MARDI involvement in microorganism utilization related research (other than food) is considerably at infancy stage**.**

Current Status of Culture Collections or BRC

Since 2002 MARDI have pun and effort to enhance the activities related on microbial collection. MARDI have registered the collection to WDCM-MIRCEN database in 2012. The collection is growing up slowly from 105 (mostly are plant pathogen) in 1984 to 1993 in 2013 and recent number in the collection was 2137. MARDI are now working closely with BIOTEC Thailand to build up capacity for improving the microbial preservation techniques through special technical collaboration under the Ministry of Foreign Affair (MoFA). The importance to develop a good microbial culture collection was realized by MoFA, beside other ministries such as the Ministry of Agriculture and Agro-Based Industry (MOA), Ministry of Science Technology and Innovation (MOSTI) and Ministry of Natural Resources and Environment (NRE). MARDI is now working closely with MOA to enhance R&D activities, capacity building and legal issues through the establishment of Malaysian National Strategies and Action Plans (NSAP) on agricultural biodiversity conservation and sustainable utilization.

Research Highlight

MARDI has been working very hard to bid for grants from the government and for last two years, we managed to receive some funding to enhance our research on microbial utilization. Some of the achievements were purification of selected mushroom for fresh mushroom industry, fungi with ability to produce omega-3 fatty acids, biofertilizers, protocols for xylanase and cellulase production from Malaysia indigenous microbe species and others.

MARDI also focuses on controlling crop diseases by applying advance molecular techniques to search of gene of interest which responsible for the diseases. Beside the utilization, basic studies to understand the microbial taxonomy was also carried out and for the time being we are focusing the work on *Trichoderma* spp

Conclusion

MARDI will continue the efforts to improve and enhance R&D on microbial utilization and increase awareness activities of the stakeholders and smallholders (policy makers, ministries and public) on the important of conserving our biodiversity heritage not only microbes but other biodiversity components i.e. plants and arthropods.

S10-8

Korea National Research Resource Centers (KNRRC)

Kyungsook Ahn, Yeonhee Lee

http://www.knrrc.or.kr

Supported by the Ministry of Science, ICT and Future Planning (MSIP) and National Research Foundation of Korea (NRF), Korea National Research Resource Center (KNRRC) project aims to lead the creative scientific technology by securing and providing reliable resources and associated information for scientific researches and applications. Currently, KNRRC consists of a headquarters (HQ), 5 core centers, 11 microorganism, 6 human-origin, 7 animal, 4 plants, and 8 fusion-matter research resource centers (RRCs). This year, Korean Cell Line Bank was converted to a RC for special purposes and three new members joined the project; Neuromarker Resource Bank, Porous Nanoparticle Bank and Noncentrosymmetric Materials Bank.

In 2013, KNRRC collected more than 100,000 new resources and distributed 60,399 resources to universities, research institutes and industry. Also, KNRRC provided 123 technical services and signed 13 memorandum of understanding (MOU) with 10 domestic institutes and 3 international institutes for cooperation. Using the distributed KNRRC resources, 678 SCI papers were published and 20 patents were applied or registered. Among 36 RRCs, 11 microbial resource centers collected 8,354 resources and distributed 4,358 resources in 2013.

KNRRC HQ provides education programs and cross training programs for RRC staffs to improve resource management and to initiate collaborative researches. In 2014, KNRRC HQ supported four RRC staffs for short-term visits to RIKEN-BRC, National Institute for Longevity Science, Tokyo Metropolitan Institute of Gerontology in Japan, and Centre for Cancer Biology, University of Adelaide in Australia. In addition, two Chinese scientists from Institute of Hydrobiology, Chinese Academy of Sciences (IHB, CAS) were invited for a two week training program at Korea Marine Microalgae Culture Center in July, 2014. As a part of community education program, KNRRC offers science classes “Observation and Exploration of Research Resources” for 4^{th}~6^{th} grade elementary school students at the Seoul National Science Museum. The classes consist of hands-on experiments using various research resources and introduction of related scientific theory.

KNRRC organizes International Symposium and Exhibition of RRCs at the National Assembly. This year, the invited speakers for the 8^{th} symposium include the president of WFCC, the president of ISBER, and the executive director of BIOTEC (Thailand). The Tanzania Wildlife Research Institute (TAWIRI)-KNRRC cooperation project on biological resources is in 5^{th} year and a workshop is being planned in Arusha, Tanzania early next year. KNRRC will host the 7^{th} Asian Network of Research Resource Centers (ANRRC) international meeting in Korea in September, 2015.

S10-9

Biological Resource Center, National Institute of Technology and Evaluation (NBRC)

Manabu Suto

Yasushi Noto, Katsuhiko Ando, Ken-ichiro Suzuki, Hiroko Kawasaki, Manabu Suto, Atsushi Yamazoe, Rie Funabiki and Akane Kimura

http://www.bio.nite.go.jp/e/

(1) Introduction

NBRC is one of the leading public biological resource centers in the world established in 2002 under the support of Ministry of Economy, Trade and Industry (METI).

The mission of NBRC is to provide infrastructure necessary for promoting biotechnology by supplying microbiological resources, genome analysis, international cooperation, supporting for legitimate industrial use of LMOs and patent depository services.

(2) Current Status of NBRC culture collection (Data as of March 31, 2014)

NBRC has socked approximately 85K strains. NBRC Culture Collection holds 29,176 strains of bacteria including actinomycetes, archaea, fungi including yeasts, microalgae, and bacteriophages. Among them, 18,404 strains are available for distribution and listed in the on-line catalogue of NBRC. In the fiscal year of 2013, 683 microbial strains were newly accepted in the NBRC Culture Collection. Regarding the number of distribution in the fiscal year of 2013, 8,019 microbial strains, 91 genome DNAs and 30,287 human cDNA clones (including 30,070 clones/set) were distributed.

(3) Research Highlight

i) Taxonomic Studies: We have published a number of papers on taxonomy of fungi, yeasts, bacteria, actinomycetes, archaea and algae including isolation and proposal of new taxa. In addition, we have collected MALDI-TOFMS profile of NBRC strains for the purpose of rapid identification and quality control. ***ii) Development of Database:*** MiFuP (**Mi**crobial **Fu**nctional **P**otential) is a newly launched database of functional potentials deduced from microbial genomes. It enables users to easily search for existing microbes with potential functions fitted for specific interest. For detail, please visit <http://www.bio.nite.go.jp/ngac/e/mifup-e.html>. ***iii) Whole Genome Analysis:*** The whole genome shotgun approach has been adopted to sequence the genome of various organisms of significant importance to public health, food safety, energy production, environmental management, and taxonomic criteria. The sequenced data is published on the DDBJ (DNA Data Bank of Japan). For detail, please visit http://www.bio.nite.go.jp/ngac/e/project_wgs-e.html. We also constructed a literature-based database on secondary metabolite biosynthetic gene clusters named DoBISCUIT (http://www.bio.nite.go.jp/pks/). ***iv) International Collaborative Projects:*** At present, we have concluded the Memorandum of Understanding (MOU) and the Project Agreement (PA) with 7 Asian countries. These projects are supported by the collaborative efforts in consideration of the Convention on Biological Diversity (CBD). ***v) Isolation of Potential Useful Microbes:*** We have isolated and characterized variable microbes for food production, energy production,

secondary metabolic production, agriculture and biosafety.

(4) Conclusion

Having recognized the fact that microorganisms in various natural environments in Asia are rich in biological diversity, NBRC commits to continue the collaboration with ACM members to contribute to the conservation of biodiversity and promote sustainable use of the resources, with fair and equitable benefit sharing under the Convention on Biological Diversity (CBD).

S10-10

Korean Agricultural Culture Collection (KACC), National Academy of Agricultural Science (Korea)

Soon-Wo Kwon

http://www.genebank.go.kr

Korean Agricultural Culture Collection (KACC), an affiliate of National Academy of Agricultural Science (NASS), was established in 1995. By "Act on the Preservation, Management and Use of Agro-fishery Bioresources", Recently, KACC was relocated from Suwon city, Gyeonggi province to Wanju-gun, Jeollabuk province, Republic of Korea. KACC was authorized as the national center for the management of agricultural microorganisms. Accordingly, KACC has collected, characterized and preserved bacteria, filamentous fungi, yeasts and mushrooms. For the quality control of microorganisms, KACC identifies microorganisms through the 16S rRNA genes of bacterial strains and ITS regions of fungal strains. Taxonomically characterized microorganisms were databased (http://www.genebank.go.kr/) and open to the scientific circles. Until now, KACC registered a total of more than 18 thousand strains, and every year more than 2 thousands strains were provided for the research organizations such as universities, research institutes and private companies. Under the Budapest treaty and domestic patent law, KACC acts as the local depository for patent purposes. Each year, more than 100 patent strains are deposited in KACC. From this year, KACC plays the role of the national integrated patent depository, which backup all the patent microorganisms preserved in four patent depositories in Korea. Around 10 thousands patent microorganisms will be duplicated and preserved in KACC for the safer management. Furthermore, KACC studies the taxonomic characterization of the isolates from soil, air and Korean traditional fermented foods, and characterizes the mushrooms collected from Korean national parks. Every year, over 10 novel species of bacteria and fungi are reported by KACC.

S10-11

Laboratory of Microbiology, Institute of Biology, MAS

Tsetseg Baljinova

http://www.mas.ac.mn

Introduction of the Institute. The Institute of Biology – one of the research centres of Mongolian Academy of Sciences – was established in 1965. The main goal of the Institute is to study the biological resources and biodiversity of Mongolian ecosystems, development of scientific justification for conservation and sustainable use of bio-resources and application of research results into practice to achieve the sustainable development of the country. The Institute employs 76 people from which 63 are the researchers. Among them there are academicians – 4, ScD – 6 and PhD – 19. At present the Institute consists of 9 laboratories and a small pilot-plant. The Institute publishes annually "The Proceedings of the Institute of Biology".

Current Status of Culture Collections or BRC (in your institute). The Laboratory of Microbiology maintains about 6,000 strains. It is the largest in Mongolia culture collection. We isolate and preserve indigenous microbial cultures for *ex-situ* conservation and future distribution for R&D in biotechnology and environmental protection. At the moment we are not a service collection but we hope to receive this status in future. In 2013 within Mongolia-Japan joint research project we isolated and enriched our collection with 1045 strains of actinomycetes, bacteria, fungi and yeast.

Research Highlight:

a) Taxonomic Classification and Identification.

Within Mongolia-Japan joint research project isolates of 2013 were assigned to 16 genera of actinobacteria, 76 genera of fungi, 6 genera of bacteria and 16 genera of yeast based on sequencing results and morphology. By invitation of the NITE 2 mongolian researchers made taxonomic study of 47 endophytic fungi isolated from 17 plants. Based on phylogenetic analysis of ITS and D1D2 regions of DNA they were assigned to 13 genera. The genera *Didymella, Stagonospora, Leptosphaeria, Neosetophoma, Coniochaete, Neofabraea* were registered for the first time in Mongolia. The 16S rDNA sequencing of 50 bacteria isolated from the plant rhizosphere and nodules revealed that they belonged to 10 genera. For the first time in Mongolia *nif*H genes were found in isolates of the genus *Mesorhizobium* and in 1 strain of *Rhizobium*. The *nif*H gene of this strain had the same phylogenetic posisiton with the *nif*H genes of *Mesorhizobium* strains. To train researchers in morphological and molecular taxonomy methods 2 workshops titled "Identification of mitosporic fungi" and "Introduction of molecular phylogenetic analysis of microorganisms" were carried out in our Laboratory by Dr. Ando K. and Dr. Sekimoto S. (NITE) on 21-23 July, 2014.

b) Utilization of Microbial Resources.

Evaluation of microbial resources maintained in our culture collection revealed their high biotechnological potential. Some lactic acid bacteria had antimutagenic activity, endophytic fungi and

actinomycetes showed L-asparaginase and fibrinolytic activities.

Conclusion. Mongolian microorganisms isolated and preserved in our culture collection represent a valuable source for R&D and taxonomic study. Despite of extreme environments, microbial diversity of Mongolia is quite rich. For example, actinobacteria found in Mongolia represent 42% of families and 25% of genera of the Class *Actinobacteria* published in Bergey's Manual of Systematic Bacteriology (2012).

S10-12

Microbial Culture Collection at the National Institute for Environmental Studies

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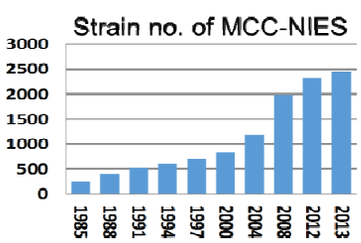
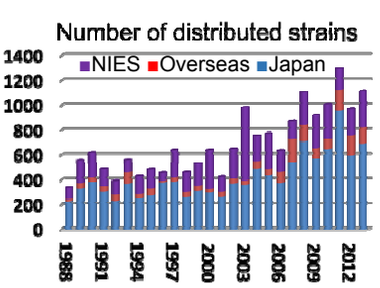


The Microbial Culture Collection at the National Institute for Environmental Studies (MCC-NIES), located at Tsukuba, Japan, was founded as an "environmental study-oriented" culture collection in 1983 when eutrophication of lakes and rivers, air and water pollution, were severe in Japan. The MCC-NIES started with ca. 250 strains mainly of red-tide-forming algae and water-bloom-forming cyanobacteria. Although MCC-NIES is still characterized by such strains, the collection now holds variety of species. At present the collection includes more than 50 classes, 375 genera, 752 species and 2,453 strains (Sep. 2014). MCC is also the only culture collection holding the endangered algae. In the list of endangered Japanese wildlife (the Red List) compiled by the Ministry of Environment (2012), charophytes (66 spp.) and red algae (32 spp.) were listed as extinct, extinct in the wild and/or endangered in Japan. MCC now maintained 39 species (421 strains) of the endangered algal species.

In 2002 the MCC-NIES was designated as a core collection for algae in the National BioResource Project (NBRP, http://www.nbrp.jp) conducted by the Ministry of Education, Culture, Sports, Science and Technology of Japan. In this framework, more than 200 strains of *Microcystis* and *Anabaena*, collected from representative eutrophic lakes all over Japan were deposited by the National Science Museum, together with phylogenetically diverse strains of microalgae and colorless protists deposited by the University of Tsukuba. In addition, more than 300 strains of cyanobacteria and eukaryotic microalgae maintained at the IAM Collection (University of Tokyo) had been transferred to the MCC-NIES up until the end of FY 2006, when the IAM Collection was closed. MCC-NIES now covers, 1) evolutionarily important species, 2) experimental materials that have been well-studied in genomic, genetic, molecular, and physiological terms, 3) ecologically significant species, 4) harmful algal species, and 5) commercially useful strains.

Highlights in 2013-2014



- As a public collection, MCC has distributed 1,113 strains in 2013 and 612 strains in 2014 (Sep.) for various purposes.
- MCC has developed cryopreservation and culturing techniques, PC programs for efficient maintenance of collection. In addition, MCC has accumulated DNA barcoding data and physiological data (e.g. photosynthetic pigments, fatty acids), resulting re-evaluation of the taxonomy and the characterizations.
- Collaborative works and projects: backups of the collection in Kobe Univ.

and Hokkaido Univ., molecular phylogeny and evolutionary works, genomic studies on cyanobacteria, metagenomics for biodiversity and environmental assessment, commercialization project targeting DHA/oil producing algae, etc.

S10-13

Philippine National Collection of Microorganisms (PNCM), National Institute of Molecular Biology and Biotechnology (BIOTECH)

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www.biotech.uplb.edu.ph

The Philippine National Collection of Microorganisms (PNCM) started as an in-house culture collection of the National Institute of Molecular Biology and Biotechnology (BIOTECH) in 1981, a year after the Institute was established, to have a permanent facility for the cultures isolated/studied by the different programs of the Institute. The culture collection was given the national repository status by the Department of Science and Technology (DOST) in 1996 and renamed PNCM. Aside from culture deposit and distribution, it has expanded its services and included various microbiological analyses to help the food and agriculture sector, and other industries. The PNCM has a current culture holding of 4,248 accessions consisting of bacteria, yeasts, molds, mushrooms and a few algae. It distributes ~600 cultures yearly to different research groups of the Institute for their research needs, to the academe, other research institutions and industries throughout the country.

Recent research undertaking of the PNCM is the identification of new isolates which have been screened by the other projects of the Institute for biofertilizer, biostimulant and biopesticide production, and the identification of lactic acid and acetic bacteria, as well as yeasts from local fruits which have been isolated by PNCM researchers. Moreover, the PNCM also identifies bacteria, yeasts and mold cultures for the private sector seeking assistance in their microbial identification needs.

BIOTECH which serves as the national research and development (R&D) organization under the University of the Philippines Los Banos specializing in agricultural, environmental, food and feeds, and health biotechnology, is continually searching for new bacterial and fungal strains for biotechnological innovations. It capitalizes on the use of the country's diverse collection of microorganisms, rich natural resources and agro-industrial wastes and by-products to develop and advance alternative technologies and products towards improved agro-industrial productivity.

Some new isolates are already in the pipeline for second generation inoculant production for agriculture. Several products which are already in the local market are biofertilizers (Bio-N, Nitroplus, Biogroe, VAM Root Inoculant, Mycovam, Brown Magic, Cocogroe, Biogreen and Bio-Fix), farm residue decomposer (Bioquick), animal vaccines (Biovac-HS and Biovac-FC), biopesticides (Pelmictrol, NPV and Bactrolep), antibiotic (tylosin), food and feed enzymes (alpha-amylase, celluase, glucoamylase, lipase, pectinase, protease and xylanase), animal probiotics, and microbial rennet.

S10-14

Thailand Bioresource Research Center (TBRC) and the Mission to Encourage International Cooperation for Utilization of Microbial Resources in Asia

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http://www.biotec.or.th/en

Thailand and other Asian countries represent a huge share of the planet's biodiversity. Many countries respect this unique, rich and diverse natural environment by establishing bioreseource centers for conservation and sustainable use of such resources. Thailand Bioresource Research Center (TBRC) also recognizes the important of microbial and genetic resources for the fundamental role in encouraging research and development as well as applications in biotechnology and industry. In the process of continuous integration in 2015 with the ASEAN Economic Community's single market, TBRC aims to coordinate the exchange and utilization of biological materials, information, and regulation among members.

TBRC has been actively engaging in many activities to strengthen and enhanced ASEAN research connectivity towards sustainable utilization of biological resources. TBRC launched the ASEAN Network on Microbial Utilization (AnMicro) with academic and research institutes in ASEAN and ASEAN Centre for Biodiversity during the first ASEAN Microbial Biotechnology Conference (AMBC2014) in February 2014. This activity is funded by the Ministry of Science and Technology, Thailand. Current activities of AnMicro to promote ASEAN connectivity included (1) capacity building through training course of data management in ASEAN, (2) creation of the microbial biotechnological-based forum as a platform for scientific exchange in ASEAN, and (3) establishment of knowledge exchanging network in ASEAN on microbial biotechnology.

TBRC and NITE Biological Resource Center (NBRC) have initiated the Curation Course for Microbial Resource Management as part of human resources development in 2013. The objective of this course is to develop skills in the management of biological resources including storage and identification of microorganisms and information management. For the past two years, this course has gained attention among Thai researchers in both public and private sectors and hopefully will expand internationally next year.

Given the increasing amount of biological resources used for the scientific and industrial purposes and internationalization of R&D, TBRC, Korea Research Institute of Bioscience and Biotechnology (KRIBB), NBRC, and World Data Centre for Microorganisms (WDCM) have developed the "Network of International Exchange of Microbes under ACM (NIEMA)" scheme to facilitate the international exchange, utilization, and distribution of microbial and their genetic resources. This international agreed best practice is in compliance with the Convention on Biological Diversity (CBD) and the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from Their Utilization (ABS).

Recently, TBRC has been intensively working on developing Thailand ABS guideline to facilitate international access, transfer, and share benefit arising from the utilization of the microbe for sustainable use of

its components and eventually for biological diversity conservation. This project is part of the ASEAN-U.S Science and Technology Fellows Program funded by USAID and U.S. Mission to ASEAN.

Under the collaborative efforts between TBRC and its partners, TBRC attempts to utilize its expertise in bioresource utilization to connect research and analysis in many scientific disciplines as well as in biotechnology and industry for conservation and sustainable future.

Keywords: Bioresource research center, microbial utilization, ASEAN, Convention on Biodiversity

S10-15

Updates of Activities of University of Santo Tomas Collection of Microbial Strains

Gina Rio Dedeles

www.ustcms.webs.com

The University of Santo Tomas Collection of Microbial Strains (USTCMS), an academic, research and community service-oriented unit affiliated with the Basic and Applied Microbiology Laboratory of the Research Center for the Natural and Applied Sciences, acts as the university main repository of strains dedicated to supply authentic microbial cultures to student-researchers, faculty researchers and scientists, as well as to student-researchers in neighboring universities.

USTCMS further provides microbiological services such as identification, characterization, and safe-keeping of bacteria and fungi to the student and faculty researchers from different colleges and research units of the university. A large number of microbial strains have been utilized in various research activities of the students and faculty researchers.

The major activities of USTCMS are currently focused on the development of preservation techniques and distribution of microbial cultures as well as on the on-going microbiological and analytical services catered to the needs of a private company.

At present, it has maintained several bacterial, filamentous fungal and yeast cultures obtained from students and faculty researchers as well. There have been still a large number of yeasts and actinomycetes that are to be extensively examined for proper identification.

The USTCMS website has been useful for those who may check, inquire and order on-line organisms for research activities.

S10-16

TISTR Culture Collection, Thailand Institute of Scientific and Technological Research

Chatrudee Suwannachart*, Chantara Phoonsiri, Aparat Mahakhant, Bhusita Wannissorn, Lawan Chatanon, Jaruwan Sidthipol, Somporn Moonmangmee, Premsuda Saman, Achara Chaiongkarn, Pongsatorn Prapakrangkul, Sirithon Teeravet, Mayuree Tungthananuwat, Chutima Klayprayoon, Suksan Srichuay, Pirawan Srisin and Kanjana Rueangyotchanthana

www.tistr.or.th/tistr_culture

Thailand Institute of Scientific and Technological Research, Thailand

TISTR Culture Collection or Bangkok (MIRCENs), Bio-Science Department, Thailand Institute of Scientific and Technological Research (TISTR) is one of the main service culture collections in Thailand. It was created with UNESCO/UNEP support in 976 with the aim of establishing a holding center for agriculturally and industrially useful microbial strains. In the year 2000, the quality management system of TISTR Culture Collection services were audited and issued complaint with quality standards ISO 900:2008 on supply, preservation and distribution of microorganisms.

Recently, TISTR Culture Collection collected more than 6,000 strains. TISTR strains especially bacteria, yeast, fungi and microalgae covered a wide range of known and potential applications for biotechnology, bio-control, agriculture, environment and industry. The cultures are distributed about ,000 strains per year. TISTR Culture Collection not only provides cultures but also offers safe deposit for microorganisms with high potential application. Therefore, TISTR Culture Collection also provides services on identification and characterization of microorganisms by using biochemical analysis and molecular based methods. In addition, TISTR Culture Collection has established many researches on application of local microorganisms. It is one of the aims of our culture collection to further develop its culture collection activities.

S10-17

Vietnam Type Culture Collection (VTCC), Institute of Microbiology and Biotechnology (IMBT), Vietnam National University, Hanoi, Vietnam (VNU)

Assoc. Prof. Dr. Duong Van Hop

http://www.imbt.vnu.edu.vn/vtcc/

Vietnam Type Culture Collection (VTCC) was established in 995 as part of the Institute of Microbiology and Biotechnology, Vietnam National University Hanoi (VNUH). Recently, VTCC was given the mission to establish a National Biological Resources Center (NBRC) Network for all 8 Biological Resources Centers (BRCs) in Vietnam. The main objectives of VTCC are:

- Enrich and maintain useful microorganisms by using standard methods
- Carry out taxonomical research of microorganisms
- Study diversity of microorganisms and their utilization (bioactive compounds)
- Provide pure cultures of microorganisms and related information as well as consultation in the field of microbiology
- Training young scientists in the fields of microbial diversity and culture collection management
- Establish a database for NBRC including all 8 BRCs in Vietnam

Current status of the Culture Collection at VTCC:

The online catalogue of VTCC has been updated with total 300 strains among 9,230 currently preserved cultures (filamentous fungi: 962; yeast: 886; acninomycetes: 57; bacteria: 68) for public users. Recently, a number of 2082 VTCC cultures were registered in the Global Catalogue of Microorganisms.

VTCC has proposed a project to the Vietnam government to establish a NBRC at the new campus, Hoa Lac.

Research Highlight:

- In 203, in cooperation with Japanese scientists from NITE, sampling trips were conducted in several ecological areas: Hanoi, Ho Chi Minh City, Hue, Ninh Binh and Tay Nguyen. From total 232 samples (soil, litter, plant), 575 isolates were obtained for further study (filamentous fungi: 000; yeast: 225; acninomycetes: 50, bacteria: 300).
- The biodiversity of lactic acid bacteria in Thanh Hoa fermented meat, yeasts in Phu Quoc and filament fungi in Bach Ma National park have been studied.
- VTCC was recognized as the best culture collection in Vietnam and obtained the prize from the minister of MOST, Vietnam.
- In collaboration with ICBiotech, Osaka University and Toyama Prefectural University, Japan, VTCC started to establish a database of bioactive compounds from VTCC cultures.
- Applied research activities at VTCC in 203 focused on several topics, including () enzymes (amylase, cellulose, lipase, phytase, protease, xylanase…) and probiotics for animal feeds, (2) environmental pollution treatment, (3) bioactive compounds from microbes against pathogenic microbes in rice (*Xoo*), foot mouth disease virus, (4)

bioactive compounds from *Bacillus sensu lato* species against multidrug resistant indicators.
- In the period 203-204, IMBT has successfully operared fermentation pilot at Hoa Lac.
- In 204 VTCC submitted the proposal of establishing national center of bioresources (BRC) as national key lab. The proposal recently has been adopted by VNU scientific committee.

S10-18

ACM Member Report

Bioresource Collection and Research Center (BCRC), Food Industry R&D Institute, Taiwan

Gwo-Fang Yuan, BCRC Director

www.bcrc.firdi.org.tw

Introduction of BCRC

BCRC, a working group in the non-profit Food Industry R&D Institute, has been the only centralized microbial resource center in Taiwan since 982, and originally aimed to preserve cultures for industry. BCRC has kept expanding its mission. It is now also entrusted by government authorities to operate agricultural microorganism bank, national authority for the deposit of patent biological materials, Cell Bank for National Health Research Institute, Taiwan Stem Cell Bank, etc.

Current Status of BCRC

BCRC's functions include: (1)collection, preservation, and distribution of microbial, cell, and gene resources; (2)services for preservation, identification, patent and safe deposit, information, consultation, training, contract tests, open lab; (3)research on biological material isolation, classification, identification, and improvement and development; (4)transfer technology resulted from research on biological material improvement and development; (5)provision of resources for public information and policy formulation.

Up to 204, BCRC has collected microorganisms of >26,200 strains, animal and human cells of >9,500 lines, and DNAs of >80,000 clones. The collection keeps expanding to include microalgae as well. And, more than 60 service items are available for the public. To ensure the quality of services, BCRC introduced ISO900 management system in 200, ISO/IEC 7025 in 2007, and ISO Guide 34 in 202.

Research Highlight

Taxonomic Classification and Identification

For microbial identification, a multiphasic approach platform established by BCRC has kept providing service to industries. Examples are identification and strain typing of lactic acid bacteria for food companies, identification of medicinal mushroom (such as *Antrodia cinnamomea*, *Ganonderma*, and *Cordyceps*) for biotech companies, and identification of microorganisms for environmental monitoring purposes for pharmaceutical companies. To distinguish closely related microorganisms, multi-locus sequence typing technology is the current research focus of BCRC for microbial identification and strain typing.

For human cell authentication, short tandem repeat profiling analysis service has been provided by BCRC since 2009. BCRC is also one of accredited institute by International Journal of Cancer to provide DNA profiles for cell authentication.

Utilization of Microbial Resources

Fermentation banks of microorganisms isolated from Taiwan were constructed available for further exploitation. Constructed fermentation banks include metabolites from marine fungi, thermophilic actinomycetes, freshwater fungi, yeast like fungi, waste water fungi, etc.

Cell-based screening assays were established for screening bioactive metabolites from fermentation banks. Established assays include binding assays to sex hormone and metabolic hormone, and cancer biomarker assays.

Conclusion

Recognizing that collaboration among biological resource centers (BRCs) is important for promoting conservation and sustainable use of microbial resources, BCRC being the only centralized microbial resource center in Taiwan, also one of active Asian BRCs, feels obligated to dedicate itself to the regional joint efforts in ACM. BCRC expresses its intent to become an ACM member. BCRC will work with ACM members and continue to promoting conservation and sustainable use of microbial resources.

Symposium [S11]

Microbial Stress Response

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S11-1

Endeavors of Proteomic Studies in the Research of Microbial Stress Response with the Gap Between Gene Expression and Function

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Bacteria regulate the expression of many genes for survival and growth under stressful conditions. The stress-related genes are tuned to maintain their expression levels by distinct signaling transduction and transcriptional mechanisms, thereby resulting in a significant noise in their expression patterns in response to environments [1]. A range of proteomics studies provide insights into the phenotypic plasticity for the adaptation to environment changes by keeping the gene expression levels or protein activities with physiological needs [2]. Most bacteria show a great phenotypic plasticity for physiological acclimation to changes in their habitat conditions, and they use these changes to generate phenotypic variation and evolutionary diversity. We introduced here a gap between genetic and proteomic studies for the evolution of non-inherited aminoglycoside resistance in *Escherichia coli*. The isolation rate of aminoglycoside-resistant bacteria increases in death phase. Even though population dynamics and antimicrobial susceptibility of the original strains vary with different genotypes, most of the resistant bacteria, once selected by an aminoglycoside drug, are able to develop a wide range of resistance to aminoglycosides, and result in a tradeoff with the sensitivity to other antibiotic classes. The aminoglycoside-resistant bacteria are differentiated into two phenotypes by growth-rate-dependent control. They all exhibit variable frequencies of point mutations in a noncoding region containing cos sequence of cryptic prophage DLP2. During aminoglycoside treatment, but not starvation, the resistant bacteria induce a cos cleavage activity, showing strong relationship between cos cleavage and resistance. Furthermore, the resistant bacteria are susceptible to accumulation of protein-coding mutations under stressful conditions. These changes are responsible for the phenotypic variation and genetic diversity of the resistant bacteria.

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Keywords: antibiotic resistance, proteomics, phenotypic variation, noncoding mutation, cos cleavage

S11-2

Cordycepin is A New Chemical Suppressor of EBV Replication.

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Cordyceps is known to produce many kinds of active components and used for a diversity of medicinal purpose due to various physiological activities. The physiological activities are anticancer activity, protection of liver damage, antidepressant effects, anti-inflammatory effects, hypoglycemic effect, antimicrobial activity, and etc. Cordycepin is a derivative of adenosine, differing from adenosine in that cordycepin lacks oxygen in the 3' position of its ribose part. Several research groups reported anti-viral activities of cordycepin to influenza virus, plant viruses, HIV, murine leukemia virus, Epstein-Barr virus (EBV), and etc. This study would address to define how cordycepin makes anti-gammaherpesviral effects using epigenetic approaches. Our study demonstrated that cordycepin contains antitumor and antiviral activity against gastric carcinoma and EBV, respectively. First, comparison of CD_{50} between cordycepin and its analogues indicated that loss of 2'-hydroxyl group in cordycepin was critical of cordycepin to produce stronger cytotoxicity than its analogues. Treatment of cordycepin suppressed early apoptosis up to 64%, yet it increased late apoptosis/necrosis up to 3% in SNU79 cells. Interestingly, cordycepin induced methylation up to 58% and suppressed unmethylation up to 37% on BCL7A in SUN79 cells. Consistent with methylation, cordycepin treatment demonstrated to significantly downregulate most EBV genes tested. In same context, cordycepin significantly decreased frequencies of Qp and Fp promoter usages and H3K4me3 histone enrichment was significantly reduced by cordycepin from several important EBV genomic locuses. Extracellular and intracellular EBV genome copy numbers were significantly reduced up to 55% and 30% at 25 μM treatment of cordycepin, respectively. Finally, cordycepin significantly suppressed the transfer of EBV from LCL-EBV-GFP cells to AGS cells, meaning significant inhibition of EBV infection to gastric epithelial cells. These results suggested that cordycepin is antiviral and antitumor against gammaherpesviruses and host cells virus latently infected.

Keywords: cordycepin, Epstein-Barr virus, gastric carcinoma, antiviral agents

S11-3

Role and Application of Two-Component Systems (TCS) of Radiation-Resistant Bacterium, *Deinococcus radiodurans*

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Deinococcus radiodurans R, a small, red-pigmented, and tetrad-forming gram positive soil bacterium, is one of the most radiation-resistant organisms known. *D. radiodurans* is also capable of withstanding desiccation, UV-C radiation, and various DNA damaging chemicals such as mitomycin C (MMC). Therefore, the mechanisms that underlie the extreme multiple stress tolerances of this organism are primary topics of interest for researchers. Although previous studies indicate that the extraordinary resistance of *D. radiodurans* to γ-radiation might result from a combination of different molecular mechanisms and physiological determinants, including efficient DNA repair, protection of proteins against oxidation, and a highly condensed nucleoid structure, the molecular mechanisms underlying this phenotype have not been fully revealed.

Bacteria are able to adapt to changes in the environment using two-component signal transduction systems (TCSs) composed of a histidine kinase (HK) and a response regulator (RR). *D. radiodurans* has 20 putative HKs and 25 putative RRs. In this study, we constructed 2 *D. radiodurans* mutant strains lacking a gene encoding a HK and surveyed their resistance to γ-radiation, UV radiation, MMC, and H_2O_2. Among them, the dr246 mutant strain showed decreases in resistance to DNA-damaging agents than the wild type. Reductions in the resistance to γ-radiation and H_2O_2 were observed in the absence of DR245, which seems to be a cognate RR of DR246. This result suggests that DR245/DR246 (DrtR/S: DNA damage response TCS) may be another TCS responsible for the extreme resistance of *D. radiodurans* to DNA-damaging agents. DR0053 belongs to the DinB/YfiT protein family, which is one of the over-represented protein families in *D. radiodurans*. We found that the *dr0053* transcript level was highly induced in response to gamma radiation (γ-radiation) and mitomycin C (MMC) depending on the RecA and DrtR/S. A *dr0053* mutant strain displayed sensitivity to γ-radiation and MMC and produced significantly higher levels of exopolysaccharide (EPS) and biofilm compared with the wild-type. Taken together, the results indicate that DR0053 may be involved not only in the DNA repair system but also in EPS synthesis in *D. radiodurans*.

We cloned and expressed a radiation inducible response regulator, DR558, from *D. radiodurans* into *Escherichia coli* and found that the stress tolerance of this recombinant strain (EC-558) was remarkably improved. EC-558 cells showed incredible stress tolerance with almost no loss of viability even at 20 mM of H_2O_2, while control cells showed 3-log cycle reduction at 0mM H_2O_2. The EC-558 cells were also tested for their ability to withstand acid (pH 2.5, 2h), high salt (2.5 M NaCl, 2h), and heat (54°C, 40 mins) stresses. Under all tested conditions, the engineered EC-558 cells showed remarkable tolerance with hardly any loss of viability as compared to the control cells (over 2-4 log cycles reduction), thus making it a multi-stress resistant strain. We employed a microarray analysis to study the regulatory network in EC-558 cells. Several genes implicated in the stress response were up-regulated, which are modulated by RpoS (σs) that is a general stress response sigma factor of *E. coli*. Although we do not rule out the possibility of DR558 targeting other potential genes in an RpoS-independent manner, it is likely that DR558 rewires the stress regulatory network of *E. coli* by favoring the

accumulation of RpoS.

Reference

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Keywords: *Deinococcus radiodurans*, gamma radiation, two-component system

S11-4

Non-replicative Helicases, UvrD and DinG, Regulate the Replication of Theta (θ)-replicative Plasmids in *Escherichia coli*

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When *Escherichia coli* cell cultures are transferred from 37°C to 5°C, *E. coli* experiences cold-shock stress. During cold-shock response, *E. coli* stops growing temporarily and the synthesis of most cellular proteins is dramatically reduced. However, the cold shock-inducible proteins are specifically expressed, and among these proteins, CspA has been identified as a major cold-shock protein in *E. coli*. CspA binds to RNAs which were abnormally folded by cold-shock, and acts as an RNA chaperone destabilizing RNAs. The dramatic expression of *cspA* at low temperature occurs temporarily because this gene has strong upstream box, SD sequence and downstream box. These factors are complementary to the 6S rRNA sequence and directly interact with ribosome. Interestingly, when *cspA* mRNA encoding a premature nonsense codon was overexpressed at low temperature, cell growth was completely inhibited. This phenotype was termed LACE (the low temperature-dependent antibiotic effect of truncated *cspA* expression), and this lethality results from an exclusive stalling of most of ribosomes on mutant *cspA* mRNA. In a previous study, we found that trans-factors which suppressed the growth inhibition caused by mutant *cspA* mRNA and they were ATP-dependent DNA helicases, UvrD and DinG. While both UvrD and DinG possess DNA-dependent ATPase and helicase activities, they are generally recognized to be involved in DNA repair. UvrD of *Esherichia coli* is originally identified for its crucial role in nucleotide excision repair and mismatch repair. *E. coli* DinG is a DNA damage-inducible protein (SOS-inducible protein), although its function *in vivo* is still unknown. From our preliminary experiments, we found that UvrD or DinG seems to have an ability to down-regulate the replication of high copy plasmid. In plasmid copy number tests, copy numbers of theta (θ)-replicative plasmids harboring mutant *cspA* were reduced by 3~0-folds when either UvrD or DinG was expressed. Through β-galactosidase activity assay, we also confirmed that the expression of gene inserted in the plasmid was reduced due to the down-regulation of plasmid replication. These results imply that UvrD and DinG, known as non-replicative helicase, have a novel role in the regulation of theta-plasmid replication.

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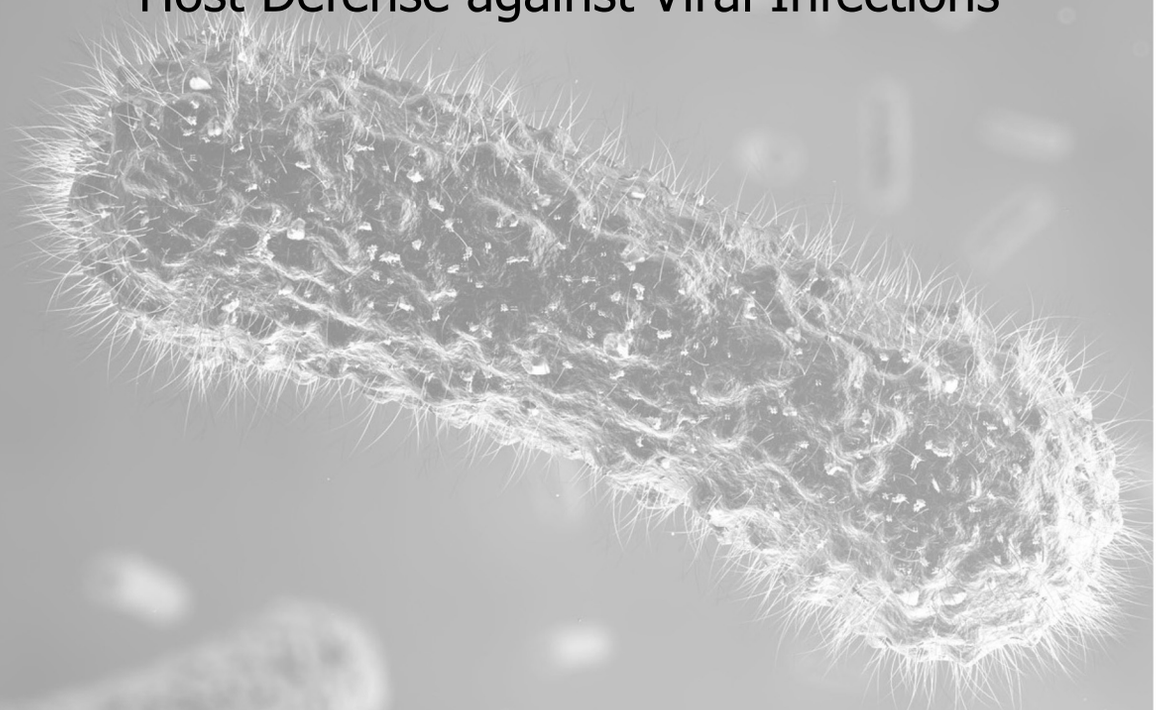
Key words: cold-shock response, CspA, UvrD, DinG, DNA helicase, LACE, theta-replication of plasmid

Symposium [S12]

Host Defense against Viral Infections



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S12-1

Upregulation of PD- on Regulatory T Cells Potentiates Their Suppressive Function during Chronic Viral Infection

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Regulatory T (T_{reg}) cells act as terminators in the case of T cell immunity during the acute phase of viral infection; however, their roles in chronic viral infection are not completely understood. Here, we compared the phenotype and function of T_{reg} cells during acute and chronic viral infection using lymphocytic choriomeningitis virus-infected mouse models. Chronic infection, unlike acute infection, led to induction of T_{reg} cells and upregulation of programmed death- (PD-). T_{reg} cells isolated from chronically infected mice displayed greater suppressive capacity for inhibiting T cell proliferation and subsequent cytokine production than those from naïve or acutely infected mice. Suppression was contact-dependent and required PD- expression. We found that PD- signaling in T_{reg} cells enhanced suppressive function by upregulating IL-0 and granzyme B. These findings establish PD- as a mediator of T_{reg} cell suppressive function in the regulation of T cell responses and suggest a role of PD- expression on T_{reg} cells, in addition to that on exhausted T cells, during chronic viral infection.

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Keywords: chronic virus infection, PD-, T_{reg} cells

S12-2

Development of a Novel Therapeutic DNA Vaccine against Human Papillomavirus-Associated Pre-Malignant Lesions; from Bench to Bedside

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Persistent human papillomavirus (HPV) infections, high risk HPV6 and HPV8 in particular, have been proven to be causative and necessary for the development of cervical dysplasia and cancer. Since current preventive vaccines against HPV are not likely to be effective for preventing progression in pre-existing HPV infections or HPV-associated lesions, there is still urgent need to develop therapeutic HPV vaccines. Cervical intraepithelial neoplasia 3 (CIN3) is pre-malignant lesions induced by persistent HPV infection, which, if not treated, progress to invasive cancer. Thus, CIN3 is a major therapeutic target for preventing severe complication by a persistent HPV infection. Several types of HPV therapeutic vaccines have been evaluated in patients with HPV6 and HPV8 positive CIN3 using viral vector, peptide, protein, and plasmid DNA. DNA vaccines have been proven to be highly advantageous in safety, versatility, and stability in many studies. However, a consistent theme in human DNA vaccine trials has been their suboptimal immunogenicity when compared to traditional vaccines. GX-88E is a strategically developed HPV DNA vaccine to enhance immunogenic potency and therapeutic efficacy against established HPV infection and high-grade lesions at cervix. Herein, I first introduce the proof of concept study in animal, and then provide an overview of pre-clinical studies to evaluate activity and safety profiles in developing the GX-88E therapeutic DNA vaccine as a new drug. In phase clinical study, we demonstrate that immunization with a GX-88E vaccine elicits a significant E6/E7-specific IFN-γ-producing T cell response in all 9 CIN3 patients. Importantly, 8 of 9 patients exhibit an enhanced polyfunctional HPV-specific CD8 T cell response as shown by an increase in cytolytic activity, proliferative capacity, and secretion of effector molecules. Among the 8 patients, all except one with the largest lesion size and the weakest polyfunctional CD8 T cell response show complete regression of high-grade lesions and clearance of HPV. Moreover, GX-88E administration does not elicit serious vaccine-associated adverse events at all administered doses. These findings indicate that the magnitude of systemic polyfunctional CD8 T cell response is the main contributing factor for histological, cytological, and virological responses, providing valuable insights into the design of therapeutic vaccines for effectively treating persistent infections and cancers in humans.

Keywords: vaccine, human papillomavirus, cervical intraepithelial lesion

S12-3

Cytokine-mediated Suppression of Hepatitis B Virus

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Cytokines are involved in the early host defense against pathogen infections. In particular, tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) are known to play a critical role in the non-cytopatic elimination of HBV in hepatocytes. Numerous studies have reported that TNF- α and IFN-γ inhibit HBV gene expression and replication *in vitro* and *in vivo*, however, the molecular mechanism and mediator molecules are unclear.

Cellular FLICE-inhibitory protein (c-FLIP), an anti-apoptotic protein, is known to be induced by TNF-a. We recently showed that p22-FLIP, a newly discovered c-FLIP variant, is constitutively generated in hepatocytes and interacts with HBx. In this study, we found that p22-FLIP inhibits the replication of HBV. Furthermore, we found that p22-FLIP is generated through the processing of c-FLIP by the TNF-α/NF-κB pathway, and that it is involved in the TNF-α-mediated inhibition of HBV. A mechanistic study revealed that p22-FLIP inhibits HBV replication through the dysregulation of hepatocyte nuclear factor 3 beta (HNF3b) and 4 alpha (HNF4a). Finally, p22-FLIP was found to potently inhibit the replication of HBV in a mouse model of HBV infection. These findings suggest that the anti-apoptotic p22-FLIP exerts a novel function in hepatocytes as a natural inhibitor against HBV infection and may provide a novel mechanism to explain the TNF-α-mediated suppression of HBV.

Keywords: hepatitis B virus, cytokine, c-FLIP, TNF-α, IFN-γ

S12-4

Deregulation of Immune Response Genes in Epstein-Barr Virus Associated Gastric Cancer Reflects Favorable Prognosis

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Background & Aims: Patients with Epstein-Barr virus (EBV)-associated gastric carcinoma (EBVaGC) have a better prognosis than those with EBV-non associated GC (EBVnGC). This is partly because EBV infection recruits lymphocytes, which infiltrate the tumor. High degree of tumor heterogeneity is likely associated with poor response. We investigated differences in gene expression patterns between EBVaGC and EBVnGC.

Methods: We used gene expression profile analysis to compare tumor and non-tumor gastric tissues from 2 patients with EBVaGC and 4 patients with EBVnGC. Findings were validated by whole transcriptome RNAseq and real-time quantitative PCR analyses using EBV-infected and -noninfected GC cell lines. $CD3^+$ primary T cells were isolated from human blood samples; migrations of these cells and of Jurkat T cells were measured in culture with EBV-infected and uninfected gastric cancer cells.

Results: Based on Pearson correlation matrix analysis, EBVaGC had a higher degree of homogeneity than EBVnGC. Although 4550 genes were differentially expressed between tumor and non-tumor gastric tissues of patients with EBVnGC, only 86 genes were deregulated in EBVaGC (P<.00). This finding supports the concept that EBVaGC has fewer genetic and epigenetic alterations than EBVnGC. Expression of MHC-class II genes and genes that regulate chemokine activity were more often deregulated in EBVaGCs, compared with non-tumor tissues. In culture, more T cells migrated to EBV-infected gastric cancer cells than to uninfected cells; migration was blocked with a neutralizing antibody against CXCR3, a receptor for many chemokines.

Conclusions: Fewer genes are deregulated in EBVaGC than in EBVnGC. Most frequent expressional changes in EBVaGC occurred in immune response genes. We propose these changes allow EBVaGC to recruit reactive immune cells; this might contribute to the better outcomes of these patients, compared to those with EBVnGC.

Symposium [S13]

Fungal Taxonomy and Diversity

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S13-1

Are Cryptic Species Real?

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Since Darwin and Wallace introduced the concept on the evolution of species, scientists have been furiously debating what species are, and how to define them. This basic yet intriguing question has bothered us ever since, as communicating to fellow biologists about fungal species is the very cornerstone of mycology. For the species presently known, this has largely been accomplished via Latin binomials linked to morphology in the absence of DNA barcodes. In recent years mycologists have embraced the ribosomal ITS as official barcode region for Fungi, and this locus is also mainly used in environmental pyrosequencing studies. Furthermore, DNA data can now also be used to describe sterile species in the absence or lack of distinct morphological structures. Recent developments such as the registration of names in MycoBank, and linking the phenotype to the genotype, have significantly changed the face of fungal systematics. By employing the Consolidated Species Concept, incorporating genealogical concordance, ecology and morphology, robust species recognition is now possible. Several international initiatives have since built on these developments, such as the DNA barcoding of holdings of Biological Resource Centres, followed by the Genera of Fungi Project, aiming to recollect, and epitypify all type species of all genera. What these data have revealed, is that most genera are poly- and paraphyletic, and that morphological species normally encompass several genetic entities, which may be cryptic species. Once we provide a stable genetic backbone capturing our existing knowledge of the past 250 years, we will be able to accommodate novelties obtained via environmental sequencing platforms. Being able to communicate these species to other biologists in a clear manner that is DNA-based, will enable scientists to elucidate the importance, role and ecological interactions that these fungi have on our planet.

Keywords: barcoding gap, ecology, genealogical concordance, morphology, MycoBank, registration, speciation, systematics

S13-2

The Origin of *Meju* Fungi - Fungal Diversity of Soybean, Rice Straw and Air for *Meju* Fermentation

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Meju is a brick of dried fermented soybeans and is the core material for *Jang* such as *Doenjang* and *Ganjang*. *Jang* is produced by addition of salty water to *Meju* and is considered the essential sauces of authentic Korean cuisine. *Meju* is fermented by diverse microorganisms such as bacteria, fungi and yeasts. It is known that fungi play an important role in the *Meju* fermentation and they degrade macromolecules of the soybeans into small nutrient molecules. In previous study, 26 genera and 0 species were reported as *Meju* fungi. However, it is not comprehensively examined where the fungi present on the *Meju* are originated. In order to elucidate the origin of the fungi present on the *Meju*, the mycobiota of 500 samples soybean kernels, 296 rice straw pieces and air samples of *Jang* factories was determined in 0, 2 and 7 *Jang* factories respectively.

Forty-one genera covering 86 species were isolated from the soybeans and 33 species were identical with the species from *Meju*. From sodium hypochlorite untreated soybeans, *Eurotium herbariorum*, *Eurotium repens*, *Cladosporium tenuissimum*, *Fusarium fujikuroi*, *Aspergillus oryzae/flavus* and *Penicillium steckii* were the predominant species. In case of sodium hypochlorite-treated soybeans, *Eurotium herbariorum*, *E. repens* and *Cladosporium tenuissimum* were the predominant species. Of the 4 genera and 86 species isolated from soybeans, 3 genera and 33 species were also found in *Meju*.

Thirty-nine genera and 92 species were isolated from the rice straws and 40 species were identical with the species from *Meju*. *Fusarium asiaticum*, *Cladosporium cladosporioides*, *Aspergillus tubingensis*, *A. oryzae*, *E. repens* and *Eurotium chevalieri* were frequently isolated from the rice straw obtained from many factories. Twelve genera and 40 species of fungi that were isolated in the rice straw in this study, were also isolated from *Meju*. Especially, *A. oryzae, C. cladosporioides, E. chevalieri, E. repens, F. asiaticum* and *Penicillium polonicum* that are abundant species in *Meju*, were also isolated frequently from rice straw. *C. cladosporioides, F. asiaticum* and *P. polonicum* that are abundant in low temperature fermentation process of *Meju* fermentation, were frequently isolated from rice straw incubated at 5°C and 25°C, while *A. oryzae, E. repens* and *E. chevalieri* that are abundant in high temperature fermentation process of *Meju* fermentation, were frequently isolated from rice straw incubated at 25°C and 35°C. This suggests that the mycobiota of rice straw have a large influence in mycobiota of *Meju*.

Thirty-nine genera and 92 species were isolated from the air of *Jang* factories and 34 species were identical with the species from *Meju*. In outside air of the fermentation room, *Cladosporium* sp. and *Cladosporium cladosporioides* were the dominant species, followed by *Cladosporium tenuissimum*, *Eurotium* sp., *Phoma* sp. *Sistotrema brinkmannii*, *Alternaria* sp., *Aspergillus fumigatus*, *Schizophyllum commune*, and *Penicillium glabrum*. In inside air of the fermentation room, *Cladosporium* sp., *Aspergillus oryzae*, *Penicillium chrysogenum*, *A. nidulans*, *Aspergillus* sp., *C. cladosporioides*, *Eurotium* sp., *Penicillium* sp., *C. tenuissimum*, *A. niger*, *E. herbariorum*, *A. sydowii*, and *E. repens* were collected with high frequency. The concentrations of the genus

Aspergillus, *Eurotium* and *Penicillium* were significantly higher in inside air than outside air.

From this results, the origin of fungi present on *Meju* was inferred. Of the dominant fungal species present on *Meju*, *Lichtheimia ramosa*, *Mucor circinelloides*, *Mucor racemosus*, and *Scopulariopsis brevicaulis* are thought to be originated from outside air, because these species are not or are rarely isolated from rice straw and soybean; however, they were detected outside air of fermentation room and are species commonly found in indoor environments. However, *A. oryzae*, *P. polonicum*, *E. repens*, *P. solitum*, and *E. chevalieri*, which are frequently found on *Meju*, are common in rice straw and could be transferred from rice straw to *Meju*. The fungi grow and produce abundant spores during *Meju* fermentation, and after the spores accumulate in the air of fermentation room, they could influence mycobiota of *Meju* fermentation in the following year. This could explain why concentrations of the genus *Aspergillus*, *Eurotium*, and *Penicillium* are much higher inside than outside of the fermentation rooms.

Keywords: *Meju*, soybean, rice straw, air, fungi, origin

S13-3

Morphological and Genetic Characteristics of *Colletotrichum gloeosporioides* Isolated from Newly Emerging Static-Symptom Anthracnose in Apple

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Filamentous fungi of the genus *Colletotrichum* (teleomorph, *Glomerella*) are considered major plant pathogens worldwide. Cereals, legumes, vegetables, and fruit trees may be seriously affected by this pathogen (). *Colletotrichum* species cause typical disease symptoms known as anthracnoses, characterized by sunken necrotic tissue, where orange conidial masses are produced. Anthracnose appears in both developing and mature plant tissues (2). We investigated disease occurrence in apple orchards from 203 to 204 in northern Gyeongbuk province, Korea. Typical anthracnose with advanced symptoms was observed in all apple orchards studied. Of late, static fruit spot symptoms are being observed in apple orchards. A small lesion, which does not expand further and remains static until the harvesting season, is observed at the beginning of fruit growth period. In our study, static symptoms, together with the typical symptoms, were observed on apples. The isolated fungus was tested for pathogenicity on cv. 'Fuji apple' (fully ripe fruits, unripe fruits, and cross-section of fruits) by inoculating the fruits with a conidial suspension (0^5 conidia/ml). In apple inoculated with typical anthracnose fungus, the anthracnose symptoms progressed, and dark lesions with salmon-colored masses of conidia were observed on fruit, which were also soft and sunken. However, in apple inoculated with fungi causing static symptoms, the size of the spots did not increase. Interestingly, the shape and size of the conidia and the shape of the appressoria of both types of fungi were found to be similar. The conidia of the two types of fungi were straight and cylindrical, with an obtuse apex. The culture and morphological characteristics of the conidia were similar to those of *C. gloeosporioides* (5). The conidia of *C. gloeosporioides* germinate and form appressoria in response to chemical signals such as host surface wax and the fruit-ripening hormone ethylene (3). In this study, the spores started to germinate 4 h after incubation with an ethephon suspension. Then, the germ tubes began to swell, and subsequently, differentiation into appressoria with dark thick walls was completed by 8 h. In advanced symptoms, fungal spores of virtually all the appressoria formed primary hyphae within 6 h. However, in the static-symptom fungus spores, no primary hyphae formed by 6 h. The two types of isolates exhibited different growth rates on medium containing apple pectin, Na polypectate, or glucose as the sole carbon. Static-symptom fungi had a >0% reduction in growth (apple pectin, 4.9%; Na polypectate, 27.7%; glucose, 0.4%). The fungal isolates were also genetically characterized by sequencing. ITS regions of rDNA, chitin synthase (CHS), actin (ACT), and β-tubulin (βt) were amplified from isolates using primer pairs ITS and ITS 4 (4), CHS-79F and CHS-354R, ACT-52F and ACT-783R, and T and βt2 (5), respectively. The resulting sequences showed 00% identity with sequences of *C. gloeosporioides* at KC49356, and the sequence of the βt gene showed 00% identity with *C. gloeosporioides* at JX009557.. Therefore, sequence data from the four loci studied proves that the isolated pathogen is *C. gloeosporioides*. We also performed random amplified polymorphic DNA-PCR, which showed clearly differentiated subgroups of *C. gloeosporioides* genotypes. The clustering of these groups was highly

related to the symptom types of the individual strains.

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Keywords: anthracnose, apple, appressorium, *Colletotrichum gloeosporioides*, static symptoms

S13-4

Ecological Characteristics and Unique Diagnostic Techniques of Apple Blotch Disease Caused by *Marssonina coronaria* in Korea

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Apple blotch, caused by *Marssonina coronaria,* induce early defoliation in apple and leading to critical economic losses in apple orchards in Korea. Since *M. coronaria* is difficult to culture, we developed isolation and cultural method. We collected *M. coronaria* isolates from Gyeongbuk Province and then constructed phylogentic tree based on ITS regions. As the results, phylogenetic relationship indicated that all Korean isolates formed a same cluster and closely related to Chinese isolates [1]. Ecological characteristic of *M. coronaria* have been observed in apple orchards which located in Gyeongbuk Province from 20 to present. As the results, the typical apple blotch symptoms were observed from July, and then the infected leaves were discolored and formed acervuli on the leaves. After rainfall, severe infection of symptoms such as discoloration and early defoliation were continuously observed until October. Also overwintered conidia were observed in next March on the fallen diseased leaves [2]. In the last 5 years, ascopores of *M. coronaria* were not observed in apple orchards which were severely infected by *M. coronaria* in Korea. Thus, it is assumed that overwintered conidia could be a primary inoculum of *M. coronaria*. Meanwhile, apple blotch has long latent periods compare to other apple disease. During the latent period, early diagnosis of apple blotch is the most important to control the disease by spray fungicide. In this reason, we developed novel diagnostic method to detect *M. coronaria* during latent period using optical coherence tomography (OCT) and Loop-mediated isothermal amplification (LAMP) method [2, 3]. In this presentation, it will introduce ecological characterization of *M. coronaria* in Korea and unique detection technique of *M. coronaria* in apple. It will be helpful to develop new strategies to control apple blotch in Korea.

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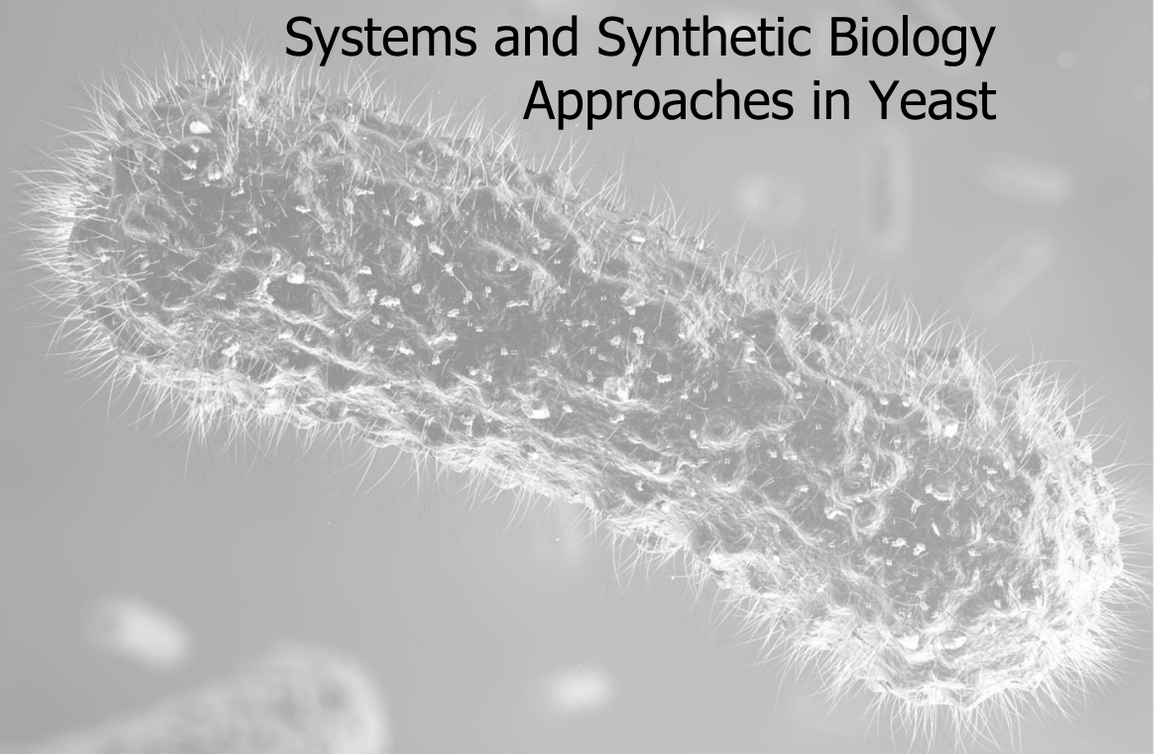
Keywords: Apple blotch, Diagnostic technique, Ecological characterization, *Marssonina coronaria*

Symposium [S14]

Systems and Synthetic Biology Approaches in Yeast



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S14-1

Nitric Oxide-mediated Antioxidative Mechanism in *Saccharomyces Cerevisiae* and its Application to Baker's Yeast

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Nitric oxide (NO) is a signaling molecule involved in the regulation of many cellular functions. In the unicellular eukaryote yeast, the role of NO is poorly understood due to the lack of mammalian NO synthase (NOS) orthologues. In yeast *Saccharomyces cerevisiae*, we found that increased conversion of proline into arginine led to NO production in response to elevated temperature that increases reactive oxygen species (ROS) level [1], [2]. We also showed that the flavoprotein Tah8, which was reported to transfer electrons to the Fe-S cluster protein Dre2, was involved in NO synthesis in yeast. Gene knockdown analysis demonstrated that Tah8-dependent NO synthesis confers high-temperature stress tolerance on yeast cells [3].

Currently, we focus on the antioxidative mechanism by NO, such as cGMP-mediated signal transduction and protein activation via S-nitrosylation found in mammals. Our microarray analysis revealed that NO up-regulates the expression of genes involved in copper uptake, which are regulated by the transcriptional activator Mac. Our hypothesis is that NO can directly modify the Cys residue in Mac via S-nitrosylation, leading to a conformational change for its activation. In fact, NO increases intracellular copper level. Interestingly, NO activates the superoxide dismutase Sod that requires copper for its activity in the presence of copper, due to an increase in copper uptake. As it appears that such a cell protection mechanism is specific to yeasts and fungi, it represents a promising target for antifungal activity.

During bread-making processes, baker's yeast mostly *S. cerevisiae* cells are exposed to baking-associated stresses, such as air-drying, high-sugar concentrations and freeze-thaw stresses. Therefore, it is necessary to construct yeast strains with higher tolerance to these stresses. We showed that engineered baker's yeast strains with enhanced proline and NO synthetic ability are tolerant to multiple baking-associated stresses by reducing intracellular ROS level. The increased NO level also improved the fermentation ability after air-drying and freeze-thaw stress treatment in baker's yeast. Hence, appropriate NO production could be promising for breeding novel industrial yeast strains that are tolerant to various stresses [4].

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Keywords: yeast, saccharomyces cerevisiae, nitric oxide, oxidative stress, copper transport, superoxide dismutase, mac

S14-2

Systematic Genome Deletion of Fission Yeast and Its Applications

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The fission yeast, *Schizosaccharomyces pombe*, has served as an excellent model organism for mechanism studies of cell cycle control, mitosis and meiosis, DNA repair and recombination, the checkpoint controls and genome stability. The systematic generation of deletion mutants by targeted mutagenesis accelerates the use of *S. pombe* for functional and comparative studies of eukaryotic cell processes. We have reported that more than 95% of total genes of *S. pombe* have been deleted by using PCR-generated deletion cassettes, which were designed to facilitate the later High-Throughput Screening (HCS) procedures. The previous serial- or block-PCR method for the systematic gene deletion requires elaborate skill. In this study, we developed a novel gene synthesis method for the systematic preparation of deletion cassettes on a 96-well basis in fission yeast.

Lowering the dosage of a single gene from two copies to one copy in diploid *S. pombe* results in HAPLOINSUCFFICIENCY, that is sensitized to any drug that acts on the product of this gene. With the genome-wide gene deletion heterozygotic mutants, we have setup a microarray system, which is useful when only small amount of chemical is available. The HCS method using the systematic *S. pombe* deletion mutants can be exploited for the identification of drug targets. Despite its convenience, it still has many empirical problems of artifacts, so it needs to be improved. In this study, we report a novel method keeping up with cutting-edge technology. Details will be presented in the presentation.

Keywords: gene deletion, fission yeast, haploinsufficiency, microarray

S14-3

Regulatory Proteolysis by N-Terminal Acetylation and the N-end Rule Pathway

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Department of Life Sciences, POSTECH

The N-end rule relates in vivo half-life of a protein to the identity of its N-terminal residue. The N-end rule pathway comprises two branches: the Ac/N-end rule pathway and the Arg/N-end rule pathway. We recently discovered the crosstalk of the Ac/N-end rule and Arg/N-end rule pathway for the elimination of both N-terminally acetylated proteins and their unacetylated counterparts. In addition, we found that the N-terminal acetylation and the N-end rule pathway control G-protein signaling by accelerating degradation of RGS (Regulators of G-protein Signaling) proteins, which play a pivotal role in the maintenance of blood pressure. Our findings will provide new strategies for the treatment and prevention of hypertension and cardiovascular diseases.

S14-4

Synthetic Glyco-engineering of Yeast for Production of Therapeutic Enzymes

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Lysosomal storage diseases are treated with enzyme replacement therapies with recombinant enzymes produced from mammalian cells. These enzymes require the addition of *N*-glycans containing mannose-6-phosphates (M-6-Ps), which is recognized by M-6-P receptors on plasma membrane for cellular uptake and targeting to lysosomes. Although M-6-P-containing glycans are found only in mammalian cells, the mannosylphosphorylated mannose structure (mannose--phosphate-6-*O*-mannose) of *N*-glcyans in yeast can be converted to M-6-P structure (phosphate-6-*O*-mannose) by uncapping the outer mannose residue. In the traditional yeast *Saccharomyces cerevisiae*, both *ScMNN4* and *ScMNN6* genes are required for efficient mannosylphosphorylation [1]. ScMnn4 protein has been known to be a positive regulator of ScMnn6p, a real enzyme for mannosylphosphorylation. On the other hand, YlMpop, a ScMnn4p homologue, mediates mannosylphosphorylation in *Yarrowia lypolytica* without the involvement of ScMnn6p homologues [2]. In this study, we show that heterologous expression of YlMpop can perform mannosylphosphorylation in *S. cerevisiae* in the absence of ScMnn4p and ScMnn6p. Moreover, mannosylphosphorylation of *N*-glycans enhanced by YlMpop overexpression is much higher than that with ScMnn4p overexpression, and this is highlighted further in *Scmnn4*- and *Scmnn6*-disrupted mutants (*S. cerevisiae mnn4Δ*, *mnn6Δ*, and *mnn4Δmnn6Δ* strains). We applied the strategy of mannosylphosphorylation enhancement by YlMpop overexpression to a glyco-engineered *S. cerevisiae* (*ScochΔmnnΔ* strain) in which the synthesis of yeast-specific immunogenic glycans is blocked. When compared to ScMnn4p overexpression, a great increase of bi-mannosylphosphorylated glycan is observed. Through an *in vitro* process involving the uncapping of the outer mannose residue, this bi-mannosylphosphorylated structure is changed to a bi-phosphorylated structure with high affinity for mannose-6-phosphate receptor. The superior ability of YlMpop to increase bi-mannosylphosphorylated glycan in yeast shows promise for the production of therapeutic enzymes with improved lysosomal targeting capability. However, since the production yields of therapeutic enzymes in yeast were very low, we also developed the screening method to select a yeast with improved secretion. Gas protein is a beta(,3)-glucanosyltransglycosylase playing an essential role in the assembly of cell wall as localized on the yeast surface through a glycosylphsphatidylinositol (GPI) anchor [3]. When *GAS* gene was disrupted in yeasts, the resulting mutant strain were reported to exhibit hypersensitivity to cell wall-perturbing reagents together with increased capability of protein secretion due to the loosed cell wall structure [4-5]. Functional complementation of cell wall-defective phenotype of *Gas*-deletion mutant using recombinant expression of Gas protein was employed to generate a screening system for a strain with improved capability of protein secretion. We constructed the expression vectors encoding fusion proteins with N-terminal secretory protein of interest linked to Gasp without signal sequence. After these vectors were transformed into *Gas*-deletion mutant, the growths of the resulting transformants were tested on the agar plates containing cell

wall-perturbing reagents. Only the strains expressing Gasp fused to well secreted proteins showed restored growth phenotype under cell-wall stress condition. This system can be used to enrich yeasts with improved secretion capability after genome-wide random mutagenesis, which would contribute to the development of super secretory yeasts. All these efforts would contribute to generating 'smart super secretory yeast' producing therapeutic enzymes attached with the glycans optimized for lysosomal targeting.

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S14-5

Metabolic Engineering of *Saccharomyces cerevisiae* for Production of Lactic Acid

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Lactic acid has wide industrial applications and one of its attractive uses is as a monomer for polylactic acid (PLA) which is then used as raw material for a biodegradable plastics. Lactic acid has been mainly produced using lactic acid bacteria, the natural host for lactate fermentation. However, yeasts such as *Saccharomyces cerevisiae* receive increasing attention recently as proper microorganism for industrial lactate production because of their acid tolerance [1].

In order to generate lactic acid producing yeast, acid-tolerant *S. cerevisiae* CEN.PK2 strain was genetically engineered. *S. cerevisiae* does not produce lactic acid naturally, therefore expression of heterologous L-lactate dehydrogenase (*L-LDH*) genes enabled production of L-lactic acid by the recombinant strain [2]. Since the yeast has strong tendency for ethanol production, reduction or inactivation of the ethanol synthetic pathway is required for the increase in lactic acid yield. We constructed high yield lactic acid-producing yeast by disruption of pyruvate decarboxylase (*PDC*) and alcohol dehydrogenase (*ADH*) genes. However, the mutation in the ethanol pathway genes in *S. cerevisiae* caused decrease in growth rate and glucose consumption resulting the reduced productivity despite high yield [3, 4, 5]. Hence overcome the defect of ethanol pathway mutation is one of the critical issues on the engineering of industrial yeast strains. In spite of various attempts including evolutionary engineering, the rational approaches for the improvement of their productivity have been still limited. By metabolic engineering of acetyl-CoA biosynthetic pathway, we successfully increased the glucose consumption rate and lactic acid productivity. This study demonstrates a novel approach that develops the industrial strain producing biochemical in *S. cerevisiae*.

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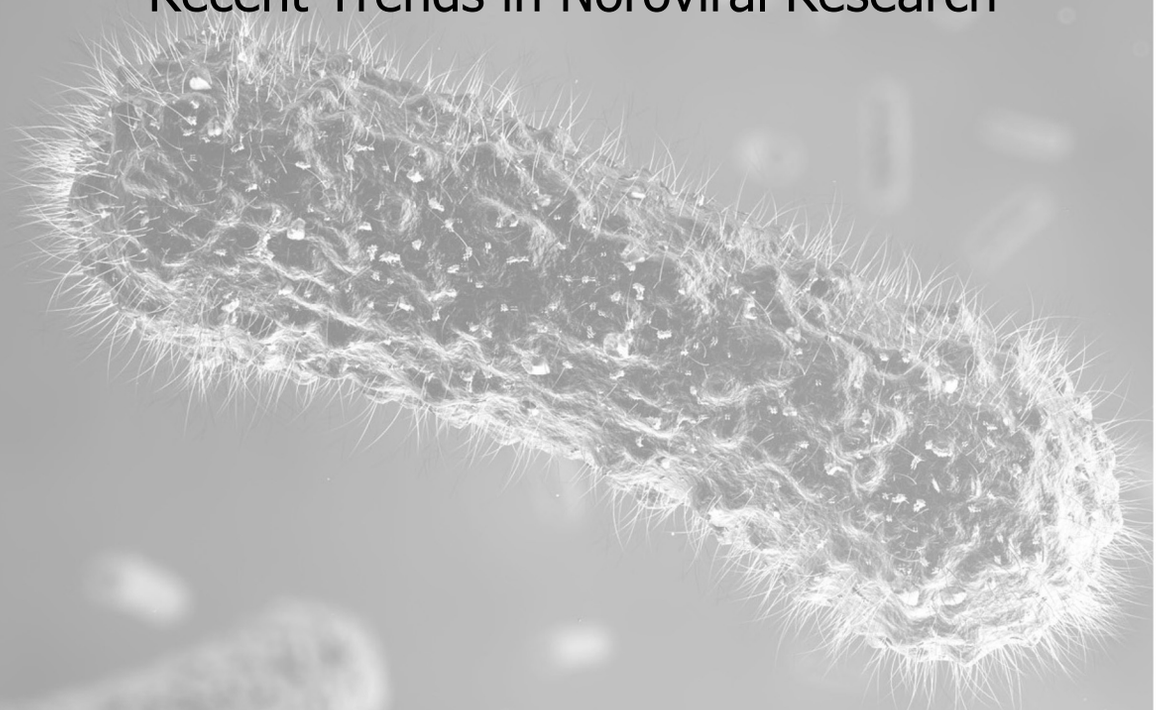
Keywords: lactic acid, *Saccharomyces cerevisiae,* alcohol dehydrogenase, acetyl-CoA, metabolic engineering

Symposium [S15]

Recent Trends in Noroviral Research



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S15-1

Insight into VPg-mediated RNA Synthesis in Norovirus

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Norovirus is the leading cause of epidemic acute, nonbacterial gastroenteritis and adopts *de novo* and VPg (Virion protein genome linked)-primed RNA synthesis by an RNA-dependent RNA polymerase (RdRp). To understand the interaction between RdRp and VPg in replication of murine norovirus- (MNV-), we determined the crystal structure of MNV- RdRp-VPg(-73) complex in the presence of RNA. VPg was bound to the base of the palm domain and the tip of the fingers domain of RdRp, but the RNA template could not be modeled. The affinity constant K_D of VPg to RdRp was 3.6±.3 nM and VPg(-73) showed approximately 20-fold lower affinity to RdRp than that of full-length VPg. In addition to this multiple binding mode, VPg enhanced the interactions of RdRp hexamers, leading to the formation of high-order multimers or tubular fibrils with significantly increased polymerase activity, confirmed by electron microscopic and biochemical-studies. Our data suggested that VPg plays a crucial architectural role in the organization of RdRp complexes, inducing high-order multimers in the presence of RNA. The multimers of RdRp-VPg-RNA can provide a mechanistic understanding of viral polymerase multimeric arrays and a new tool for development of antivirals to control norovirus outbreaks.

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[3] RNA synthesis is enhanced by VPg-mediated multimerization of norovirus polymerase. PLoS Pathogens (204) In revision.

Keywords: norovirus, protein structure, multimerization, VPg, RdRp

S15-2

Application of Omics Technology for Food Material Development

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Lectins found in fish tissues play an important role in the innate immune response against viral infection. A fucose-binding type lectin, RbFTL-3, from rock bream (*Oplegnathus fasciatus*) was identified using expressed sequence tag (EST) analysis. The expression of RbFTL-3 mRNA was higher in intestine than other tissues of rock bream. To determine the function of RbFTL-3, VHSV-susceptible fathead minnow (FHM) cells were transfected with pcDNA3.(+) or pcDNA3.(+)-RbFTL-3 and further infected with VHSV. The results show that the viability of FHM cells transfected with pcDNA3.(+)-RbFTL-3 is higher than that of cells transfected with pcDNA3.(+) (relative cell viability: 28.9% vs 56.2%). A comparative proteomic analysis, performed to explore the proteins related to the protective effect of RbFTL-3 in the cells during VHSV infection, identified 90 proteins differentially expressed in VHSV-infected FHM cells transfected with pcDNA3.(+) or pcDNA3.(+)-RbFTL-3. The expression of RbFTL-3 inhibits a vascular-sorting protein (SNF8) and diminishes the loss of prothrombin, which are closely associated with controlling viral budding and hemorrhage in fish cells, respectively. Subsequent Ingenuity Pathways Analysis enabled prediction of their biofunctional groupings and interaction networks. The results suggest RbFTL-3 modulates the expression of proteins related to viral budding (SNF8, CCT5 and TUBB) and thrombin signaling (F2) to increase the viability of VHSV infected cells.

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Keywords: proteomics, lectin, VHSV, fish, disease

S15-3

Prevention of Norovirus Associated Foodborne Outbreak: Diagnostics and Viral Control

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Human norovirus (HuNoV) is important in public health worldwide but greatly understudied virus. Recently, Korean Food and Drug Administration (KFDA) initiated the program, so called NoroTECL (TEam for ControL of Noroviral Foodborne Outbreaks), for preventing the outbreaks caused by norovirus in South Korea. This initiative program includes the surveillance of seafood and agricultural foods, the identification of source of viral contamination in major food producing area, development of novel diagnostic and control techniques, and development of tools for noroviral research. Various sampling including vegetables, soils, groundwater, seawater, surface water and fecal samples have been collected from South Korea and subsequently analyzed. The presence of noroviral RNA and fecal indicators in collected samples were measured using novel molecular techniques. In addition, fecal indicators (total coliforms, male-specific coliphages, etc) and bacterial communities based on next-generation sequencing (NGS) were assessed in order to identify the molecular markers of norovirus, and then source identification by spatial analysis based on geographic information system (GIS), environmental parameters and meteoroidal data will be combined with these data in future. Finally, we have investigated potential measures to prevent or reduce noroviral outbreaks. For example, various phytochemicals, physical and chemical tools have been characterized for potential inhibitors of viral replication. In summary, we have performed survey and tried to identify tools for preventing the noroviral outbreaks in South Korea.

Keywords: norovirus, acute gastroenteritis, diagnostic technique, foodborne diseases, DNA barcode, control, prevention.

S15-4

An Plasmid Based Human Norovirus Reverse Genetics System

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Human norovirus (HuNoV) is the predominant viral cause of acute gastroenteritis and outbreaks of foodborne illness. It is challenging to study these viruses because they cannot be grown in cultured cells in the laboratory, and no small animal infection model exists. Therefore, knowledge about what controls HuNoV infection and replication and how these viruses cause disease is limited. Research and development of ways to prevent and treat HuNoV infections, such as an antiviral drugs and methods to inactivate virus and vaccines remains difficult.

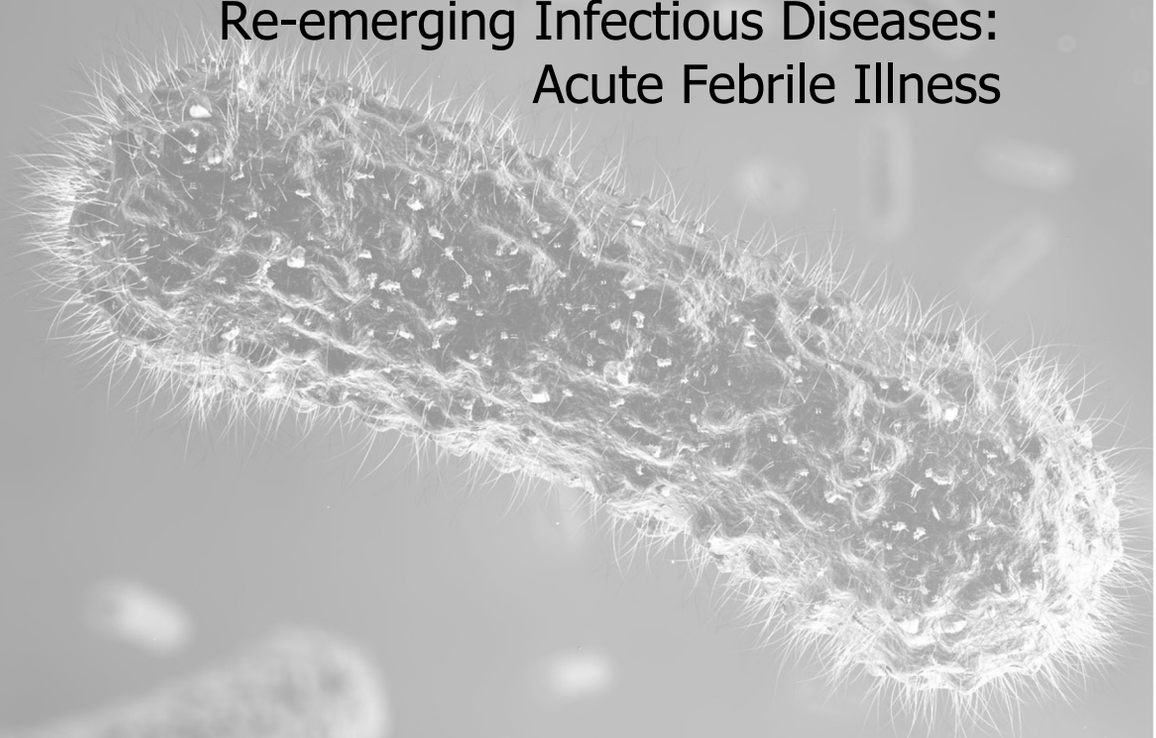
Investigators at the National Institute of Infectious Diseases in Japan (Chief Kazuhiko Katayama), Baylor College of Medicine in the United States of America (Professor Mary K. Estes), the National Center for Geriatrics and Gerontology in Japan (Chief Akira Nakanishi) cloned a HuNoV genome into a plasmid expression vector and were successful in producing HuNoV particles using mammalian cell lines. This technique is called reverse genetics and allows detailed understanding of how the virus genome functions. Furthermore, a green fluorescence protein (GFP) gene was incorporated into a gene of the HuNoV genome and HuNoV particles were made that produced this fluorescent protein inside transfected cells. In addition, the same system was used to produce infectious murine norovirus (MNV).

This new reverse genetics system will now be used to manipulate the HuNoV and MNV genes for new studies designed to understand how the HuNoV replicates and how the virus causes disease. One longterm goal is to alter the genome to produce HuNoVs that do not cause disease in humans because such particles might be useful to develop a live, attenuated, oral vaccine. This system also is able to help evaluate antiviral drugs and disinfectants, and to develop new cell or small animal models to culture these important viruses.

Keywords: Human norovirus, reverse genetics system, GFP tagged virus

Symposium [S16]

Re-emerging Infectious Diseases: Acute Febrile Illness



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INTERNATIONAL MEETING OF

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S16-1

Epidemiology of Scrub Typhus: Current Issues on Environmental Factors

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Scrub typhus is an acute febrile illness caused by *Orientia tsutsugamushi* infection. The bacterium is an obligate intracellular pathogen maintained through transovarian transmission in trombiculid mites that serve as vectors for the disease. Humans are accidental host while the infected larval mites feeding tissue fluids for their development. Endemic region of scrub typhus is geographically confined to south-eastern Asian area extending from the Far East Russia and Korea in the north, to northern Australia in the south and Afghanistan in the west, and Japan and the western Pacific islands in the east. It has been estimated more than a million cases occur annually within the endemic region and scrub typhus occupies up to 20% of febrile hospital admissions in rural area of southern Asia. In addition, the rapid increase of scrub typhus incidence in China and South Korea, coupled with sporadic outbreaks in several other countries, has become a serious public health issue in the areas of disease endemicity. It has been estimated that changes in the habitats of mites and human activities are the key factors affecting the prevalence of scrub typhus. In order to elucidate the environmental factors affecting the epidemiological characteristics in detail, I'll overview epidemiological features of scrub typhus in endemic regions of Asia and suggest a potential factors affecting the disease endemicity.

S16-2

Rickettsial Diseases in Korea

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Department of Microbiology, School of Medicine, Konkuk University

Rickettsioses are some of the emerging infectious diseases. The diseases are arthropod-borne diseases caused by obligate intracellular bacteria belonging to the genus *Rickettsia*. The most common clinical features of rickettsioses in human are fever, headache, rash, and inoculation eschar. Members of the genus *Rickettsia* (family: *Rickettsiaceae*, order: *Rickettsiales*) are obligate intracellular gram-negative like bacteria. *Rickettsia* spp. are classified into the spotted fever group (SFG) (including *R. rickettsii*, *R. massiliae*, *R. helvetica*, and *R. akari* subgroups), typhus group (TG) (including *R. prowazekii* and *R. typhi*), and *R. canadensis*, *R. bellii*, and other groups.

Human rickettsioses, known to occur in Korea, include mainly scrub typhus, murine typhus, and epidemic typhus. Scrub typhus, caused by *Orientia tsutsugamushi*, the only species of the genus *Orientia*, which was formally known as the scrub typhus group rickettsia, is a major rickettsial disease in Korea, and is transmitted through the bites of mite larvae. It has been reported that 34.3% of febrile hospital patients in autumn were seropositive for the disease. *Rickettsia typhi,* transmitted by the fleas of various rodents, causes murine typhus, which is a mild form of typhus in humans. The first patient with murine typhus in Korea was reported in 959. Since then, few cases have been reported. Epidemic typhus is caused by *R. prowazekii* and is transmitted by the body louse. The disease is fatal in 0–30% of patients, depending on underlying diseases and the nutritional state of the host. It appeared after the end of the Korean War. Thereafter, however, no other cases have been reported in Korea.

SFG rickettsioses are widely distributed throughout the world in endemic foci, occurring sporadic outbreak. These occur in a worldwide geographic distribution that includes Japan, Southern China, and Eastern Russia, countries that surround Korea. The previous report demonstrated the possibility of the existence of SFG rickettsiosis in Korea through serological methods using SFG rickettsial antigens and molecular detection methods. As serologic evidence, antibodies to *R. japonica* in a patient with acute febrile illness were observed by indirect immunefluorescence assay technique (IFAT) in 2004. Additionally, a serologic assay by IFAT showed the prevalence of antibodies against *R. sibirica*, *R. conorii*, *R. akari* in sera of patients with acute febrile illness in 2005. The rickettsial DNAs related to *R. japonica*, *R. conorii* and other SFG rickettsiae have been detected in *Haemaphysalis* spp. collected in Chungju-si (city) in 2003 and 2005. Furthermore, *R. akari*, *R. japonica*, *R. sibirica*, *R. conorii*, and *R. felis* were detected by PCR in human sera in 2005. Recently, SFG rickettsiae that were closely related to *R. japonica* and *R. monacensis* were also observed in *Haemaphysalis* spp. from Jeju Island. Recently, one human case of spotted fever rickettsiosis caused by *R. japonica*, has been reported (2006) in Korea. In addition, *R. monacensis* has been detected and isolated from *I. nipponensis* in Korea. More recently, human cases of *R. monacensis* were found in Daejeon metropolitan and Gwangju Province, Korea. *R. monacensis* has been described in ticks from around the world, beginning with its initial isolation from *I. ricinus* in Munich, Germany (2002), over the past 0 years only two cases of *R. monacensis* infections in humans from Spain (2007) and Italy (202) have been described.

In conclusion, the findings in previous studies suggest that various rickettsial diseases should be considered early in the differential diagnosis of febrile infections for persons exposed to ticks through agricultural practices or recreational and military activities in Korea.

S16-3

Re-emerging Infections in Sri Lanka-the Challenge

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Sri Lanka is facing a transition of diseases patterns from infectious diseases to non-communicable diseases. However infectious diseases still contribuite to a significant burden to the health care system. While dengue is in the war front and once notorious malaria seems to be entring history books since 2008, infectious diseases of yester year, are noted to raise their heads due to unkown reasons and encounter significant proportion of acute febrile illness. Knowledge on these illnesses, availability of novel rapid diagnostic facilities together with therapeutic options is the main requirement in order to reduce morbidity and mortality associated with re-emerging infections.

In late 2002, a patient presented with fever headache and body-aches for 0 days. She became increasingly drowsy, restless and agitated. A working diagnosis of encephalitis, meningo-encephalitis or cerebral malaria was made however was negative for all investigations and rapidly deteriorated despite triple therapy with iv quinine, ceftriaxone and acyclovir. She became delerious with complete hearing loss. A careful clinical examination revealed an eschar hidden in the right axilla. A prompt diagnosis of scrub typhus was made and she had a dramatic recovery with anti-rickettsial antibiotics. Her illness was later confirmed serologically as scrub typhus caused by *Orientia tsutsugamushi.*

In Sri Lanka, the history of typhus fever dates back to 930-940s. A dramatic outbreak of Scrub typhus occured in over 750 East African and British troops during a 4 day military exercise carrying a high morbidity. Thereafter, the disease was within main differentials for few decades however it remained silent or not prominent apart from few intermittent reports for the next five to six decades.

In 2003, several patients presented with fever and acute reversible deafness reminding the yester years known clinical combination in the prediction of re-emerging ST. Thereafter further studies revealed that rickettsial infections caused by both *Orientia tsutsugamushi* and SFG are re-emerging in most parts of the country and delay in diagnosis may result in complications such as myocarditis, encephalitis and severe multi-organ failure. In a study of PUO and who responded to empirical doxycycline, rickettsiosis accounted for a majority justifying the use of empirical anti-rickettsial antibiotics, such as doxycycline in patients with undiagnosed fever in settings where rickettsial infections are endemic or re-emerging.

Recent studies reveal all three main OT genotypes in Sri Lanka, and the majority falling into Thai Karp related clade demonstrating great antigenic diversity of OT within the country. Furthermore, a very high background sero prevalence against rickettsial species was noted and therefore clinically helpful rickettsial disease diagnostic algorhythm was established to interprit a single titre of IFA-IgG based on the duration of illness; if sample is obtained ≤7 day of illness, an IgG titer of </28 requires a follow up sample in the diagnosis and > 7 days of illness, a single ≥/256 titer is diagnostic for all ST and 90% of SFG in Sri Lanka.

In order to overcome delay in the diagnosis of ST, ImmuneMed Scrub typhus Rapid card test (IStR)- Korea was validated in the diagnosis of scrub typhus in Sri Lanka using stored sera of patients confirmed having ST. The mean duration of illness at time of sample collection was 2.3 days(SD 3.5). IStR-IgM test gave 96%

specificity [CI:88-98], and 92% sensitivity [CI: 84-97] making IStR-IgM to be a useful test to diagnose acute SF in Sri Lanka, especially in the context of samples being collected after 7 days of onset of illness.

Keywords: Scrub typhus, Sri Lanka

S16-4

Clinical Validation of Scrub Typhus Diagnosis Kit in Asian Countries

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Institute of Medical Science, School of Medicine, Hallym University, [2]Department of Microbiology, College of Medicine, Hallym University, Chuncheon, [3]ImmuneMed, Chuncheon, [4]Department of Laboratory Medicine, Hallym University

Scrub typhus is the endemic febrile disease which has more than million outbreaks every year in the triangle from Southeast Asia, India to Australia. Scrub typhus is an infectious disease and is caused by the intracellular parasite *Orientia tsutsugamushi,* Gram-negative bacteria. Scrub typhus is transmitted by some species of trombiculid mites ("chiggers", particularly *Leptotrombidium pallidium*). The bite of this mite infected with *O. tsutsugamushi* causes a characteristic black eschar that is useful to the doctor for making the diagnosis. Symptoms include fever, headache, muscle pain, cough, and gastrointestinal complain. These symptoms are in common among other acute febrile illnesses causing that doctors have difficulties to differentiate this scrub typhus from others. If without early treatment, the disease is often fatal. Fatal cases have increased to 30%. Therefore, early and accurate diagnosis is very important for the treatment of patient. Currently, the gold standard method for diagnosis is indirect immunofluorescence assay (IFA), but the main limitation of this method is the requirement of expensive devices such as fluorescent microscopy, CO_2 incubator and so on with well trained personnel. Since it is difficult to interpret IFA, false positive or false negative is frequent. In this study, we and ImmuneMed developed a rapid and accurate diagnostics test for scrub typhus to overcome these difficulties. This kit is based on lateral flow immunochromatographic assay and it takes only 5 minutes to interpret the result. The antigen used for ImmuneMed kit is a mixture composed of chimeric antigen from 3 major serotypes, Gilliam, Karp and Kato and 2 antigens from Korean epidemic serotypes, Boryong and Kangwon. Then, we mixed these recombinant antigens according to optimized ratio between antigens, resulting to show high sensitivity. This study is to validate the domestic and international clinical evaluation of the rapid diagnostics kit for scrub typhus. In domestic evaluation, the sensitivity and specificity are 97.3% and 00%. From international clinical evaluations done in Thailand and South India, each sensitivity and specificity is 70.5% and 85.7%, and 94% and ?% respectively. The some low sensitivity of ImmuneMed kit against Thailand sera presumably is due to the various endemic serotypes of Thailand other than serotypes used in ImmuneMed kit.

Judging from this result, the performance of ImmuneMed Scrub Typhus Rapid Kit is excellent. In addition we plan to do clinical evaluation to validate this kit in several countries adding the above for the global usage in future.

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Keywords: scrub typhus, rapid rit, lateral flow assay kit, immunofluorescence assay (IFA), clinical evaluation

Symposium [S18]

KNMRRC Session I

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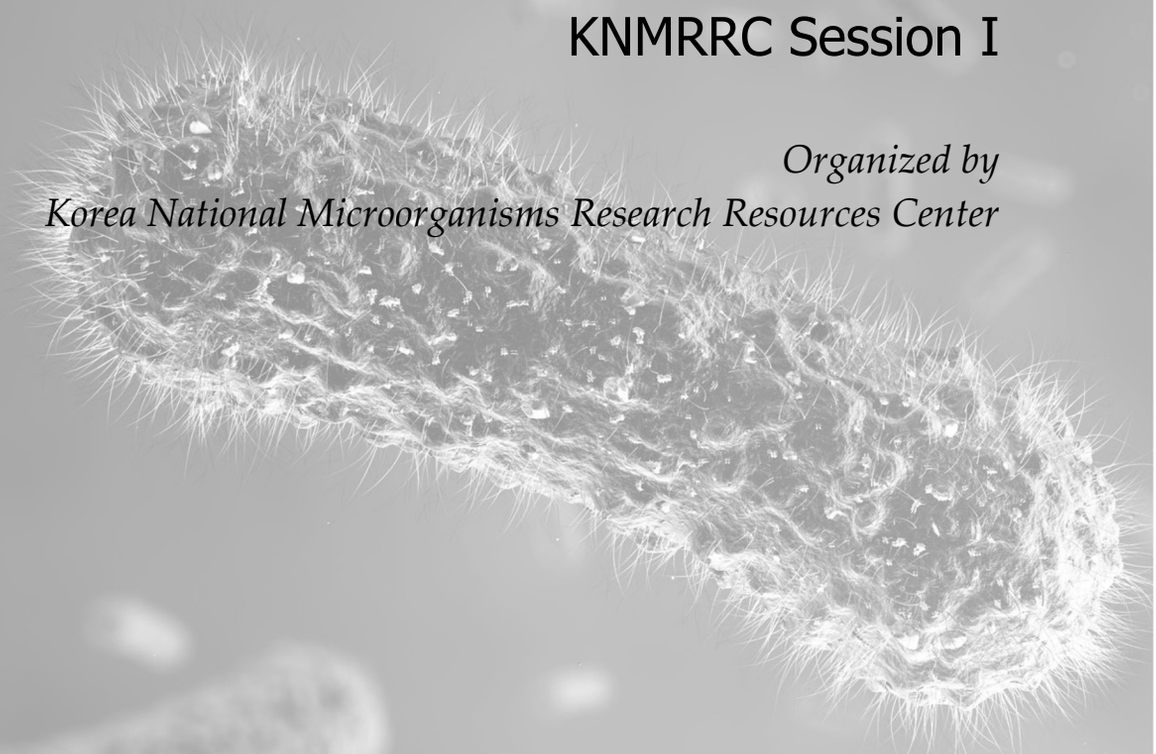

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S18-1

Korea Bank for Pathogenic Viruses

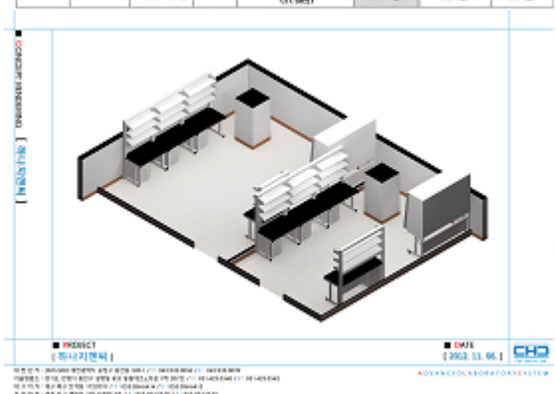
Song Ki-Joon

Department of Microbiology, College of Medicine, Korea University, Korea

* First isolation and identification of human metapneumovirus
* Genetic analysis of human parainfluenza viruses circulating in Korea
* Accomplishment of Korea bank for pathogenic viruses

	2010	2011	2012	2013	2014.9
Distribution (cases)	57	38	41	38	50
Distribution (vials)	1388	497	289	391	317
Collection	1529	103	131	110	5
Service (Consulting, Training)	25	6	3	12	9
Advertisement	12	12	13	9	9

* Movement to new facilities and operation of BSL-3 lab.



S18-2

Campylobacter in Korea: Their Relateness

Eunju Shin

Culture Collection of Antimicrobial Resistant Microbes, Department of Horticulture, Biotechnology, and Landscape Architecture, Seoul Women's University

Campylobacter jejuni and *Campylobacter coli* are the most common bacterial species associated with diarrhea in humans. Food animals are considered to be the primary reservoirs of the *Campylobacter* species which induce infections in humans. However, few studies have compared *Campylobacter* from different sources. This study aimed to analyze and compare antimicrobial susceptibility, molecular characteristics and molecular relatedness of *Campylobacter* isolates from humans and animals-swines and chickens. *Campylobacter* isolates from humans (2 strains of *C. jejuni*), swines (4 strains of *C. coli*), and chickens (50 strains of *C. jejuni*) collected during last 0 years were identified and characterized by biochemical methods, PCR, and multilocus sequence typing (MLST). Antimicrobial susceptibilities were determined with the agar dilution method. The proportion of porcine isolates resistant to each antimicrobial agent was as follows: 28.9% for ampicillin, 2.6% for chloramphenicol, 84.2% for ciprofloxacin, 83.3% for enrofloxacin, 46.5% for erythromycin, 20.2% for gentamicin, and 56.% for tetracycline. Resistant rates of human isolates were as follows: .9% for ampicillin, 0.8% for chloramphenicol, 24% for ciprofloxacin, 46.3% for enrofloxacin, 0.8% for erythromycin, 6.6% for gentamicin, and 46.3% for tetracycline. Resistant rates of chicken isolates were as follows: 88% for ampicillin, 0% for chloramphenicol, 78% for ciprofloxacin, 92% for enrofloxacin, 0% for erythromycin, 0% for gentamicin, and 96% for tetracycline. Sixty-one porcine isolates (53.5%) were found to be multi-drug resistant (resistant to more than three antimicrobial agents in different classes). Eleven human isolates (9.%) and 44 chicken isolates (88%) were multi-drug resistant. Especially one isolate (*C. jejuni* CCARM 3322) which was obtained from a Korean who suffered diarrhea after travelled Philippine in the year 2008 was found to be resistant to all seven antimicrobials tested in this study. *C. coil* porcine isolates with high level resistance (HLR) to erythromycin were identified as 28 different sequence types (STs) including 0 new STs by MLST. Human isolates (all *C. jejuni*) were determined as 23 different STs including nine new STs. The majority of the *Campylobacter* isolates from porcine were *C. coli* with ST-828 CC while human isolates were *C. jejuni* with ST-45 CC. *C. jejuni* CCARM 3322 resistant to all seven antimicrobials was determined as a novel MLST type (ST 5075). This is the first report from Korea providing an overview of the genotypes of *Campylobaceter* human and animal isolates identified by PCR and MLST analysis and their antimicrobial susceptibilities.

Keywords: *Campylobacter*, antimicrobial drug resistance, MLST, extensively drug resistant (XDR)

S18-3

Development of Transformation System of Lichen-Forming Fungus, *Umbilicaria muehlenbergii*

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Deciphering function of predicted genes can provide useful resources in the whole genome scale. It is therefore frequently used to disrupt their genes via transformation in both targeted and random manner. However, there has been no transformation system developed for lichen-forming fungi, one of largest groups of Ascomycetes, so far. Here we reported *Agrbacterium tumefaciens* mediated transformation of lichen-forming fungus, *Umbricaria muehelenbergii*. We generated a total 98 transformants employing the binary vector pYL63, which carries the hygromycin B phosphotransferase gene and enhanced green fluorescence protein gene under the control of the *Aspergillus nidulans* trpC promoter and *Cochliobolus heterostrophus* GAPD promoter, respectively. Randomly selected fifty transformants were showed mitotically stable, maintaining hygromycin B resistance after several generations of growth. A genomic Southern blot analysis showed that 88% of the 784 transformants contained a single T-DNA insert on their genome. A number of phenotype-defective mutants were found in their color, growth and morphologies compared to wild-type, suggesting highly efficient means for identifying the important genes of lichen-forming fungi. Our transformation system will provide valuable tool for functional characterization of lichen-forming fungal genes.

S18-4

Helicobacter pylori Korean Type Culture Collection

Hyung-Lyun Kang

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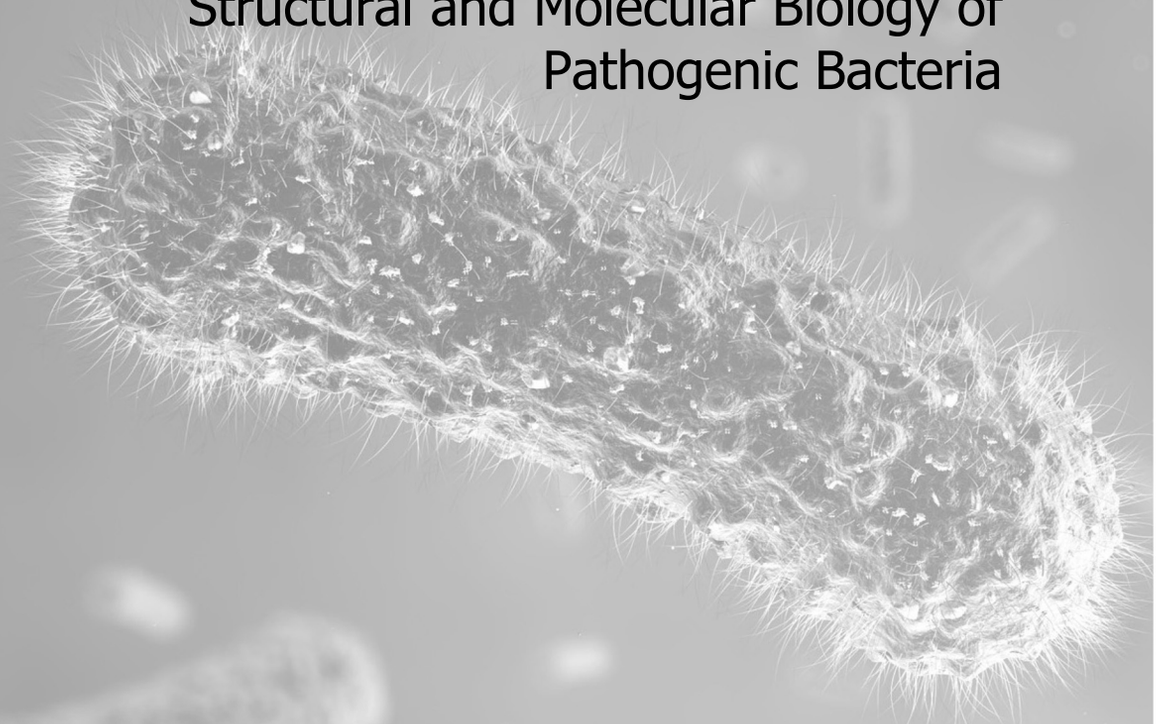
Dr. Kwang-Ho Rhee started study on bacteriology of *Helicobacter pylori* since 1987, and established *Helicobacter pylori* research center in 1999 in the Gyeongsang university school of medicine. In 2005, *Helicobacter pylori* Korean Type Culture Collection (HpKTCC, http://hpktcc.knrrc.or.kr) was launched and succeeded to the research center in 1999 supported by the National Research Foundation of Korea (NRF).

The first clinical strain of *H. pylori* was isolated and cultured from a korean patient with gastric disease in 1987. We reported prevalence and bacteriological characteristics of *H. pylori* on korean people in 1988. In 1989, bacteriological study including antibiotic resistance and antigenic properties of *H. pylori* were reported. In 1990, we reported that *H. pylori* infection caused DNA alteration on the gastric tissue. In 2002, we sequenced and released whole genomes of two korean clinical isolates. These days we are focusing on screening both virulence factors and functions of genes of *H. pylori*.

We stocked 3,171 clinical isolates and modified strains from korean patients with various gastric diseases. We have distributed 6,486 *H. pylori* strains to the researchers and have held workshops for 10 years.

Symposium [S20]

Structural and Molecular Biology of Pathogenic Bacteria



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S20-1

Structure of the Tripartite Multidrug Efflux Pump AcrAB-TolC Shows an Intermeshing Cogwheel Interaction between AcrA and TolC

Nam-Chul Ha, Jin-Sik Kim, Hyeong-Seop Jeong[2], Saemee Song, Kangseok Lee[3], and Jaekyung Hyun[2]

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Escherichia coli AcrAB-TolC is a multidrug efflux pump that expels a wide range of toxic substrates. The dynamic nature of the binding or low affinity between the components has impeded elucidation of how the three components assemble in the functional state. Here, we created fusion proteins composed of AcrB, a transmembrane linker, and two copies of AcrA. The fusion protein exhibited acridine pumping activity, which suggests that the protein reflects the functional structure in vivo. To discern the assembling mode with TolC, the AcrBA fusion protein was incubated with TolC or a chimeric protein containing the TolC aperture tip region. Three-dimensional structures of the complex proteins were determined through transmission electron microscopy. The overall structure exemplifies the adaptor bridging model, wherein the funnel-like AcrA hexamer forms an intermeshing cogwheel interaction with the α-barrel tip region of TolC, and a direct interaction between AcrB and TolC is not allowed. These observations provide a structural blueprint for understanding multidrug resistance in pathogenic Gram-negative bacteria.

Keywords: multidrug efflux pump, multidrug resistance, X-ray crystallography, electron microscopy

S20-2

Reciprocal Regulation of the Autophosphorylation of Enzyme I^{Ntr} by Glutamine and α-Ketoglutarate in *Escherichia coli*

Chang-Ro Lee, Young-Ha Park[2], Yeon-Ran Kim[2], Soyoung Park[2], Alan Peterkofsky[3] and Yeong-Jae Seok[2,4]

Department of Biological Sciences, Myongji University, [2b]Department of Biological Sciences and Institute of Microbiology, Seoul National University, [3c]Laboratory of Cell Biology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, USA, [4]Department of Biophysics and Chemical Biology, Seoul National University

In addition to the phosphoenolpyruvate:sugar phosphotransferase system (sugar PTS), most proteobacteria possess a paralogous system (nitrogen phosphotransferase system, PTS^{Ntr}). The first proteins in both pathways are enzymes (enzyme I^{sugar} and enzyme I^{Ntr}) that can be autophosphorylated by phosphoenolpyruvate. The most striking difference between enzyme I^{sugar} and enzyme I^{Ntr} is the presence of a GAF domain at the N-terminus of enzyme I^{Ntr}. Since the PTS^{Ntr} was identified in 995, it has been implicated in a variety of cellular processes in many proteobacteria and many of these regulations have been shown to be dependent on the phosphorylation state of PTS^{Ntr} components. However, there has been little evidence that any component of this so-called PTS^{Ntr} is directly involved in nitrogen metabolism. Moreover, a signal regulating the phosphorylation state of the PTS^{Ntr} had not been uncovered. Here, we demonstrate that glutamine and α-ketoglutarate, the canonical signals of nitrogen availability, reciprocally regulate the phosphorylation state of the PTS^{Ntr} by direct effects on enzyme I^{Ntr} autophosphorylation and the GAF signal transduction domain is necessary for the regulation of enzyme I^{Ntr} activity by the two signal molecules. Taken together, our results suggest that the PTS^{Ntr} senses nitrogen availability.

Keyword: GAF domain, glutamine, α–ketoglutarate, nitrogen PTS, phosphorylation-dependent mobility shift

S20-3

FeoC Regulation of Fe(II) Uptake in *Salmonella enterica*

Dongwoo Shin and Hyunkeun Kim

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In the gammaproteobacteria, the FeoA, FeoB, and FeoC proteins constitute the Feo system, which mediates ferrous iron [Fe(II)] import. Of these Feo proteins, FeoB is an inner membrane Fe(II) transporter that is aided by the small protein FeoA. However, the role of another small protein, FeoC, has remained unknown. Here we report that the FeoC protein is necessary for FeoB protein-mediated Fe(II) uptake in *Salmonella* experiencing low-oxygen/low-iron conditions. The FeoC protein was found to directly bind to the FeoB transporter, leading to high cellular levels of FeoB. Depletion of the FtsH protease enabled high levels of FeoB in the absence of FeoC, suggesting that the FeoC protein protects the FeoB transporter from FtsH-mediated proteolysis.

We also report proteolytic regulation of FeoC that occurs in an oxygen-dependent fashion. While relatively stable under low-oxygen conditions, FeoC was rapidly degraded by the Lon protease under high-oxygen conditions. *Salmonella* ectopically expressing the *feoB* and *feoC* genes was able to accumulate FeoB and FeoC only under low-oxygen conditions, suggesting that FeoC proteolysis prevents *Salmonella* from accumulating the FeoB transporter under high-oxygen conditions. Finally, we propose that Lon-mediated FeoC proteolysis followed by FtsH-mediated FeoB proteolysis helps *Salmonella* to avoid uncontrolled Fe(II) uptake during the radical environmental changes encountered when shifting from low-iron anaerobic conditions to high-iron aerobic conditions.

Keywords: Fe(II) uptake, Feo system, FeoB transporter, FeoC protein, proteolysis, *Salmonella*

S20-4

Stringent Control of EPEC Pathogenesis

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In nature, living cells need to sense and respond to their surroundings properly for thriving in various environmental conditions. One of those adaptive responses in bacteria and some plant cells, but not animal cells, is the stringent response, which has been initially characterized as a bacterial hunger response against depletion of amino acids. Although amino acid starvation has been well established as an environmental trigger for the stringent response, recent studies demonstrated that many other signals can also evoke the stringent response, such as limitation of carbon sources, fatty acids, iron, nitrogen, or phosphate as well as a down-shift of pH, osmotic shock, and oxidative stresses. Guanosine 3',5'-bispyrophosphate (ppGpp) is an alarmone that induces the stringent response in bacteria and plants, but not in animals, under starved and stressful conditions. As a stringent response regulator, ppGpp is rapidly synthesized and hydrolyzed in cells depending on their surrounding environments and involves in many cellular processes such as growth, secondary metabolism, and virulence. To define the role of ppGpp in enteropathogenic *Escherichia coli* (EPEC), a major cause of infant diarrhea especially in developing countries, a ppGpp-defective mutant of the EPEC E2348/69 strain was created by inactivating both *relA* and *spoT* genes, previously known to encode a functional ppGpp synthetase in *E. coli* K-2, and further characterized. Our experimental analyses demonstrated that the lack of ppGpp in the EPEC E2348/69 strain (i) derepressed the expression of Type IV bundle forming pili (BFP), (ii) repressed the locus of enterocyte and effacement (LEE) pathogenicity island encoding a functional Type III secretion system (TTSS), (iii) could not induce the EPEC-mediated killing of *Caenorhabditis elegans*, and (iv) altered the outer membrane structure and integrity. The whole genome-scale transcriptomic analysis revealed the 854 EPEC genes that were differentially expressed by ppGpp (cut-offs of > 2 folds), including the *LEE*, *bfp*, *per*, and *gad* operons. Collectively, our results imply that ppGpp signaling in EPEC is important for switching between the expression of two major virulence determinants, BFP and TTSS on the LEE island, as well as for optimizing *in vivo* pathophysiology while EPEC survives passage through diverse microenvironments in the gastrointestinal tracts during a host infection.

Reference

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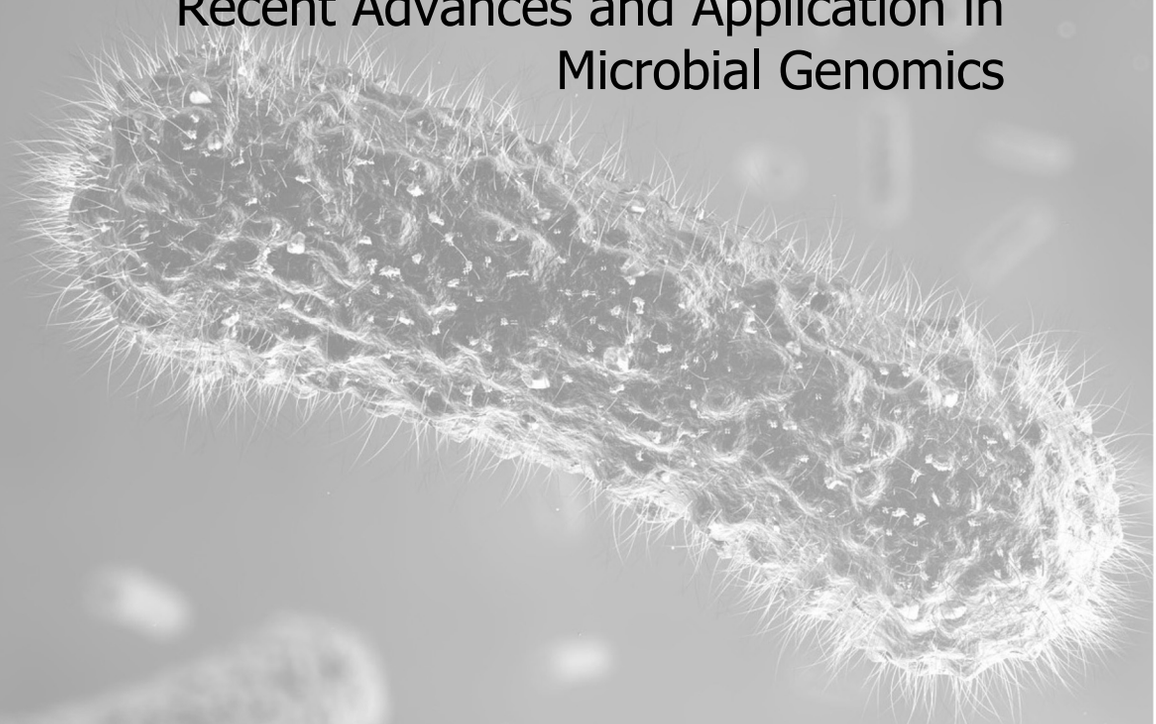
Keywords: stringent control, ppGpp, EPEC, pathogenesis

Symposium [S21]

Recent Advances and Application in Microbial Genomics



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S21-1

Small Yet Big: Interpretation of Microbial Big Data

Changhoon Kim

Bioinformatics Institutes, Macrogen Inc.

Rapid growth in NGS (Next-Generation-Sequencing) genome sequencing data exposes the researcher to un-experienced territories, where data volumes are too big to be efficiently handled in a small laboratory with a decent personal computer. In microbial gnome research, individual genome sizes are relatively small, compared to higher eukaryotic organisms, but collective size of microbial genomic data is also big. Such changes in the research environment require new ways of thinking for data management and new algorithms for data analyses. In this talk, the nature of microbial genome sequencing data as a "big data" will be touched and cloud solutions for big data available to public will be discussed.

Keywords : big data, cloud, microbial genome, next-generation-sequencing

S21-2

Harnessing the Power of Adaptive Laboratory Evolution and Genome-scale Sciences for Strain Engineering

Daehee Lee

Synthetic Biology and Bioengineering Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB)

Adaptive laboratory evolution (ALE) studies provide key information to address a wide range of issues in evolution through direct observation of the evolution process with microorganisms in laboratory. They not only allow us to test evolutionary theory and principles, but also to have applications to metabolic engineering. ALE strategies enable combining genetic variation with the selection of beneficial mutations in an unbiased fashion, which can open new way to the metabolic engineering and have been proven highly effective in the optimization of production strain. In contrast to rational engineering strategies, ALE has the advantage of letting nonintuitive beneficial mutations occur in many different structural and regulatory proteins in parallel. Genome-scale tools with next-generation sequencing are revolutionizing studies of ALE by providing complete determination of the genetic basis of adaptation and the phenotypic changes in the microorganisms.

Escherichia coli is a widely used workhorse for production of heterologous proteins. Often, however, membrane protein overexpression, which is essential for structural and functional studies, in *E. coli* is not successful and yields are frequently too low because it has adverse effects on host cells, ending in cell death. An understanding of the genetic basis and physiological response to overexpression of membrane proteins is needed to improve such yields. In this study, the genetic and biochemical bases for bacterial adaptation to membrane protein overexpression were determined to gain understating of the plasticity of bacterial genomes and to engineer *E. coli* cells for better production of toxic membrane proteins. Using next-generation sequencing technology, we identified all accumulated mutations that appear during ALE of an *E. coli* BL2(DE3) strain expressing membrane proteins. We obtained proof that the observed spontaneous mutations were responsible for improved overexpression of membrane proteins by creating defined site-directed mutants. To elucidate the mechanisms underlying the adaptation to membrane protein overexpression, we generated a series of evolved *E. coli* mutants by overexpressing membrane proteins. Once we resequenced the genomes of evolved E. coli strains, comparative genome analysis with their ancestral strain *E. coli* BL2(DE3) was conducted to reveal genetic changes. We found significant mutations in *lacI* or *lac*UV5 promoter region that can controls the expression of toxic proteins, which is linked to reduction of host toxicity caused by overexpression of membrane proteins. Crucial mutations in *lacI* conferring the host cell tolerance to membrane protein overexpression were experimentally validated. Based on this result, we have engineered the *E. coli* BL2(DE3) strain, named OPTE(DE3), in which the activity of the T7 RNAP can be precisely controlled by *lac* repressor mutant supplied from the compatible pmLacI plasmid that is tightly regulated by L-rhamnose. The OPTE(DE3) is suitable for tuning and optimizing of membrane protein overexpression by varying concentration of a inducer L-rhamnose.

Keywords: adaptive laboratory evolution, *Escherichia coli*, membrane protein overexpression, next-generation sequencing, *lac* repressor

S21-3

Genome-wide Analysis of the Extremophilic Bacterium *Fervidobacterium islandicum* AW- Revealed the Degradation Mechanism of Feather Keratin

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School of Applied Biosciences, Kyungpook National University

The extremophilic bacterium *Fervidobacterium islandicum* AW- isolated from a geothermal hot stream in Indonesia could degrade native feathers (0.8%, w/v) completely at 70°C and pH 7 in the modified *Thermotoga-Fervidobacterium* (TF) medium. After 24 h of culture, feather degradation led to an increase in free amino acids such as histidine, cysteine and lysine. Moreover, nutritionally essential amino acids such as tryptophan and methionine, which are rare in feather keratin, were also produced as microbial metabolites. To better understand the mechanism of native feather-degradation, we first sequenced the 2.72-Mb genome of *F. islandicum* AW- that contains 2,938 protein-coding genes including 68 genes encoding proteolytic and redox-related enzymes. Genomic comparison of *F. islandicum* AW- with the closely-related *F. nodosum* which cannot degrade a native feather suggested that several protein-coding genes might be highly involved in keratin degradation, which was further investigated using the next-generation sequencing-aided RNA-seq. Based on the transcriptome data, we chose several putative genes for keratin degradation, overexpressed them in *Escherichia coli* and characterized the recombinant enzymes in detail. Consequently, this study provide the basis of identification of keratinolytic enzymes, which is potentially applicable for development of novel biomaterials for cosmetics as well as treatment of poultry wastes.

Keywords: extremophilies, *Fervidobacterium islandicum* AW-, feather-degradation, RNA-seq, proteolytic, redox-related enzymes

S21-4

Comparative Genomic Analysis of Complete Genome Sequence of 4 Microbes, Isolated from the Real Food-Borne Outbreak in South Korea

Heebal Kim, Sang-Ho Choi, Sangryeol Ryu, and Ju-Hoon Lee[2]

Department of Agricultural Biotechnology, Seoul National University,

[2]Department of Food Science and Biotechnology and Institute of Life Science and Resources, Kyung Hee University

Bacteria are the most common cause of foodborne gastrointestinal infections worldwide, usually transmitted through contaminated food or untreated water. The exact infection mechanisms of food borne bacteria invading into host are not yet fully elucidated. In this study, the food borne bacteria such as Staphylococcus aureus FORC_00, *Yersinia enterocolitica* FORC_002, *Clostridium perfringens* FORC_003, and *Vibrio parahaemolyticus* FORC_004 were isolated from a contaminated food.

To extend our understanding of pathogenesis, we sequenced the genome of food borne bacteria by Pacific Biosciences RSII sequencer and compared the genome data with previously published strains of *S.aureus*, *Y.enterocolitica*, *C.perfringens*, and *V.parahaemolyticus*. Genome annotation was carried by using Rapid Annotation using Subsystem Technology (RAST). The genome tree was constructed for all the strains to find the closest genome sequences using average nucleotide identity (ANI) values. Genomic islands and virulence factors were identified for determining the pathogenicity of food borne strains.

This genome sequence would be useful in many application including, elucidate the uncover mechanisms of its successful worldwide distribution, facilitate the development of new molecular diagnostic tools and revealing the virulence factors responsible for host-gene expression. In addition, detection and characterization of VFs would be useful to provide information for epidemiological survey and for development of new biomarkers for rapid detection of this pathogen in foods.

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Keywords: food-poisoning, genome, comparative genomics, virulence factors, genomic island

Symposium [S22]

Molecular Basis of Bacterial Pathogenesis

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S22-1

An Anti-virulence Strategy to Combat *Pseudomonas aeruginosa* Infection

Sang Sun Yoon

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Pseudomonas aeruginosa, a Gram-negative bacterium of clinical significance, produces elastase as a predominant exoprotease. Here, we screened a library of chemical compounds currently used for human medication and identified diethylene triamine pentaacetic acid (DTPA, pentetic acid) as an agent that suppresses the production of elastase. Elastase activity found in the prototype *P. aeruginosa* strain PAO was significantly decreased when grown with as low as 20 μM DTPA. Supplementation with Zn^{2+} or Mn^{2+} ions restored the suppressive effect of DTPA suggesting that the DTPA-mediated decrease in elastase activity is associated with ion-chelating activity. In DTPA-treated PAO cells, transcription of the elastase-encoding *lasB* gene and levels of pseudomonas quinolone signal (PQS), a molecule that mediates *P. aeruginosa* quorum sensing (QS), were significantly downregulated, reflecting the potential involvement of the PQS QS system in DTPA-mediated elastase suppression. Biofilm formation was also decreased by DTPA treatment. When A549 alveolar type II-like adenocarcinoma cells were infected with PAO cells in the presence of DTPA, A549 cell viability was substantially increased. Furthermore, intranasal delivery of DTPA to PAO-infected mice alleviated the pathogenic effects of PAO cells in the animals. Together, our results revealed a novel function for a known molecule that may help treat *P. aeruginosa* airway infection.

S22-2

Host Partners of An RTX Toxin in *Vibrio vulnificus* Cytotoxicity

Young Ran Kim, BoA Kim, and Joon Haeng Rhee[2]

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[2]Department of Microbiology, Chonnam National University Medical School

Vibrio vulnificus, a halophilic estuarine bacterium causing fatal septicemia and necrotic wound infection, is highly cytotoxic to eukaryotic cells. We have reported that RtxA kills host cells only after they come into contact with bacteria and plays an essential role in the pathogenesis of *V. vulnificus* [1]. RtxA toxin is responsible for cytoskeletal rearrangement, contact cytotoxicity, hemolysis, tissue invasion, and lethality to mice. We conducted the real-time quantitative analysis of *V. vulnificus*-infected host cells using quantitative phase microscopy (QPM) based on interferometric techniques to study dynamic cell morphologic changes and to noninvasively quantify the cell volumes of RBL-2H3 cells infected with *V. vulnificus* strains. During the process of *V. vulnificus* wild type infection in RBL-2H3 cells, the dynamic changes of quantitative phase images, cell volumes, and areas were observed in QPM. In contrast, dramatic changes were not detected in RBL-2H3 cells infected with the noncytotoxic *rtxA* mutant strain [2]. By using confocal microscopy and immunoblot analysis, we show that the 50-kDa RtxA toxin is processed into 2 fragments after its secretion into host cells. The larger N-terminal fragment (RtxA-N; approximately 370 kDa) remained at the host cell membrane, whereas the smaller C-terminal fragment (RtxA-C; approximately 30 kDa) was internalized into the host cell cytoplasm. RtxA-N is believed to polymerize and form pores at the host cell membrane and to induce an increase in necrotic volume related to calcium. The RtxA toxin caused an increase in the intracellular calcium concentration and the subsequent activation of JNK. The cell death mechanism occurred via calcium-dependent mitochondrial pathways, which caused calcium sequestration in the mitochondria, accompanied by irreversible mitochondrial membrane dysfunction and adenosine triphosphate depletion, and was later accompanied by the disruption of the integrity of the plasma membrane [3].

Bioinformatic analysis of *rtxA* gene predicted 4 functional domains that presumably exert discrete functions during host cell killing. Although *V. vulnificus* RtxA shows high homology in amino acid sequences to *V. cholerae* MARTX, a unique domain encoded by amino acids 95-2574 (Domain 2, designated as RtxA-D2) is absent in *V. cholerae* MARTX, suggesting this domain may confer biological activities specific to *V. vulnificus* RtxA. HeLa cells expressing GFP-RtxA-D2 become rounded and lost viability. Using a yeast two-hybrid system, prohibitin was screened as a RtxA-D2 partner in HeLa cells. The specific interaction of RtxA-D2 and prohibitin was confirmed by immunoprecipitation and immunocytochemistry. Interestingly, *V. vulnificus* RtxA induced the upregulation of prohibitin expression in association with the cytoplasmic membrane of HeLa cells, which was dependent upon p38 mitogen-activated protein kinase activation. Down-regulation of prohibitin by siRNAs resulted in a decreased cytotoxicity of HeLa cells infected with *V. vulnificus* wild type strain. These results suggest that prohibitin should be an important host partner of *V. vulnificus* RtxA toxin.

Referene

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Keywords: *Vibrio vulnificus*, RtxA toxin, calcium, mitochondria, prohibitin

S22-3

Virulence Factors of *Acinetobacter baumannii* as Promising New Drug Target

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Department of Microbiology and Research Institute for Medical Sciences, School of Medicine, Chungnam National University

Acinetobacter baumannii has emerged as an opportunistic pathogen causing nosocomial infection in immunocompromised patients. A major factor in prominence of *A. baumannii* as a pathogen is highly resistant to various antibiotics. The characteristic of *A. baumannii* restricts the therapeutic options that target bacterial viability to treat infections. Therefore, development of effective alternative drugs, which target virulence factors while minimizing antibiotic resistance, is constantly required for the treatment of multi-drug resistant *A. baumannii* infection. Quorum sensing, cell to cell communication system by which bacteria coordinate virulence gene expression, is an increasingly interesting target for developing alternative drugs for the treatment of multi-drug resistant *A. baumannii* infection. However, the role of quorum sensing has not been characterized in *A. baumannii*. Recently, in some studies, quorum sensing inhibitory compounds on *A. baumannii* have been identified by chemical screening. The present study describes the role and regulatory characteristics of quorum sensing system in *A. baumannii* pathogenesis. Furthermore, small molecules that inhibit the quorum sensing of *A. baumannii* will be delineated in this seminar.

Keywords: *Acinetobacter baumannii*, multi-drug resistance, quorum sensing, virulence factor

S22-4

Differential Modulation of Cytokine Production in Gram-negative and Gram-positive Bacterial Infection by ATF3, a Stress Inducible Eukaryotic Gene

Dong-Kwon Rhee, Cuong Thach Nguyen, and Truc Thanh Luong

School of Pharmacy, Sungkyunkwan University

Background. Activating transcription factor-3 (ATF3) is known as a suppressor of cytokine production after exposure to lipopolysaccharide or during Gram-negative bacterial infection. However, the mechanism by which ATF3 regulates innate immunity against Gram-positive bacterial infection, particularly *Streptococcus pneumoniae*, remains unknown.

Methods. The wild-type and ATF3 knock-out (KO) mice were infected intranasally (*i.n*) or intraperitoneally with *S. pneumoniae,* and bacterial colonization or survival rate was determined. Pneumococcal pneumonia was induced by *i.n* infection, and ATF3 level was determined by Western blot. ATF3 KO cells or ATF3 siRNA transfection were used to determine expression of ATF3 downstream genes. ELISA was used to examine cytokines levels.

Results. ATF3 was highly expressed in various cell lines *in vitro* and in many organs *in vivo*. Pneumolysin (PLY) was a novel inducer of ATF3. Pneumococcal infection induced ATF3, which subsequently stimulated production of cytokines (TNF-α, IL-β, and IFN-γ). ATF3-mediated cytokine induction protected the host from pneumococcal infection. In the pneumonia infection model, the bacterial clearance of wild-type mice was more efficient than those of ATF3 KO mice.

Conclusions. Taken together, we can conclude that ATF3 regulates innate immunity positively upon pneumococcus infection by enhancing TNF-α, IL-β, and IFN-γ expression and modulating bacterial clearance.

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Keywords: ATF3, cytokine, *S. pneumoniae*, TNF-α, infection.

Symposium [S24]

KNMRRC Session II

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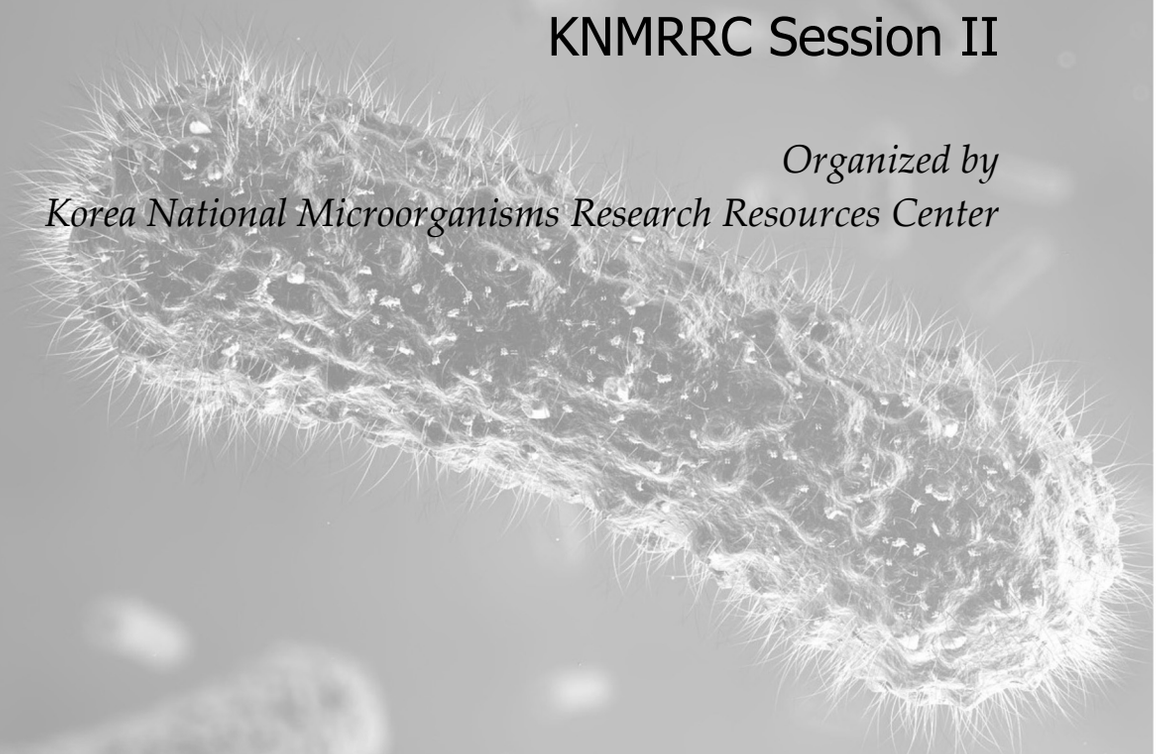

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S24-1

Importance of Isolating Bacterial Viruses for the Interpretation of Metagenomes

Ilnam Kang Jang-Cheon Cho*

Department of Biological Sciences, Inha University

Recent ocean metagenomic studies have revealed the numerous novel genetic repertoires of marine viromes but most genome fragments in the viromes are hard to be categorized into known viral groups. The reason of the poor assignment of virome sequences to viral genomes has been thought to be the low number of phage isolates infecting major bacterial groups. Since cultivation of bacterial strains is the only way of isolating bacteriophages, there is an urgent requirement for the isolation of phages that infect important marine bacterial groups. In this presentation, we summarize the isolation of several bacteriophages from the euphotic zone of the Yellow Sea and the East Sea of Korea, genome characteristics, and distribution of their genome sequences by virome binning. We obtained 8 phages infecting 5 strains of bacteria: HMO-20 and P322B from *Ca*. "*Puniceispirillum marinum*" IMCC322, P2024L and P2024S from *Persicivirga* sp., P2026 from *Marinomonas* sp., P2053L from *Celeribacter* sp., and P2559S and P2559Y of *Croceibacter atlanticus*. Except for HMO-20 and P322B, genome sequences of all other phages showed limited similarity to other bacteriophage genomes in public databases. Fragment recruitment analyses were performed to gain insights on the distribution of phages related to HMO-20 and/or P322B. When the two phage genomes were used as queries in BLAST to recruit marine viral metagenome (virome) sequences, both the phages recruited a large number of virome reads from the euphotic zone, suggesting the prevalence of phage types related to HMO-20 and P322B in the surface ocean. Most of recruited reads showed similarities to the genome regions shared between two phages, implicating that the genes common to the two phages may represent core genes of phages infecting the SAR6 clade. Overall distribution pattern of HMO-20 type phages was similar to that of the SAR6 clade, suggesting that the dynamics of SAR6 bacteria may be under the influence of infection by phages coexisting in their habitats.

S24-2

The Applications of Genome Information to Understand Biology

Ik-Young Choi

National Instrumentation Center for Environmental Management, College of Agriculture and Life Sciences, Seoul National University

The NGS technologies of genome DNA structure, expression profiling and epigenome elements have been used widely as approaches in the expertise of genome biology and genetics. The application to genome study has been particularly developed with the introduction of the next-generation DNA sequencer (NGS) Roche/454, Illumina/Solexa and PacBio systems along with bioinformation analysis technologies of whole-genome *de novo* assembly, expression profiling, DNA variation discovery, and genotyping. One of the advantages of the NGS systems is the cost-effectiveness to obtain the result of high-throughput DNA sequencing for genome, RNAnome, and miRNAnome studies. Both massive whole-genome shotgun paired-end sequencing and mate paired-end sequencing data are important steps for constructing *de novo* assembly of novel genome sequencing data and for resequencing the samples with a reference genome DNA sequence. To construct high-quality contig consensus sequences, each DNA fragment read length is important to obtain *de novo* assembly with long reading sequences of the Roche/454 and PacBio systems. It is necessary to have DNA sequence information from a multiplatform NGS with at least 2x and 30x depth sequence of genome coverage using Roche/454 and Illumina/Solexa, respectively, for effective a way of *de novo* assembly, as hybrid assembly for novel genome sequencing would be cost-effective. In some cases, Illumina/Solexa data are used to construct scaffolds through *de novo* assembly with high coverage depth and large diverse fragment mate paired-end information, even though they are already participating in assembly and have made many contigs. Massive short-length reading data from the Illumina/Solexa system is enough to discover DNA variation, resulting in reducing the cost of DNA sequencing. MAQ and CLC software are useful to both SNP discovery and genotyping through a comparison of resequencing data to a reference genome. Whole-genome expression profile data are useful to approach genome system biology with quantification of expressed RNAs from a whole-genome transcriptome, depending on the tissue samples, such as control and exposed tissue. The long read sequence data of PacBio are more powerful to find full length cDNA sequence through *de novo* assembly in any whole-genome sequenced species. An average 30x coverage of a transcriptome with short read sequences of Illumina/Solexa is enough to check expression quantification, compared to the reference EST sequence. In an *in silico* method, conserved miRNA and novel miRNA discovery is available on massive miRNAnome data in any species. Particularly, the discovered target genes of miRNA could be robust to approach genome biology study.

S24-4

Detection of Coliforms in Drinking Water Using Skin Patches: A Rapid, Reliable Method that Does Not Require An External Energy Source

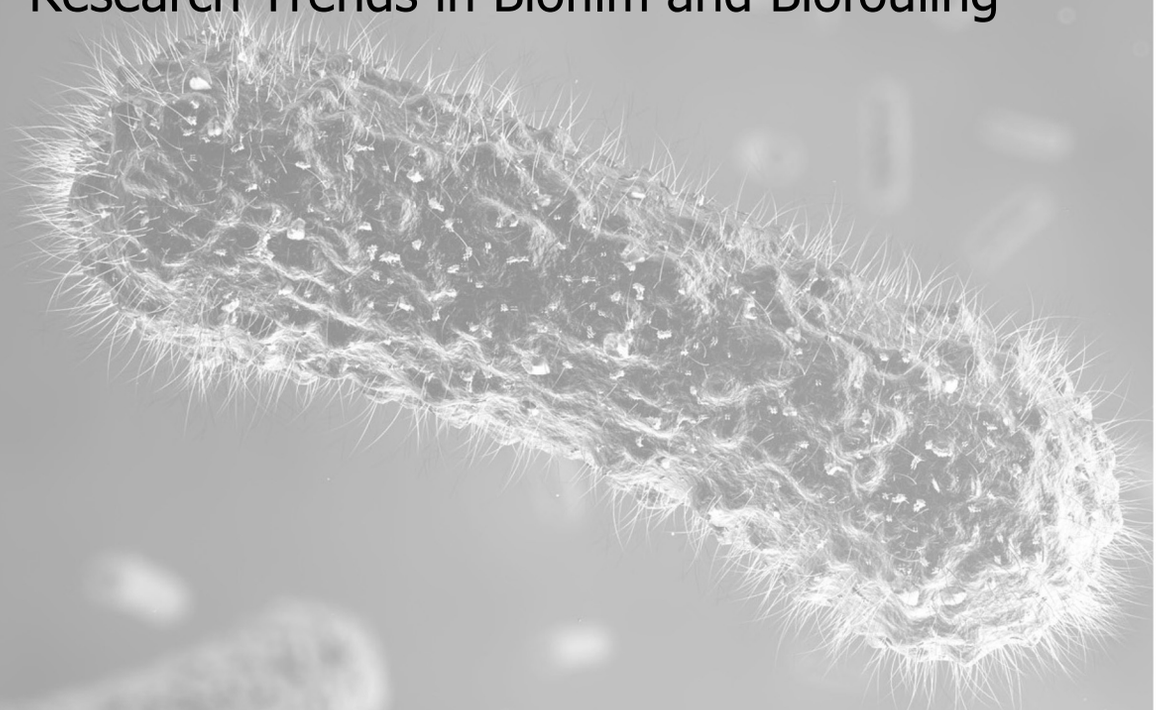
Min-jeong Kim, Yu-jin Lee, Sung-Ae Oh, and Gyu-Cheol Lee*

Water Quality Research Center, K-water

The detection of coliforms requires incubation in a laboratory, generally powered using electricity. In many parts of the developing world, however, external energy sources such as electricity are not readily available. To develop a fast, reliable method for detecting coliforms in water without an external energy source, we assessed the efficacy of six test kits for the identification of coliforms in water samples. To assess the possibility of using body temperature as the sole source of heat for incubation, bacterial samples were mixed with the enzymatic test kit reagent and attached to the human body surface using a patch system. The patches were attached to the bodies of volunteers for 24 hours and the practicality and accuracy of the patches were assessed. Coliforms were detected within 24 hours in all patches. This innovation will facilitate the testing of water quality by researchers and by economically disadvantaged people without electricity.

Symposium [S25]

Research Trends in Biofilm and Biofouling



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S25-1

Studying Mechanical Properties of Bacterial Appendages Using Atomic Force Microscopy and Traction Force Microscopy

Sungsu Park

School of Mechanical Engineering
Sungkyunkwan University

The bacterial cell surface including curli fibers is responsible for many important biological functions. It plays a structural role by helping to maintain cellular structure and resisting turgor pressure, it sustains changes made by growth and division, and it makes a way to transfers information about the environment into the cell. These functions not only suggest that the cell wall is dynamic, but that its mechanical properties are of significant importance. To understand physical properties of curli and its relation to adherence on the surface, we used an atomic force microscopy (AFM) to quantify the mechanical behavior and the structure of culri strain surfaces. Even after bacteria are attached to the surface, some of them can be motile for a while. To measure the force generated by motile bacteria, we developed traction force microscopy (TRM) using soft nanopillars made of elastic polymer. Using TRM, we can detect bacteria and also measure the force generated by bacteria tethered to each pillar.

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Keywords: AFM, soft nanopillar, bacterial adhesion, curli, nanomechanical properties

S25-2

The Cocci-shaped *Escherichia coli*: Biofilm Formation and Antibiotics Resistance

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It has been well-established that bacterial shape influences on their biological and physiological characteristics. In recent, RodZ (formally YfgA) was identified as a putative cytoskelectal anchoring protein and the deletion of *rodZ* dramatically changed cell morphology from rod cells to round cells [1]. Here we investigate on the novel role of RodZ in biofilm formation and antibiotics resistance of *E. coli* K-2 strain BW253. Consistently, we confirmed that cells lacking *rodZ* no longer had rod shape but rather were round or oval by the scanning electron microscopy (SEM). Interestingly, curli production was critically reduced by the deletion of *rodZ* gene and that was recovered by the complementary of RodZ. Importantly, biofilm formation (both on the polystyrene and mucus layer) and antibiotic resistance were significantly regulated by the presence of RodZ. Whole transcriptome analysis showed that curli production-, cell division-, and biofilm-associated genes as well as phage-related genes. Taken together, these results indicated that RodZ play a critical role in morphology of *E. coli* and is important on the biofilm formation and antibiotic resistance. These findings provide new insight in discovering novel target gene for antibacterial agents.

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Keywords: RodZ, cell morphology, *E. coli*, biofilm formation, antibiotic resistance

S25-3

Inhibition of Biofilm Formation using Ginger Chemicals: 6-gingerol and Raffinose

Han-Shin Kim, Sang-Hoon Lee, Eun Ji Cha, Youngjoo Byun[2] and Hee-Deung Park

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Ginger extract showed anti-biofilm effect in various bacteria (1), but it has not been identified ginger's active chemicals inhibiting biofilm formation. We hypothesized that ginger extract contains chemicals effective in inhibiting biofilm formation, and screened several ginger chemicals demonstrating anti-biofilm effect using a static biofilm assay. 6-Gingerol and raffinose were studied in detail for elucidating molecular anti-biofilm mechanisms using *Pseudomonas aeruginosa* as a model bacterium (2, 3). 6-Gingerol is similar to a *P. aeruginosa* quorum-sensing signal molecule, *N*-(3-oxododecanoyl)-L-homoserine lactone, in chemical structure, and showed a competitive binding to cognate receptor molecule in inhibiting biofilm formation. Analyses of a global transcriptome and virulence factors production also confirmed the anti-biofilm mechanism. Moreover, 6-gingerol was effective to reduce mortality of mice infected with *P. aeruginosa*. On the other hand, raffinose was effective to inhibit biofilm formation by reducing the level of bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP). Phenotypes of *P. aeruginosa* treated with raffinose were characterized by decreased production of extracellular polymeric substances, inhibition of rugose colony, and increased swarming motility. All these results were related to decreased level of cellular c-di-GMP. Notably, raffinose was effective to reduce biofilm formation in both Gram-negative and positive bacteria, unlike 6-gingerol's effectiveness to Gram-negative bacteria.

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Keywords: biofilm, biofilm inhibitor, c-di-GMP, *Pseudomonas aeruginosa* , quorum sensing

S25-4

Diverse Quorum Quenching Bacteria Isolated from Wastewater Treatment Plant, and Their Application for Controlling Biofilm Formation

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Bacteria recognize changes in their population density by sensing the concentration of signal molecules, *N*-acyl-homoserine lactones (AHLs). AHL-mediated quorum sensing (QS) plays a key role in biofilm formation, so the interference of QS, referred as quorum quenching (QQ), has received greate deal of attention. A QQ strategy can be applied to membrane bioreactors (MBR) for advanced wastewater treatment to control biofouling. The purpose of our study is to isolate indigenous quorum quenching bacteria that can inhibit biofilm formation and eventually be used to reduce biofouling in MBR systems of wastewater treatment plant. To isolate QQ bacteria that can inhibit biofilm formation, we isolated diverse AHL degrading bacteria from a lab-scale MBR and sludge from real wastewater treatment plants. A total of 225 AHL-degrading bacteria were isolated from the sludge sample by enrichment culture. To identify the enzyme responsible for AHL degradation in QQ bacteria, AHL-degrading activities were analyzed using cell-free lysate, culture supernatant and whole cells. *Afipia* sp. and *Acinetobacter* sp. strains produced the intracellular QQ enzyme, while *Pseudomonas* sp. and *Micrococcus* sp. produced the extracellular QQ enzyme that was most likely to produce AHL-acylase. AHL-degrading activity was observed in whole-cell assay with the *Microbacterium* sp. and *Rhodococcus* sp. strains. In the meantime, we cloned and expressed a AHL-lactonase and AHL-acylase genes from the isolates in *E. coli*. A cloned AHL-lactonase gene from *Rhodococcus* sp. shared 90% DNA sequence identity with the AHL-lactonase gene (*qsdA*) of *R. erythropolis* W2. In addition, three genes encoding putative AHL-acylases were identified in *Pseudomonas* sp. The deduced amino acid sequence of the three acylase genes shared 52%, 63%, and 68% identities with that of *pvdQ*, *quiP*, and *hacB* from *P. aeruginosa* PAO, respectively. These three AHL-acylases genes of *Pseudomonas* sp. strain were expressed in *Bacillus thuringiensis* as well.

Finally, inhibition of biofilm formation by isolated QQ bacteria or enzymes was observed on glass slides and 96-well microtiter plates using crystal violet staining. Interestingly, the QQ strains or enzymes not only inhibited initial biofilm development but also reduced established biofilms. This study shows the potential of AHLs degradation by QQ enzymes of isolated bacteria and inhibition of QS regulated biofilm formation.

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Keywords : quorum sensing, quorum quenching, AHL, biofilm, wastewater treatment

Symposium [S26]

Immunity to Infection

2014 한국미생물학회연합 국제학술대회
INTERNATIONAL MEETING OF
THE FEDERATION OF KOREAN MICROBIOLOGICAL SOCIETIES

S26-1

Preconditioning of High Mobility Group Box Protein (HMGB) Attenuates TLR4-mediated Inflammatory Response

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Activation of innate immunity via TLRs plays a pivotal role in host defense against pathogens through the recognition of PAMPs. TLR signaling should be tightly regulated since uncontrolled immune response leads to various kinds of autoimmune and inflammatory diseases. Recent studies have shown that HMGB could be critical for the control of TLR signaling. In this study, we demonstrate that HMGB mediates negative regulating signals of TLR activation. Preconditioning of HMGB attenuated TLR activation by LPS in murine, human macrophage cell lines and mouse bone marrow derived macrophages (BMDMs). Treatment of HMGB followed by LPS stimulation decreased production of TNF-α and IL-6 in mouse BMDM compared with LPS alone stimulation. Pretreatment of HMGB promoted rapid termination of signaling in macrophage cells through transcription factor NF-κB by augmenting negative regulators of TLRs such as TRIM30α and SHIP-. Knockdown experiments using TRIM30α-specific small interfering RNA impair HMGB-mediated TLR tolerance. We identified that HMGB bound to lymphotoxin β receptor (LTβR) and induced its downstream signaling of TIRM30α expression. HMGB preconditioning induced LPS tolerance in wild type mice but not in LTβR knock-out mice. Collectively, our results demonstrate that preconditioning of HMGB induces tolerance mechanism against excessive TLR-mediated inflammation via LTβR.

Keywords: HMGB, tolerance, lymphotoxin β receptor (LTβR), TLR

† This research was supported by grants from the NRF (20-0076 and 204RA4A008625) funded by the Korea government (MEST).

S26-2

Helicobacter pylori-mediated Inflammation in Host Cells: Mechanism for VEGF and IL-1b Production

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Colonization of the stomach by *Helicobacter pylori* affects about half of the world population and is associated with the development of gastritis, ulcers and cancer. Polymorphisms in the *IL1B* gene are linked to an increased risk of *H. pylori*-associated cancer, but the bacterial and host factors that regulate IL-1β production in response to *H. pylori* infection remain largely unknown. As well, although *H. pylori* have been known to induce VEGF production in gastric epithelial cells, the precise mechanism for cellular signaling is incompletely understood. In our recent studies, we investigated the role of bacterial virulence factor and host cellular signaling in IL-1β production of DCs and VEGF production of gastric epithelial cells in response to *H. pylori*. We show here that bacterial *cag*PAI and the cooperative interaction among the host innate receptors TLR2, NOD2 and NLRP3 are important regulators of IL-1β production in *H. pylori*-infected DCs. In addition, we defined the important role of ROS-HIF-1α axis in VEGF production of *H. pylori*-infected gastric epithelial cells and bacterial T4SS has a minor role in *H. pylori*-induced VEGF production of gastric epithelial cells.

Keywords: *Helicobacter pylori*, IL-1β, VEGF, cagPAI, HIF-1α

S26-3

Role of Streptococcal Phage Lysin$_{sm}$ in the Pathogenesis of Infective Endocarditis

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The pathogenesis of infective endocarditis is a complex process, involving numerous host-pathogen interactions (1). A key interaction for disease is the binding of microbes to human components, including platelets, fibrinogen, fibrin, and fibronectin (2,3). Although this binding appears to be a central requirement for virulence, only a limited number of endocarditis-related virulent factors has been identified (4). Among the viridans group streptococci, *Streptococcus mitis* is a leading cause of endovascular infection (5). Despite its increasing -importance as a human pathogen, relatively little is known about the virulence determinants of this organism, particularly with regard to its interaction with host blood components. We have recently shown that a cell wall anchoring protein (lysin$_{SM}$) encoded by a lysogenic bacteriophage mediates the binding of this organism to human platelets through interacting with the membrane fibrinogen. Moreover, lysin$_{SM}$ readily shed from *S. mitis* to enhance the vegetation formation by inhibiting clot lysis. Through an analysis of lysin$_{SM}$ binding with purified recombinant fibrinogen in vitro, we found that this protein can bind directly to a plasminogen binding site on the C-terminal end of the fibrinogen Aα chain. Competitive inhibition ELISA and a surface plasma resonance (SPR) assay results indicate that free lysin$_{SM}$ blocks plasminogen binding to fibrin to delay the initiation of clot lysis (fibrinolysis). Taken together, our data suggests that the binding of lysin$_{SM}$ to fibrinogen is mediated by a specific domain of the phage encoded protein (lysin$_{SM}$), and that this interaction is important for fibrinolysis and vegetation formation.

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Keywords: infective endocarditis, *Streptococcus mitis*, fibrinogen, fibrinolysis, plasminogen, bacteriophage, lysin

S26-4

A GPCR9 Agonist Alleviates Systemic Inflammation by Expansion of Immune Regulatory Cells

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Cell therapeutics using immune regulatory cells might be useful in treatment of various inflammatory diseases. However, clinical application of the immune regulatory cells is limited by low efficacy of their expansion in vivo. Here, we demonstrated that treatment of a G-protein coupled receptor 9 (GPCR9) agonist, SP0 dramatically expanded immune regulatory cells and protected mice against sepsis. SP0 treatment normalized blood pressure and inflammatory cytokine levels, protected against acute kidney and liver damages in septic mice. The putative differentially expressed proteins in immune regulatory cells from septic mice were examined using a current IPI mouse protein database and Gene Ontology annotations. Proteomic analysis revealed that over 90 proteins appear to have been differentially expressed in immune regulatory cells generated after SP0 treatment. Interestingly, these are involved in increase of cell death, bleeding, and apoptosis as well as in decrease of hematocrit. These results strongly suggested that a GPCR agonist can be considered as potential therapeutics of inflammatory disease including sepsis by increase of the immune regulatory cells.

Keywords: sepsis, LPS, GPCR9 agonist, immune regulatory cells, IPI mouse protein data

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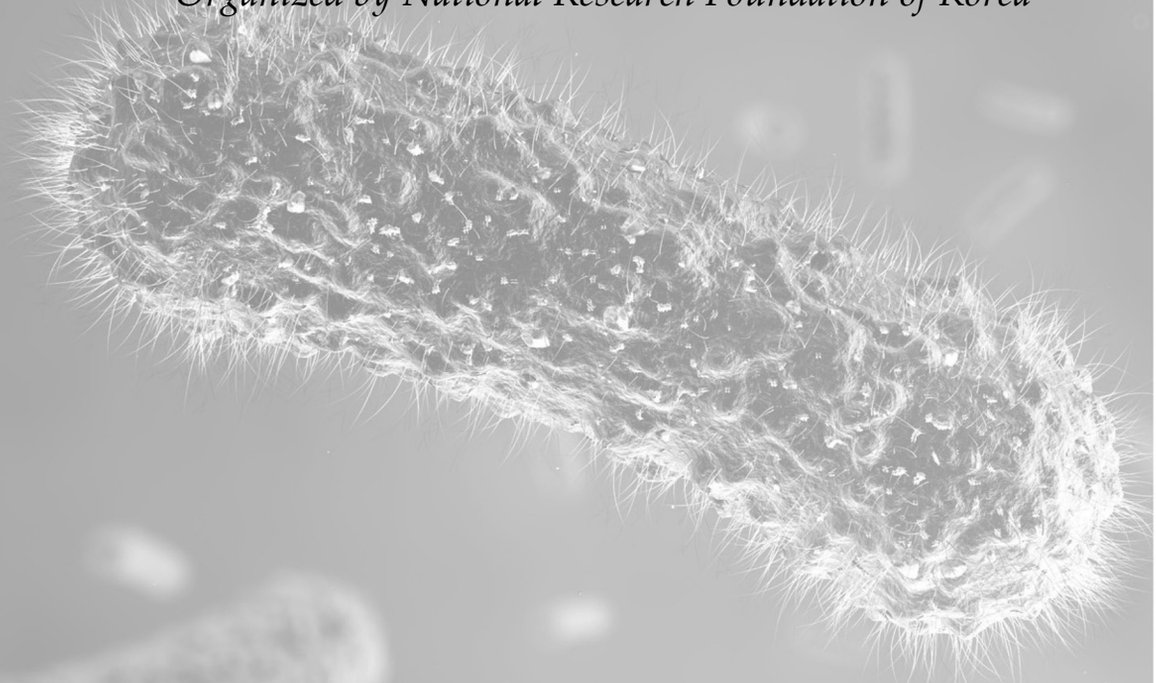

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INTERNATIONAL MEETING OF

THE FEDERATION OF KOREAN MICROBIOLOGICAL SOCIETIES

Microbiological Societies and NRF of Korea, 2015

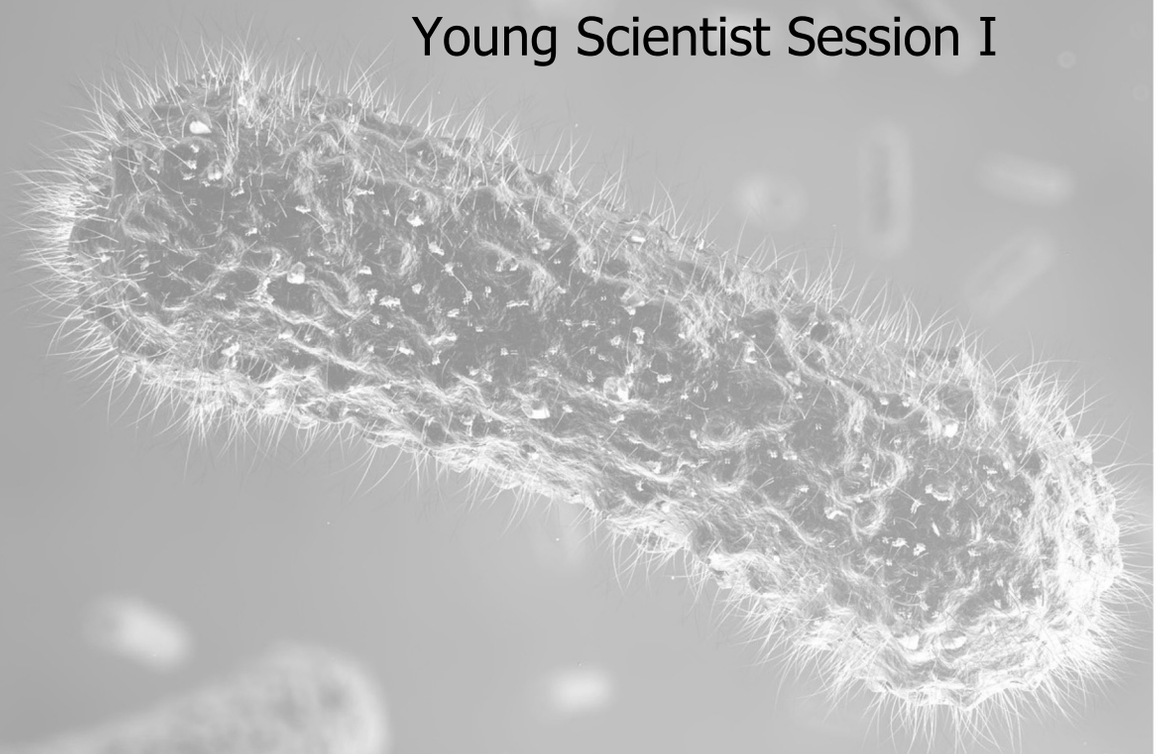
Joon Kim

Director, Division of Life Sciences, National Research Foundation of Korea
Professor of Biochemistry, Division of Life Sciences, Korea University

The National Research Foundation of Korea (NRF) is the biggest governmental funding agency of Korea which is a specialized research funding agency. This was established in 2009 which is the merge product of 3 formerly independent funding agencies such as KOSEF, KRF and KICOS. NRF supports creative academic research not only in science and technology but also in humanities and social sciences including academic studies, and interdisciplinary academic fields. Division of Life Sciences is under the Directorate for Basic Research in Science & Engineering. In the year of 2015, it will have governmental funding programs as follows. First, 'General Researcher Program' is organized for the increase of the research capacity of universities and research institutes. Second, 'Mid-career Researcher Program' is organized to enhance basic research capability and promote senior researchers' activities through creative individual or small-scale collaborative research. In 2015, the funding of this program will be increased significantly comparing to 2014. Third, 'Group Program' such as SRC, ERC and BRL will be organized to discover excellent group researchers. The funding of these programs will be increased also comparing to 2014. Fourth, 'New program will be started for the next-generation scientists and stimulate them to become global leaders and distinguished scientists to support them individually for a relatively long time. The detail of this program will be explained further in the symposium. Division of Life Sciences of NRF distributes funds to all the basic fields of life sciences including microbiology, agriculture, veterinary medicine, nutrition and food sciences. Current status of NRF and future in microbiological sciences with respect to other fields will be discussed in this session.

Symposium [S5]

Young Scientist Session I



2014 한국미생물학회연합 국제학술대회

INTERNATIONAL MEETING OF

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S5-1

Impact of Obesity on Influenza Infection

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Ewha Womans University College of Pharmacy

Obesity was considered as one of the high risk factors during the 2009 influenza pandemic. However, little is known about the mechanism to prove the relationship between obesity and influenza. We assessed general characteristics in obese mice, and whether obesity affects immune functions and mortality during influenza infection. C57BL/6 mice were fed a high fat diet (DIO) and some DIO mice performed exercise for the treating obesity. The DIO mice were immunized with an adenovirus expressing nucleoprotein (rAd/NP) and challenged with influenza virus (PR8). DIO mice showed increased airway hyperresponsiveness (AHR) and collagen levels in the lung parenchyma compared with lean mice. Pulmonary T cells were decreased in the DIO mice. Especially, naïve T cells were significantly reduced in the lung tissue of the DIO mice. In the PR8-challenged experiments, total cytotoxic T cells (CTLs) were reduced in the lung tissue of the rAd/NP-immunized DIO mice compared with the rAd/NP-lean mice. However, NP-specific CTLs were increased in the lung tissue of the DIO mice. Both NP-IgG and mortality rates were similar between rAd/NP-DIO and rAd/NP-lean mice. Treating obesity recovered the reduced total CTLs of the lung tissue of the rAd/NP-DIO mice after PR8 challenge, but NP-CTLs from the treating obesity group still up-regulated. The treating obesity delayed the mortality in the PR8-challenged DIO mice. The results demonstrate that obesity changes AHR, lung fibrosis and T cell populations in naïve mice. Obesity also changes vaccine effects according to the modifications of the pulmonary CTLs, and treating obesity restore the immune response after influenza challenge.

Keyword : adenovirus-based influenza vaccine, cytotoxic T lymphocytes, influenza virus, nucleoprotein, obesity

S5-2

Association between Respiratory Virus Specific IgE Detection in Sputa and Asthma Exacerbation

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Acute asthma exacerbation has been closely associated with respiratory viral infections. Respiratory viral detection rate, species, seasonal variation, and sequential dynamics were investigated with total 132 induced sputa samples from lower airway of adult asthmatics (n=60) and controls (n=10) with acute respiratory symptoms. Multiplex RT-PCR (mPCR) for 12 respiratory viral species including respiratory syncytial virus (RSV) were performed with those. Total 15 respiratory viral detections were observed among asthmatics and COPD controls (n= 13, and 2), and RSV was the most common (n=8). With 50 sputa supernatants, we tried to detect specific IgE (sIgE) to crude RSV and its recombinant G protein (rGp) by ImmunoCAP system (CAP), a quasi-standard for allergen sIgE. And interferon-ɣ, IL-5, and IL13 were also measured by ELISA. Among asthmatics, CAP sIgE to rGP and IL-5 in sputa were higher in the RSV-detected subjects (by mPCR) ($p<0.05$, respectively). RSV-sIgE, Interferon-ɣ and IL13 failed to show any significance. In ROC analyses with the CAP measured asthmatics, the best coordinate cutoff of rGP-sIgE, predicting RSV detection by mPCR, was 0.285 kU/L (AUC 0.73, 95% CI 0.47-0.82; SE 81.3%, SP 65.4%, PPV 59.1%, NPV 85.0%). Conclusively, RSV detection was the most common, and conventional CAP could detect the sIgE to RSV and rGp sIgE among the exacerbated asthmatic sputa. These suggested that RSV protein sensitization and Th2 pathway might have roles in asthma exacerbations.

Acknowledgement

We thank the researchers in Institute of Allergy (Internal Medicine and Pediatrics), Yonsei University College of Medicine, Research Institute of Pharmaceutical Sciences, Ewha Womans University, and Seoul Medical Science Institute. This study was partially supported by a grant from the Korean Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A040153).

Keywords: respiratory virus, asthma, exacerbation, sputum, immunoglobulin E

S5-3

Antigenic and Phylogenetic Dynamics of Influenza A(H3N2) Viruses in Korea in 2011-2012

Jin Il Kim[1], Ilseob Lee[1], Kyung-Wook Hong[3], Sehee Park[1], Joon-Yong Bae[1], Min-Woong Hwang[1], Donghwan Kim[1], Seok-Il Jang[2], Hyejin Kim[1], Mee Sook Park[1], Ji Yun Noh[4], Joon Young Song[4], Jang-Hoon Choi[5], Joo-Yeon Lee[5], Bram Vrancken[6], Philippe Lemey[6], Kisoon Kim[5], Hee Jin Cheong[4], Man-Seong Park[1,*]

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Influenza virus causes recurrent seasonal epidemics. Each year almost half a million human lives are condemned to death from influenza worldwide. In theory, vaccination should protect people from influenza, but only 60-70% vaccinees may benefit from vaccine prophylaxis. From 2010 to 2014, influenza A(H3N2) viruses continuously circulated and peaked in the 2011-2012 season in Korea, which coincided with the highest influenza-like illness rate among patients during these periods. To address the antigenic and molecular dynamics of circulating H3N2 viruses, we investigated hemagglutination inhibition reactivity of vaccine-immunized patient sera against the viruses of the 2011-2012 influenza season that were isolated from clinical specimens of Korea University Medical Center (KUMC) Guro Hospital. The patient sera exhibited extremely reduced serological reactivity to the KUMC H3N2 viruses, compared with the A/Perth/16/2009-like vaccine viruses. Phylogenetic reconstruction revealed that all of the KUMC H3N2 hemagglutinin sequences constituted two Korean subclades (group II and III) in the A/Victoria/208/2009 clade, which clustered distantly from the Perth-like vaccine and Korea group I subtree. In the neuraminidase (NA) phylogeny, KUMC H3N2 NAs also constituted the Korea group II and III subclades, but they did not cluster among the Victoria-like clade. These results suggest the dynamic nature of antigenicity and molecular evolution of influenza A(H3N2) viruses in Korea and the need of consistent surveillance to provide sufficient information for the selection of right vaccine antigens.

Keywords: influenza, H3N2, antigenicity, phylogenetic dynamics, vaccine

S5-4

Deglycosylation at Influenza A Neuraminidase Stalk Confers Enhanced Pathogenicity in Mice

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To evaluate vaccine efficacy against influenza virus *in vivo*, it is essential to have challenge virus which is lethal to mice. However, most of human influenza viruses did not kill mice without prior adaptation. It was known that aggravation of viral pathogenicity in host can be achieved by repetitive adaptation. In this study, we found a neuraminidase (NA) deletion mutant of the 2009 pandemic H1N1 (pH1N1) virus, A/Korea/01/2009 (K/09) over serial rounds of mouse lung-to-lung passage of K/09 virus. By sequence analysis, we found that this mutant has the truncated NA with deletion of 8 amino acid residues in the stalk region. Interestingly, recombinant K/09 virus with this NA stalk deletion which was generated by reverse genetics killed mice without prior adaptation. In addition, we found that there was a potential N-linked glycosylation (NLG) site in NA stalk deletion amino acids. Thus, to determine whether this NLG site plays a major role in the virulence of mouse-adapted K/09 virus, recombinant K/09 virus with NLG amino acids deletion was generated. This recombinant virus with NLG amino acids deletion also conferred severe pathogenicity on mice. Taken together, we suggest that the deletion of 8 amino acids or potential NLG sites in the N1 NA stalk region may confer fatal pathogenicity on the pH1N1 virus. It should be further investigated to determine whether these virulence markers can be applied in other NA subtypes.

Keywords: influenza, neuraminidase, glycosylation, pathogenicity, pH1N1

S5-5

A Single Amino Acid Mutation in the PA Improves Viral Yield of Influenza A Candidate Vaccine Virus

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Sustained outbreaks of highly pathogenic avian influenza H5N1 viruses in worldwide have posed concerns about a possible avian influenza pandemic. To mitigate the burden caused by this virus, candidate vaccine viruses (CVVs) have been developed against various H5 hemagglutinin (HA) clades by plasmid-based reverse genetics. However, there are several issues such as insufficient vaccine yields and HA contents, unsatisfying immunogenicity and/or suboptimal protection rates in influenza CVVs. Therefore, it is very important to develop high-yielding CVV during pandemic situation. Here we report a genetic determinant for the high-yielding influenza A vaccine backbone. H5N1 CVVs were generated by plasmid-based reverse genetics using HA and NA genes of the A/Chicken/Korea/IS/2006 (HPAI H5N1, clade 2.2.1) virus and six internal genes of the A/Puerto Rico/8/34 (PR8) human vaccine donor. Interestingly, we found a H5N1 CVV with an enhanced growth property over multiple passages in eggs. By sequence analysis, the enhanced growth of the H5N1 CVV was attributed to a single amino acid mutation in PA protein. When inserted into other subtype CVVs, CVVs with PA mutation showed enhanced growth property in cultured cells. Substantial increase in viral growth was found to be driven by increased polymerase activity. Considered all, we suggest that one amino acid mutation in PA might be applied to develop the high-yielding influenza CVVs.

Keywords: influenza, H5N1, candidate vaccine virus, high-yielding, PA

S5-6

Control of Salmonella Infection by Modulating Hepcidin

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In response to microbial infection, expression of the defensin-like peptide hepcidin (encoded by Hamp) is induced in hepatocytes to decrease iron release from macrophages1. To elucidate the mechanism by which Salmonella enterica var. Typhimurium (*S. typhimurium*), an intramacrophage bacterium, alters host iron metabolism for its own survival, we examined the role of nuclear receptor family members belonging to the NR3B subfamily in mouse hepatocytes.

Here, we report that estrogen-related receptor γ (ERRγ, encoded by Esrrγ) modulates the intramacrophage proliferation of *S. typhimurium* by altering host iron homeostasis, and we demonstrate an antimicrobial effect of an ERRγ inverse agonist. Hepatic ERRγ expression was induced by *S. typhimurium*–stimulated interleukin-6 signaling, resulting in an induction of hepcidin and eventual hypoferremia in mice. Conversely, ablation of ERRγ mRNA expression in liver attenuated the *S. typhimurium*–mediated induction of hepcidin and normalized the hypoferremia caused by *S. typhimurium* infection. An inverse agonist of ERRγ ameliorated *S. typhimurium*–mediated hypoferremia through reduction of ERRγ–mediated hepcidin mRNA expression and exerted a potent antimicrobial effect on the *S. typhimurium* infection, thereby improving host survival. Taken together, these findings suggest an alternative approach to control multidrug-resistant intracellular bacteria by modulating host iron homeostasis.

Keywords: hepcidin, infection, *Salmonella*, iron homeostasis

S5-7

A Critical Role of Defective Viral Genomes Arising *In Vivo* for the Triggering of Innate Antiviral Immunity

Karla Tapia[1,3], Won-keun Kim[2,3], Yan Sun[1], Xiomara Mercado-López[1], Emily Dunay[1], Megan Wise[1], Michael Adu[1], and Carolina B. López[1]

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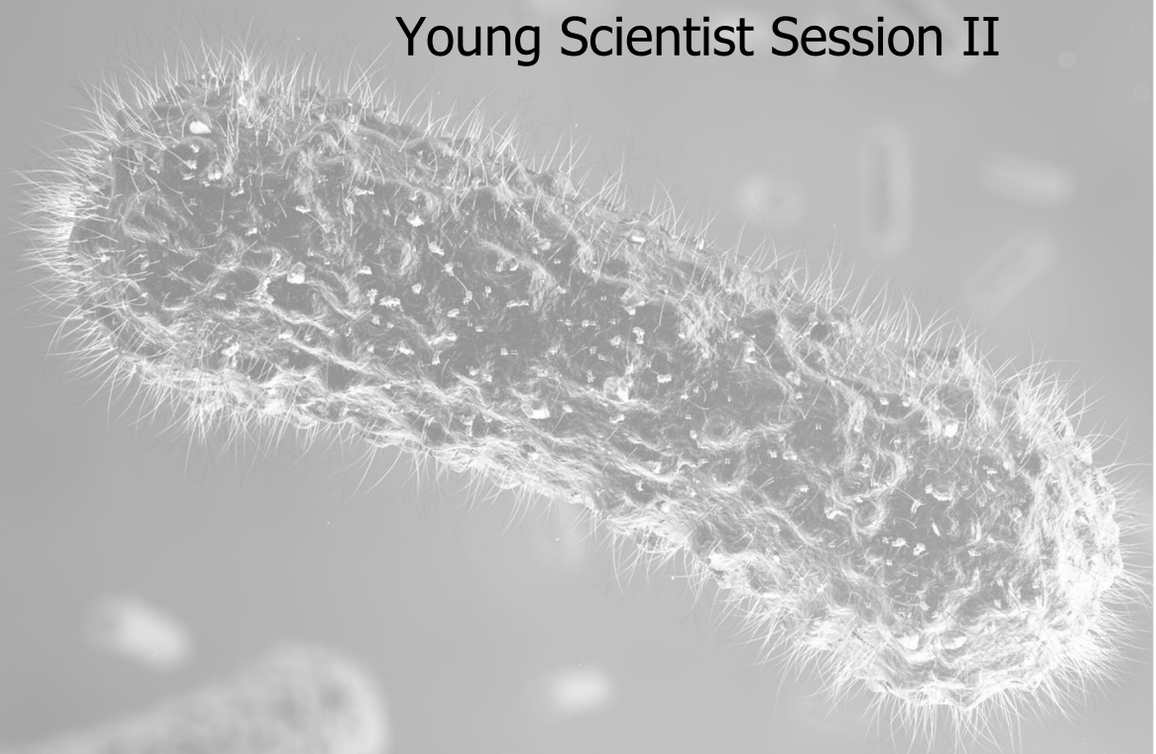
[2]Current address: Department of Microbiology, College of Medicine, Korea University,
[3]KT and WK contributed equally to this work.

The innate immune response to viruses is induced when Pattern Recognition Receptors (PRRs) recognize viral danger signals. However, most viruses produce proteins that antagonize and effectively delay signaling by the primary viral oligonucleotide sensor molecules PRRs, allowing the virus to replicate to high titers and produce large amounts of danger signals prior to host intervention. Currently, it is unclear how the host immune response overcomes viral evasion to initiate a protective antiviral response. Here we show that truncated forms of viral genomes that accumulate in infected cells potently trigger the sustained activation of the transcription factors IRF3 and NF-κB and the production of type I IFNs in a type I IFN independent manner. We demonstrate that these defective viral genomes (DVGs) are generated naturally during respiratory infections *in vivo* even in mice lacking the type I IFN receptor, and their appearance corresponds with the production of cytokines during infections with Sendai virus (SeV) or influenza A virus. Remarkably, the hallmark antiviral cytokine IFNβ is only expressed in lung epithelial cells containing DVGs, whereas cells within the lung that contain standard viral genomes alone do not express the cytokine. In conclusion, our data indicate that DVGs generated during viral replication are a primary source of danger signals for the initiation of innate antiviral immune response.

Keywords: respiratory virus, defective viral genome (DVG), danger signals, innate antiviral response

Symposium [S17]

Young Scientist Session II



2014 한국미생물학회연합 국제학술대회

INTERNATIONAL MEETING OF

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S17-1

Quorum Sensing for Biofilm Formation and Oil Degradation in *Acinetobacter oleivorans* DR

Jisun Kim and Woojun Park*

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Quorum sensing (QS) signals play an important role in biofilm formation and hexadecane biodegradation in *Acinetobacter oleivorans* DR. We constructed an *aqsR* mutant and performed RNA sequencing analysis to understand the QS system. Among up-regulated genes, both the AOLE_03905 (putative surface adhesion protein) and the AOLE_355 (L-asparaginase) genes have putative LuxR binding sites at their promoter regions. Electrophoretic mobility shift assays with purified AqsR revealed direct binding of AqsR to those promoter regions. AqsR functions as an important regulator and is associated with several phenotypes, such as hexadecane utilization, biofilm formation, and sensitivity to cumene hydroperoxide. Interestingly, QS-controlled phenotypes appeared to be inhibited by indole, and the *aqsR* mutant had the same phenotypes. We confirmed that the turnover rate of AqsR became more rapid without the QS signal and that indole could increase the expression of many protease and chaperone proteins. The addition of indole decreased the expression of two AqsR-targeted genes. The overexpression of AqsR was impossible with the indole treatment. [^{35}S]-methionine pulse labeling data demonstrated that the stability and folding of the AqsR protein decreased in the presence of indole without changing the *aqsR* mRNA expression. Here, we provided evidence for the first time showing that the indole effect on QS-controlled phenotypes is due to inhibited QS regulator folding and not a reduced QS signal.

S17-2

Genotyping of *Agaricus bisporus* Strains by PCR Fingerprints

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Agaricus bisporus, commonly known as the button mushroom, is the most widely cultivated species of edible fungi. Low frequency of recombination ratio and homokaryotic or monokaryotic spore on meiotic basidia form obstacles for breeding programs. Since the first hybrid varieties for white button mushrooms were released in Europe, new varieties released afterwards were either identical of very similar to these first hybrids on morphologies. Therefore, different DNA markers have been used to define unique varieties of *A. bisporus* strains. Aim of this study is to assess the genetic diversity of different *A. bisporus* strains in Korea. Twelve UFP (Universal fungal primer, JK BioTech. Ltd), 2 simple sequence repeat (ISSR) and 30 SSR primers were used to assess genetic diversity of monokaryotic and dikaryotic *Agaricus bisporus* strains including other 9 *Agaricus* spp. Of them, four UFP, four SSR primers, $(GA)_8T$, $(AG)_8YC$, $(GA)_8C$ and $(CTC)_6$ and seven SSR markers produced PCR polymorphic bands between the *Agaricus* species or within *A. bisporus* strains. PCR polymorphic bands were inputted for UPGMA cluster analysis. Forty five strains of *A. bisporus* are genetically clustered into 6 groups, showing coefficient similarity from 0.75 to 0.9 among them. In addition, genetic variations of monokaryotic and dikaryotic *Agaricus bisporus* strains were partially detected by PCR technologies of this study. The varieties, Saea, saedo, Saejeong and Saeyeon that have recently been developed in Korea were involved in the same group with closely genetic relationship of coefficient similarity over 0.96, whereas, other strains were genetically related to *A. bisporus* strains that were introduced from USA, Eroupe and Chinese.

Acknowledgement: This study was supported by Golden Seed Project (23-003-04--SBJ0). Kyong-Jin Min was supported by a scholarship from the BK2 Plus Program (3Z203002928), the Ministry of Education, Science and Technology, Korea.

Keywords: *Agaricus bisporus* strains, genotyping, PCR polymophism

S17-3

Analysis of Mating System in *Lentinula edodes* and Development of Mating Type-specific Markers

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Division of Applied Life Science, Gyeongsang National University

Mating of tetrapolar mushrooms is regulated by to chromosomal loci, A and B. A locus contains A gene that expresses a homeodomain protein whereas B locus contains multiple pheromones and receptor genes. In order to characterize the mating loci in Korean cultivated strains of *Lentinula edodes*, one hundred monokaryotic myclelia were isolated from the basidiospores of cultivated strains, including Cham-A-Ram, Sanjo70, and Sanjo707. Both mating loci were amplified using primer sets targeting conserved sequence regions for homeodomain (HD), pheromone, and receptor genes. Subsequent sequence analysis revealed that the Korean strains contained significant variations in the homeodomain of A locus, even within the same A or A2 mating type. Similarly, B locus was also highly diversified in the sequences of pheromones and receptors as well as gene organization. These results enabled us to design mating type-specific probes which can distinguish mating type of each strain. The specificity was confirmed by between intra- and inter-strain mating experiment.

Keywords: *Lentinula edodes*, mating gene, tetrapolar

S17-4

Invesigation of Functional Roles of A Protein Kinase in A Fungal Plant Pathogen, *Magnaporthe oryzae*

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The rice blast disease caused by of *Magnaporthe oryzae* is one of the most destructive diseases of rice. By the microarray analysis, we profiled expression changes of genes during conidiation and found out many putative genes that are up-regulated. Among those, we first selected MGG_06399 encoding a dual-specificity tyrosine-regulated protein kinase (DYRK), homologous to YAK in yeast. To investigate functional roles of MoYAK, We made *ΔMoyak* mutants by homology dependent gene replacement. The deletion mutant showed a remarkable reduction in conidiation and produced abnormally shaped conidia smaller than those of wild type. The conidia form *ΔMoyak* were able to develop a germ tube, but failed to form apppressoria on a hydrophobic coverslip. The *ΔMoyak* formed appressria on a hydrophobic cover slip when exogenous cAMP was induced, but the appressoria shape was abnormal. The *ΔMoyak* also formed appressoria abberent in shape on onion epidermis and rice sheaths and failed to penetrate the surface of the plants. These data indicate that MoYAK is associated with cAMP/PKA pathway and important for conidiation, appressorial formation and pathogenic development in *Magnaporthe oryzae*. Detailed characterization of MoYAK will be presented.

Keywords: *Magnaporthe oryzae*, conidiation, rice blast, kinase, pathogenicity

S17-5

Heterologous Expression of Der Homologues in *Escherichia coli der* Mutant and Their Functional Complementation

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Department of Integrative Biology, Pusan National University

A unique *E. coli* GTPase Der has tandemly repeated GTP-binding domains, and its essential role has been implicated in 50S subunit biogenesis. The depletion of Der accumulates 50S ribosomal subunits that are structurally unstable at lower Mg^{2+} concentration. Its homologues are ubiquitously found in eubacteria but not in archaea and eukaryotes. In this study, in order to verify their conserved role in bacterial 50S ribosome biogenesis, we cloned Der homologues from two γ-proteobacterium *Klebsiella pneumoniae* and *Salmonella typhimurium*, two pathogenic bacteria *Staphylococcus aureus* and *Neisseria gonorrhoeae* and two extremophiles *Deinococcus radiodurans* and *Thermotoga maritima*. We examined if they can functionally complement *E. coli der* null phenotype. Only *K. pneumoniae* and *S. typhimurium* Der proteins enabled *E. coli der*-deletion strains to grow under non-permissive condition, and growth rate of heterogeneously complemented *E. coli* cells was correlated with the cellular concentration of Der protein. Sucrose density gradient experiments revealed that expression of *K. pneumoniae* and *S. typhimurium* Der proteins rescued the instability of 50S ribosomal subunits which was caused either by *E. coli* Der-depletion or by *rrmJ*-deletion. Here, we propose that Der proteins of γ-proteobacterium associate with 50S ribosome subunits and play a crucial role in 50S ribosomal subunit assembly in a nucleotide dependent manner in *E. coli*.

Key words : Der, 50S ribosome, GTPase, complementation

S17-6

A Small Nucleotide Regulator (p)ppGpp Directs Metabolic Fate of Glucose in *Vibrio cholerae*

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[b] *Institute for Immunology and Immunological Diseases, Yonsei University College of Medicine*

Bacterial glucose metabolic switch from mixed organic acid fermentation to acetoin fermentation has an important role to prevent lethal acidification of their environment. However, the regulation of metabolic switch remains undefined. Here, we show that a small nucleotide regulator (p)ppGpp, the alamone of bacterial stringent response, plays a critical role on glucose metabolic change and this process is essential for maintenance of viability in *Vibrio cholerae*, the causative agent of cholera. When *V. cholerae* grown with added glucose, (p)ppGpp deficient strains (Δ*relA* Δ*relV* Δ*spoT* ; (p)ppGpp^0) generated a sharp decrease in pH of media, which resulted in loss of viability. However, the 7th pandemic strain N696 and (p)ppGpp over-producer strain (Δ*relA* Δ*spoT)* were resulted robust growth with no decrease in pH of media. To understand the (p)ppGpp-dependent glucose metabolic changes behind glucose-induced media pH drop, N696 strain and (p)ppGpp^0 strain were analyzed by RNA-sequencing analysis. The data revealed a set of genes, which involved in the biosynthesis of acetoin, neutral fermentation end products, was strongly repressed in the (p)ppGpp^0 strain.

We performed a transposon mutagenesis using the (p)ppGpp over-producer strain (Δ*relA* Δ*spoT)* to select mutants, which decrease media pH and bacterial viability during the growth with added glucose. Interestingly, we identified two tranposon insertions in promoter region of *VC589* (*alsD*) and in *VC590* (*alsS*), both genes included in the operon for biosynthesis of acetoin. Results suggest that (p)ppGpp may inhibit drop the pH of culture media through glucose metabolic change to acetoin fermentation. The ability of acetoin biosysnthesis was significantly decreased in (p)ppGpp^0 strain. And, decreased the promoter activity of *alsD* gene, the first gene of the acetoin biosynthesis operon, was observed in (p)ppGpp^0 strain. Together, results reveal that (p)ppGpp regulates glucose metabolic change to acetoin fermentation, and this process is essential to viability of *V. cholerae*.

keywords : *Vibrio cholerae*, stringent response, acetoin fermentation

S17-7

Isolation, Identification and Physiological Functionality of Yeasts from Wild Flowers in Islands and Mountains of Korea

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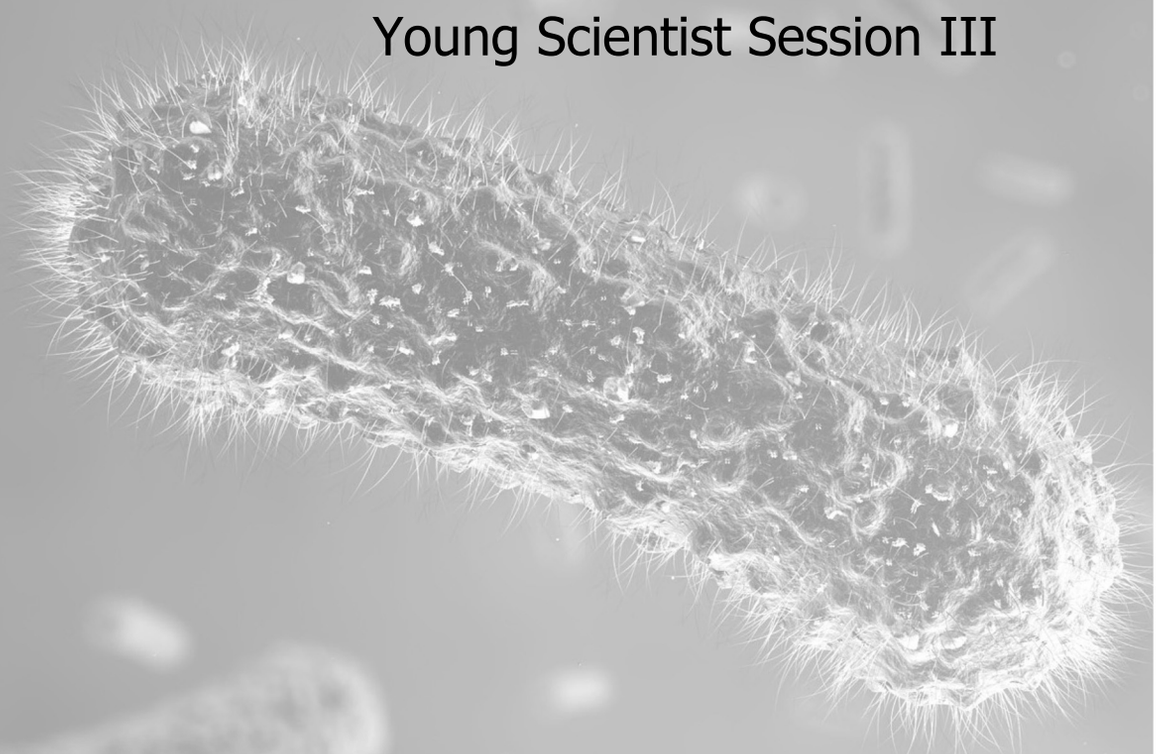
Department of Biomedicinal Science and Bioechnology Paichai University

Yeasts were isolated from wild flowers of some islands and mountains such as Jeju-do, Ulleungdo, Yokjido, Seonyudo and Gyejoksan, Oseosan, Beakamsan and Deogyusan in Korea and were identified by comparison of nucleotide sequences for PCR-amplified D/D2 region of 26S rDNA or internal transcribed pacer(ITS) and 2 including 5.8S rDNA using BLAST. Seventy two yeast strains of two hundred eighty nine species were isolated from wild flowers in islands and mountains, Korea. Among them, *Cryptococcus* species were isolated the most dominantly, and *Metschnikowia reukaufii* were also isolated thirty species, 0.3% of total strains. Twenty-three species including *Cryptococcus aureus* were overlapped between yeast strains of the islands and mountains. Some physiological functionality of the culture broth and cell-free extracts from two hundred eighty nine yeast strains were determined. The supernatant of *Candida* sp. 78-J-2 showed antioxidant activity of 22.5%, and supernatant of *Metschnilowia reukaufii* SY44-6 showed anti-gout xanthine oxidase inhibitory activity of 49.6% and whitening tyrosinase inhibitory activity of 38.4%, respectively.

keywords: islands and mountains, isolation and identification, physiological functionality, wild flowers, yeasts.

Symposium [S19]

Young Scientist Session III



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S19-1

FVB *Gulo*$^{-/-}$ Congenic Mice as An Animal Model for *H. pylori* Infection

Jong-Hun Ha, Jin-Sik Park, Neul-Bit Ha, Jae-Young Song, Kon-Ho Lee, Hyung-Lyun Kang, Seung-Chul Baik, Myung-Je Cho, Kwang-Ho Rhee and Woo-Kon Lee*

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Low dietary ascorbic acid has been proposed to negatively influence the clinical outcome of *Helicobacter pylori* infection in human studies. It was reported that ascorbic acid supplementation does not protect L-gulono-c-lactone oxidase-deficient (*gulo*$^{-/-}$) C57BL/6 mice from *H. pylori*-induced gastritis and gastric premalignancy. However, in our previous study, FVB mice were more susceptible to *H. pylori* infection than C57BL/6. In order to improve the experimental animal model to analyze whether dietary ascorbic acid would influence the outcome of *H. pylori* infection, we generated a FVB *gulo*$^{-/-}$ by backcross breeding of C57BL/6 *gulo*$^{-/-}$ and FVB wild type (*gulo*$^{+/+}$) in this study. We compared gastric colonization levels of *H. pylori* in *H. pylori*-infected *gulo*$^{-/-}$ mice supplemented with low (330mg/L) or high (3,300mg/L) ascorbic acid in drinking water for 6 or 32 weeks. This mouse model would be a useful tool for understanding of pathophysiological roles of *H. pylori* in the development of gastric disorders.

Keywords: *Helicobacter pylori*, ascorbic acid, L-gulono-c-lactone oxidase, gastric colonization level

S19-2

Mouse Susceptibility to *Vibrio cholerae* Infection is Influenced by Altered Composition of Gut Microbiota

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Department of Microbiology and Immunology, [2]Brain Korea 2 Project for Medical Sciences, Yonsei University College of Medicine

Mammalian intestine is inhabited by trillions of commensal microbes, collectively termed gut microbiota. Gut microbiota prevent pathogen infection through colonization resistance and by promoting the development of mucosal immune system. Molecular basis of such an antagonistic effect, however, remains largely unknown due to the lack of an appropriate model system. Here, we examined the effect of altered microbiota composition on mouse susceptibility to the infection by *Vibrio cholerae*, a well-known human enteric pathogen. When treated with mild concentration of streptomycin (Sm), microbes belonging to the Enterobacteriaceae family, later found to be a clonal expansion of an *Escherichia coli* variant (ECV), were abundantly recovered from the mouse intestine. Unlike the untreated control group, the Sm-treated mice became susceptible to *V. cholerae* colonization with clear manifestation of a cholera-like symptom. Likewise, *V. cholerae* infection occurred more readily in neonatal mice transplanted with the ECV strain, but not in those transplanted with a typical *E. coli* strain. The whole genome sequence of ECV revealed a wide range of atypical properties especially in carbohydrate metabolism and experiments are currently under way to understand its interaction with *V. cholerae* under host gut environment. Our results put a renewed emphasis on the role of gut commensals in regulating the extent of intestinal infections.

Keywords: gut microbiota, *Vibrio cholerae*, *Escherichia coli*, alteration, transplantation

S19-3

Mycobacterium avium MAV2054 Protein Induces Macrophage Apoptosis through Targeting to Mitochondria and Enhances Intracellular Survival of the Bacteria

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M. avium complex (MAC) and their sonic extracts induce a macrophage apoptosis. However, any components of MAC that are involved in inhibiting or triggering apoptosis are not identified. Recently we identified the immunodominant MAV2054 protein with strong antibody reactivity in MAC pulmonary disease as well as patients with tuberculosis through fractionation of *M. avium* culture filtrate protein using multistep chromatography. In this study, we investigated the biological effects of MAV2054 on murine macrophages. MAV2054 protein induced significant macrophage apoptosis via activation of caspase 3 and caspase 9, and poly (ADP-ribose) polymerase cleavage. Enhanced ROS production and JNK activation were essential of MAV2054-medicated apoptosis and induced IL-6, TNF, and MCP- production. Interestingly, MAV2054 was targeted to the mitochondrial compartment of macrophages treated with MAV-2054 and infected with *M. smegmatis* expressing MAV2054. Dissipation of the mitochondrial transmembrane potential ($\Delta\Psi_m$) and depletion of cytochrome *c*, also occurred in MAV2054-treated macrophages. A significantly increased apoptotic cells, more significant ROS production and $\Delta\Psi_m$ collapse were observed in BMDMs infected with M. *smegmatis* expressing MAV 2054 compared to *M. smegmatis* control. Furthermore, intracellular growth of *M. smegmatis* expressing MAV2054 within macrophages significantly increased, but that of *M. smegmatis* control was inhibited. Taken together, our data suggest that MAV2054 may act as a strong pathogenic factor to cause apoptosis of macrophages infected with *M. avium*.

Keywords :*Mycobacterium avium* complex, reactive oxygen species (ROS), apoptosis, mitochondria, MAV2054

S19-4

Relationship of Genotypes of *Helicobacter pylori* Korean Isolates and Clinical Entities of Gastric Diseases

Dong-Hae Lee, Jin-Sik Park, Jae-Young Song, Hee-Shang Yoon*, Kon-Ho Lee, Seung Chul Baik, Myung-Je Cho, Hyung-Lyun Kang, Kwang-Ho Rhee, Woo-Kon Lee

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H. pylori is a gram negative, spiral-shaped, and microaerophilic organism. The organisms are found to be close to the mucosal surface and in intercellular positions or caught up in the surface mucus of the stomach. H. pylori is the causative agent of acute gastritis, chronic gastritis, and peptic ulcer as well as the signification risk factor of gastric cancer. Although most Korean people become carriers of H. pylori from early childhood, only minor parts of infected persons progress to the serious symptoms. Genotyping of H. pylori isolates classified with clinical symptoms are helpful to analyze what bacterial factors contribute to the occurrence of serious gastric symptoms like gastric cancer. Here, 131 Korean isolates recovered from patients with chronic gastritis, peptic ulcer, and gastric cancer were subjected to PCR genotyping of virulence and adherence factors like CagA, VacA, AlpA, AlpB, OipA, BabA R1, BabA R2, HopZ, and SabA and vacuolating toxicity. As a result, AlpA, BabA R2, HopZ, and SabA contribute more significantly to the development of severe symptoms than chronic gastritis. Among 5 types of CagA 3'-region identified in 131 isolates, 2 types (type 4 and 5) were found in the gastric cancer. These results reveal that genotyping of *H. pylori* might be helpful to identify virulent strains associated with severe gastric disorders.

keywords : Genotyping, *Helicobacter pylori,* Gastric diseases, PCR

S19-5

Clinical Evaluation of Immunemed *Leptospira* Rapid Kit

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ImmuneMed, [2] Institute of Medical Science, Hallym University, [3]Department of Laboratory Medicine, Hallym University, [4]Department of Microbiology, College of Medicine, Hallym University

Leptospirosis is a disease of humans and animals caused by infection with the motile spirochetal bacterium of the genus, *Leptospira*. Leptospirosis is a common in world, especially in developing countries. Pathogenic leptospire, *Leptospira interrogans* lives in the proximal renal tubules of the kidneys of infected animals, and they are excreted from the kidneys intermittently or regularly in urine and contaminate soil, water, streams and rivers. In humans, many cases are mild or asymptomatic, and go unrecognized. In some patients, however, the illness may progress to kidney or liver failure, aseptic meningitis, life-threatening pulmonary hemorrhage and other symptoms. Without early treatment, the disease may be severe and often goes to death.

Therefore, early and accurate diagnosis is very important for the treatment of patient. Until now, the gold standard method of leptospirosis diagnosis is microscopic agglutination test (MAT), detecting antibody serologically against the *Leptospira* in a clinical specimen. To perform this MAT, all of the endemic or epidemic serovars of *Leptospira interrogans* should always be cultured and these serovars are mixed with patient specimens for diagnosis and examined under dark field microscopy. It is common that unskilled personnel misinterprets the result as false positive or false negative.

ImmuneMed Inc. developed a rapid and accurate diagnostics for leptospirosis to overcome these difficulties. This kit is based on lateral flow immunochromatographic assay and it takes only 5 minutes to interpret the result. The performance of rapid diagnostic test (RDT) using the genus specific antigen as a diagnostic antigen was evaluated in Korea, Malaysia, Bulgaria and Argentina, respectively. The sensitivity was 93.9, 87.7, 00 and 8.0% and the specificity was 97.9, 6.6, 00 and 95.4% in Korea (epidemic region with 2 serovars), Malaysis (endemic region with 20 serovars), Bulgaria (epidemic region with 3 serovars) and Argentina (endemic region with 9 serovars) respectively. Judging from these results, the performance of ImmuneMed Leptospira Rapid Kit shows excellency of rapid kit.

According to WHO report, it is known that over 250 serovars of *Leptospira interrogans* are widely distributed worldwide. In terms of using PS not LPS as antigen for diagnosis, it is analyzed that this antigen is a genus specific because it shows a high sensitivity against many serovars. However, we presumed that the reason of comparative low specificity in Malaysia is due to geographically endemic region of leptospirosis. For this reason, many Malaysian healthy people also have high antibody titer against leptospira.To diagnose leptospirosis correctly, we need to optimize this kit by raising the cut-off titer for diagnosis in endemic countries. In addition, we plan to expand clinical evaluation for the validation of this kit in other countries for the global usage in the near future.

Keywords: *Leptospira*, rapid kit, clinical evaluation

S19-6

Production of Reference Standard for Determining the Cut-off of the Scrub Typhus Rapid Kit

Min-Woo Kim, Jin-Woo Lee[2], Young-Jin Kim[2,3], Sungman Park[2,3], Seung-Han Kim[2], Yoon-Won Kim[3]

Department of Microbiology, College of Medicine, Hallym University, [2] ImmuneMed, [3]Medical Science Institute, Hallym University

Scrub Typhus is an endemic disease to occur in a wide range of Asia from Pakistan, through Japan to North Australia This disease is legally assigned to Group 3 infectious diseases in Korea. Because of the similarity of symptoms such as leptospira, dengue, hemorrhagic fever with cheek spelling syndrome symptoms, it is not easy to discriminate the disease. If the proper diagnosis is not carried, the disease become serious stage and goes to death. The mortality rate is 30% of patients. The most common diagnostic methods examine bite spot called a eschar, which is found only in the half of the patient. Thus, the rest patients missed the timing of the appropriate treatment of received inappropriate treatment due to misdiagnosed as other acute febrile illness. There are several diagnosis method to scrub typhus. First, Weil-Felix is easy method but low sensitivity. Second, ELISA what is need to know the serotype, has a high but limited sensitivity and specificity. IFA recommended WHO, shows sensitivity and specificity in the diagnosis. However, the performance of IFA needs skilled person and fluorescence microscopy equipment, so it is not widely used.

We developed a rapid test kit using lateral flow Immunochromatograpy. This kit can be diagnosed both IgM and IgG, and should the excellent sensitivity and specificity. Every person infected with the different scrub typhus serotype depending on geographical region, the difference is the amount of antibody or specificity. High titer in IFA or positive samples, which were selected from the Rapid kit, were prepared for reference standard.

To separate for IgM and IgG, depletion was performed and diluted with PBS and determined the limitation of minimum detection. As compare with ELISA, we determined the limitation of maximum detection of this kit using different and diluted sample. Negative sample concentrations were measured by ELISA. IgM detection strip is 7 mg/dL and IgG detection strip is 37 mg/dL. To determine Cut-off value of kit for weak positive, concentrations were measured by ELISA. IgM detection strip is 43 mg±55 mg/dL and IgG detection strip is 40 mg±50 mg/dL.

S19-7

Genipin as a Novel Chemical Activator of the Gammaherpesvirus Lytic Cycle

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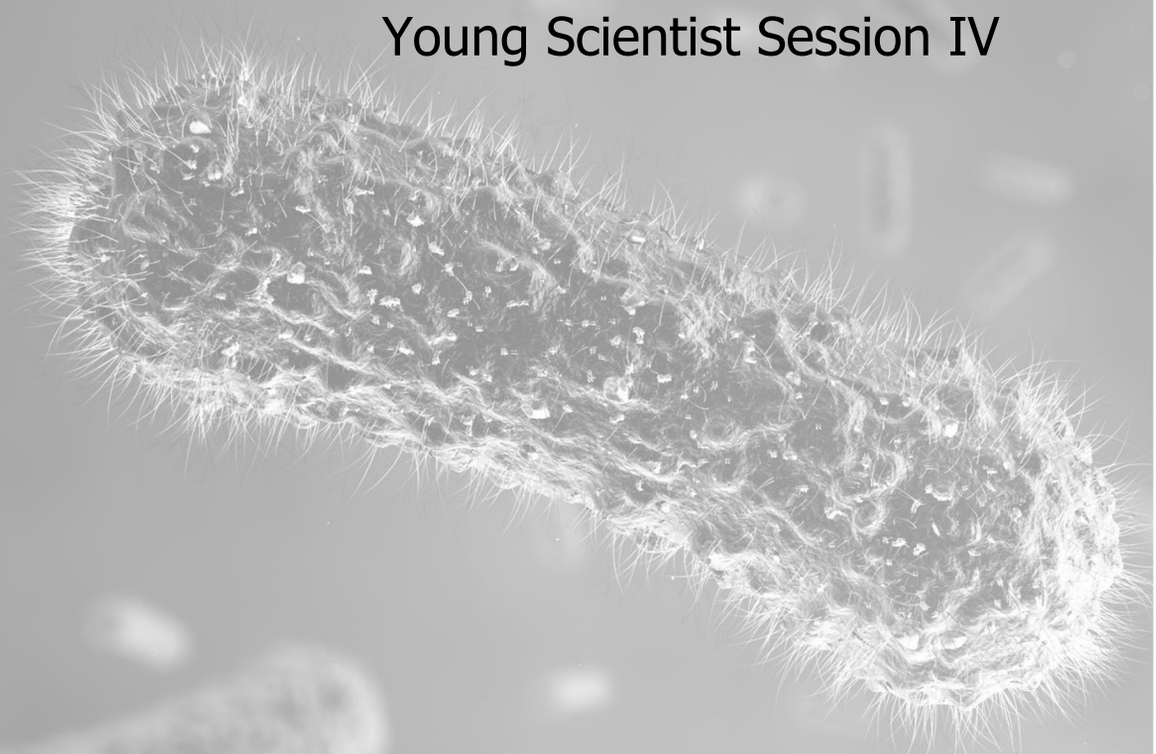
Gammaherpesviruses include Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV). EBV and KSHV cause several human cancers, including Burkitt's lymphoma, nasopharyngeal and gastric carcinoma, and Kaposi's sarcoma (KS), which is an AIDS-related form of non-Hodgkin lymphoma. Antiviral agents can be categorized as virucides, antiviral chemotherapeutic agents, and immunomodulators. Most antiviral agents affect actively replicating viruses, but not their latent forms. Novel antiviral agents must be active on both the replicating and the latent forms of the virus. *Gardenia jasminoides* is an evergreen flowering plant belonging to the *Rubiaceae* family. This plant originates in Asia and is most commonly found growing wild in Vietnam, Southern China, Taiwan, Japan, Myanmar, and India. Genipin is an aglycone derived from an iridoid glycoside called geniposide, which is present in large quantities in the fruit of *G. jasminoides*. In this study, genipin was evaluated for its role as an antiviral agent that impacts on gammaherpesviral latent and lytic replication. In host cells latently infected with gammaherpesvirus, genipin caused significant cytotoxicity (70–72.5 μM), arrested cell-cycle progress (S, G2/M phases), upregulated gammaherpesvirus gene expression (latent and lytic genes), stimulated viral progeny production, activated viral promoter for lytic gene expression (EBV Fp promoter), and suppressed viral infection. These results suggested that genipin demonstrates strong antiviral activities during the life cycle of gammaherpesvirus by stimulating the viral lytic replication cycle.

keyword: genipin, Gammaherpesvirus, lytic activation, antiviral agent, methylation

Symposium [S23]

Young Scientist Session IV



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S23-1

Ecophysiological and Genomic Characterization of Methylotrophic Bacteria Belonging to the LD28 clade from Lake Soyang

Mihye Im, Ilnam Kang, and Jang-Cheon Cho*

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Methylotrophs in freshwater can play a substantial role in the global carbon cycle although they only exist regionally. Many members of the methylotrophic bacteria have been isolated from aquatic environments, however major freshwater methylotrophic clade such as the LD28 clade do not have any cultured representatives and its marine counterpart clade, the OM43 clade, has only small number of isolates such as HTCC28. The LD28 clade of the family *Methylophilaceae* is a betIV subclass of *betaproteobacteria* widely found in freshwater environments. Here we report cultivation and genome sequencing of strain IMCC9250, chosen as a representative strain among 59 bacterial strains belonging to the LD28 clade, cultured from Lake Soyang using High-Throughput cultivation.

We found that the strain IMCC9250 is an obligate methylotroph with C metabolic pathways showing cellular growth depending on methanol, yielding 24.2μM. Optimum growth was observed at 30°C pH7, and in the presence of 0.% of NaCl. The genome of this strain contains a circular chromosome of ,30,53 bp and the overall G+C content of the chromosome is 36.63%. It includes genes for rhodopsins, but no difference in growth was observed under light and dark conditions. Through comparative genomic analysis, we could find the clue that IMCC9250 and OM43 strains originated from the same ancestor.

Keyword : methylotroph, freshwater, Lake Soyang, LD28, OM43

S23-2

Loss of Microbial Diversity Resulted in Enhanced Diesel-Bioremediation at A Cost of Trade-Off in Ecological Functions

Jaejoon Jung and Woojun Park*

Lab of Molecular Environmental Microbiology, Department of Environmental Science and Ecological Engineering, Korea University

In ecology, the relationship between microbial diversity and ecosystem function has been a unanswered question. To address this issue, soil microbial diversity was manipulated using a dilution approach in soil slurry microcosm with or without diesel-contamination along with biostimulating agent (red clay) to determine the functional consequences following the loss of microbial diversity. Dilution to $/0^2$ and $/0^5$ did not affected to the abundance of alkane degrader after 6 weeks of incubation as shown in the copy number of *alkB* encoding for alkane monooxygenase. Interestingly, GC-MS analysis indicated that diesel-biodegradation was enhanced when the microbial diversity was decreased. Community analysis showed that order *Pseudomonadales* and *Actinomycetales* was predominant in $/0^2$ and $/0^5$ dilution factors, respectively, while diesel and red clay enriched the relative abundance of *Burkholderiales* and *Caulobacterales* in $/0^2$ diluted sample, respectively. Metagenomic analysis using determined a significant difference in the 09 out of 9 KEGG pathways such as nitrogen and sulfur metabolism, biosynthesis of secondary metabolites, and xenobiotics biodegradation. These results suggested that loss of microbial diversity might maintain functional abundance whereas the actual functionality could be affected with functional trade-off of other ecosystem functions. We will discuss functional trade-off in terms of soil enzyme activity and resilience in a further environmental stress.

S23-3

Physiology and Genomic Characteristics of Strain IMCC3023, A Marine Actinobacterium Isolated from Arctic Seawater, Encoding Actinorhodopsin Gene

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It has been known that a part of marine actinobacterial populations are closely related to several freshwater lineages of actinobacteria. However, studies on those marine actinobacteria have been hampered by the lack of relevant isolates. Here we report on the polyphasic taxonomy and genome characteristics of IMCC3023 that was isolated from a coastal Arctic seawater sample collected off during glacier melting season, but was closely related to '*Candidatus* Aquiluna rubra', a freshwater actinobacterial clade. IMCC3023 was curved rod shape, strictly aerobic bacterium and contained red-orange carotenoid pigment. Optimal growth of strain IMCC3023 was observed at 5°C, pH7-8, and in the presence of % NaCl. The major fatty acid (>0%) were anteiso-$C_{5:0}$ (24.2%), iso-$C_{5:0}$(9.4%), $C_{6:0}$(3.0%), iso-$C_{6:0}$(.8%) and IMCC 3023 contained phosphatidylglycerol, diphosphatidylglycerol, glycolipid. Illumina sequencing followed by combinatorial PCR resulted in the complete genome sequence of .36Mb, the smallest genome size ever reported among free-living actinobacterial. A rhodopsin gene found in the genome was affiliated with actinorhodopsin, Functional analysis of the actinorhodopsin indicates light-driven H^+ pump like proteorhodopsin. Maximum cell densities under light condition were higher than those under dark condition. And, color of liquid media changed to pink in light condition, putatively due to the accumulation of carotenoids.

Keyword : Actinobacteria, Aquiluna, Actinorhodopsin, proton-pumping, Arctic seawater

S23-4

Diversity of Fungi from Dokdo Island Soil, Korea and Their Antimicrobial and Hydrolytic Enzyme Activity

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Dokdo island is located in the northeastern part of Ulleungdo, known as volcanic island. In total, 53 fungal isolates were isolated from Dokdo island soil sample, using dilution plate technique. The isolates were identified on the basis of morphological characteristics and rDNA ITS sequence analysis. Out of them, 4 isolates were identified at the level of species. The dominant fungal species and genera included *Fusarium* spp., *Mucor* sp., *Clonostachys* spp., and *Trichoderma* sp. The % sequence identity (the number of matches/the complete alignment length) values via NCBI BLAST searching of EML-IF9, EML-MF30- and EML-DDSF4 represented 97.9% (485/499) with *Clonostachys* cf. *rosea* (GenBank accession no. KC3307), 98.33% (472/480) with *Metarhizium guizhouense* (GenBank accession no. HM055445), and 00% (350/350) with *Mortierella oligospora* (GenBank accession no. JX976032), respectively. Three species of *C. rosea, M. guizhouense* and *M. oligospora* represented new records of fungi from Dokdo island, Korea. The antimicrobial activities of the fungal strains varied with tested. Two isolates (EML-MFS30- and EML-IF9) showed antifungal activity against several fungi including *Fusarium oxysporum* and *Rhizotonia solani. Clonostachys rosea* (EML-IF9) showed strong hydrolytic enzyme activity. Our results showed that the antagonistic fungi including *Clonostachys rosea* will be used as potential biocontrol agents for control of fungal diseases.

Keywords: fungal diversity, new record, Dokdo island, antimicrobial, hydrolytic enzyme activity.

S23-5

Intraspecific Functional Variation of Arbuscular Mycorrhizal Fungi Originated from Single Population on Plant Growth

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Arbuscular Mycorrhizal Fungi (AMF) is widespread symbiont forming mutualistic relationship with plant root in terrestrial forest in ecosystem. They provide improved absorption of nutrient and water, and enhance the resistance against plant pathogen or polluted soil, therefore AM fungi are important for survival and maintaining of individual or community of plant. For last decade, many studies about the functional variation of AM fungi on host plant growth response were showed that different geographic isolates, even same species, have different effect on host plant. However, little was known about functional variation of AM fungal isolates originated single population, which provide important insight about intraspecific diversity of AMF and their role in forest ecosystem.

In this study, four AM fungal isolates of *Rhizophagus clarus* were cultured *in vitro* using transformed carrot (*Daucus carota*) root and they showed the difference between isolates in ontogenic characteristics such as spore density and hyphal length. The plant growth response by mycorrhizas were measured also. After 20 weeks from inoculation of these isolates to host plants, dry weight, Root:Shoot ratio, colonization rates and N, P concentration of host plant showed host plant was affected differently by AM fungal isolates. This results suggest that AM fungi have high diversity in their functionality in intraspecific level, even in same population.

S23-6

Isolation and Characterization of Fungal Diversity from Crop Field Soils of Nigeria

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In order to find indigenous beneficial fungal species from crop field soils of Nigeria, 23 soil samples were collected from various places of Nigeria in June, 203 and fungi were isolated through serial dilution technique. Isolated fungi were purified and differentiated according to their morphological and microscopic characteristics. In total, 38 different representative isolates were recovered and the genomic DNA of each isolates was extracted using QIAGEN® Plasmid Mini Kit (QIAGEN Sciences, USA) and the identification of fungi was carried out by sequence analysis of internal transcribed spacer (ITS) region of the 8S ribosomal DNA (8S rDNA). Recovered isolates belonged to 9 fungal genera comprising *Fusarium*, *Aspergillus*, *Chaetomium*, *Coniothyrium*, *Dipodascaceae*, *Myrothecium*, *Neosartorya*, *Penicillium and Trichoderma*. *Aspergillus* spp., *Penicillium* spp. and *Trichoderma* spp. were the most dominant taxa in this study. The antagonistic potentiality of species belonged to *Trichoderma* against 0 phytopathogenic fungi (*F. oxysporum*, *C. gloesporoides*, *P. cytrophthora*, *A. alternata*, *A. solani*, *S. rolfsii*, *F. solani*, *R. solani*, *S. sclerotiorum and P. nicotiana*) was assessed *in vitro* using dual culture assay. The dual culture assay results showed varied degree of antagonism against the tested phytopathogens. The potential *Trichoderma* spp. will be further evaluated for their antagonistic and plant growth promotion potentiality under *in vivo* conditions.

Keywords: *Aspergillus* spp., fungal diversity, *Penicillium* spp., plant growth promotion, *Trichoderma* spp.

S23-7

Analysis of Fungal Communities on Ulleungdo and Dokdo Islands

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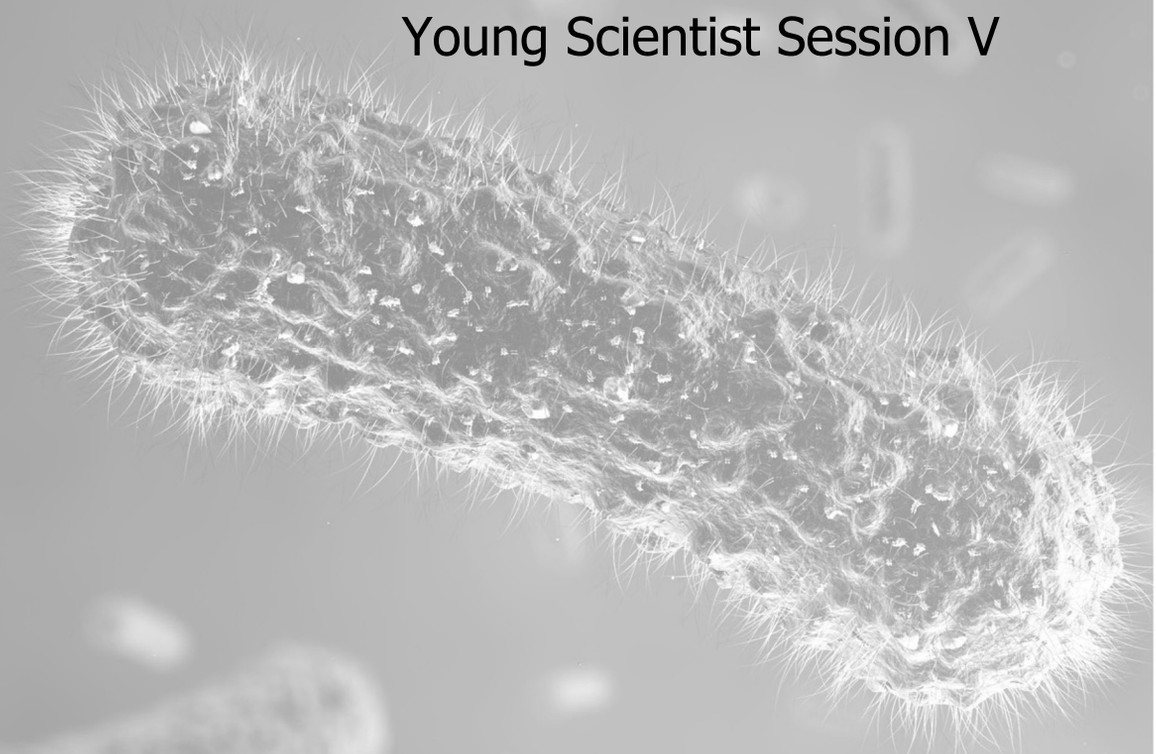
In this study, we used pyrosequencing method to analysis of soil fungal communities on the Ulleungdo and Dokdo islands. 768 operational taxonomic units (OTUs) were analyzed from the Ulleungdo sample and 640 OTUs and 382 OTUs were analyzed from the Dongdo and Seodo samples, respectively. Compared to the species richness of Ulleungdo and the Dokdo sample, the Ulleungdo sample was higher than in the Dongdo and Seodo samples. Species diversity was much the same. The phylum Basidiomycota was dominant in the Ulleungdo sample, while the phylum Ascomycota was dominant in the Dongdo sample.

Keyword: fungal community, Dokdo, Ulleungdo, pyrosequencing

Symposium [S27]

Young Scientist Session V



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S27-1

Heterologous Expression of A New Manganese-Dependent Peroxidase Gene from *Peniophora incarnata* KUC8836 *in Saccharomyces cerevisiae*

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The white rot fungus *Peniophora incarnata* KUC8836 has received attention as the greatest degrader of polycyclic aromatic hydrocarbons (PAHs) known as hazardous xenobiotics and recalcitrant pollutants. In previous study, the gene encoding manganese-dependent peroxidase (MnP) from the fungus was newly ascertained as *pimp* during degradation of anthracene. To extracellularly secrete MnP involved in the degradation of PAHs, heterologous expression of *pimp* was carried out in *Saccharomyces cerevisiae* BY474. The digested *pimp* with EcoRI and SpeI was inserted to the pESC-URA vector containing *GAL0* promoter. The transformants were then cultured with D-galactose for *GAL0* promoter. MnP was extracellularly secreted to the culture medium as an active protein with the significant efficiency (3.58 U/mL) in the transformant culture among the other transformants and the control strain carrying the empty expression plasmid pESC-URA. This transfromant also grew faster than other transformants, indicating that high growth rate resulted in significant MnP production. And the recombinant protein of MnP was revealed with western blotting analysis as 44 kDa. Consequently, *pimp* might be useful for biodegradation and gene expression technologies. To advanced bioremediation of PAHs, genetic approaches should be more improved with the excellent ligninolytic gene, *pimp* and the fungus, *P. incarnata* KUC8836.

Keywords: heterologous expression, *Peniophora incarnata*, manganese-dependent peroxidase (MnP), *Saccharomyces cerevisiae*, white rot fungi

S27-2

Cold Stress Improves the Ability of *Lactobacillus plantarum* L67 to Survive Freezing

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The stress resistance of bacteria is affected by the physiological status of the bacterial cell and environmental factors such as pH, salts and temperature. In this study, we report on the stress response of *Lactobacillus plantarum* L67 after four consecutive freeze-thawing cycles. The cold stress response of the cold-shock protein genes (*csp*C, *csp*L and *csp*P) and ATPase activities were then evaluated. The cold stress was adjusted to 5 ºC when the bacteria were growing at the mid-exponential phase. A comparative proteomic analysis was performed with two-dimensional gel electrophoresis (2D SDS-PAGE) and a Matrix Assisted Laser Desorption/ onization-Mass Spectrometer. Only 56% of the *L. plantarum* L67 cells without prior exposure to cold stress survived after four consecutive freeze-thawing cycles. However, 78% of the *L. plantarum* L67 cells that were treated with cold stress at 5 °C for 6 h survived after freeze-thawing conditions. After applying cold stress to the culture for 6 h, the cells were then stored for 60 days at 5 °C, 25 °C and 35 °C separately. The cold-stressed culture of *L. plantarum* L67 showed an 8% higher viability than the control culture. After applying cold stress for 6 h, the transcript levels of two genes (*csp*P and *csp*L) were up-regulated .4 (*csp*P) and .2 (*csp*L) times compared to the control. However, *csp*C was not up-regulated. A proteomic analysis showed that the proteins increased after a reduction of the incubation temperature to 5 °C. The importance of the expression of 3 other relevant proteins was also determined through the study. The exposure of *L. plantarum* cells to low temperatures aids their ability to survive through subsequent freeze-thawing processes and lyophilization.

Keywords: *Lactobacillus plantarum* L67, cold stress response, *csp*C, *csp*P, *csp*L

S27-3

The Acid Stress Response in *Lactobacillus rhamnosus* LGG

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Yogurt is one of low pH cultured product which affected the survival of lactic acid bacteria (LAB). The aim of this present study was to examine the survival and acid stress response in *Lactobacillus rhamnosus* GG at low pH environment. The survival of LAB in commercial yogurt was measured during long-term storage. The enumeration of viable cells of LAB was determined at a 5-day interval over 52-weeks at 5℃. *L. acidophilus*, *L. casei* and *Bifidobacterium* spp. showed low viability. However, *L. rhamnosus* GG exhibited excellent survival throughout the refrigerated storage. At the end of the 52-weeks, *L. rhamnosus* GG survived 7.0 log0 CFU/mL. F0F ATPase activity in L. rhamnosus GG at pH 4.5 was also evaluated. The ATPase activities of membrane were higher when exposed at pH 4.5 for 24 h. The survival of *L. rhamnosus* GG was attributable to induction in F0F ATPase activity. In addition, the mRNA expression levels of acid stress-inducible genes at low pH was investigated by qRT-PCR. The *clp*C and *clp*E gene were up-regulated after h of incubation at pH 4.5. The *atp*A and *dna*K gene were also up-regulated after 24 h of incubation at pH 4.5. These genes could contribute to enhancing the survival of *L. rhamnosus* GG in the acidic condition. Thus, the modulation of the enzyme or gene to assist the viability of LAB in low pH environment is thought to be important.

Keywords: acid stress response, ATPase activity, *lactobacillus rhamnosus* LGG, long-term storage

S27-4

Changes of Bacterial Communities in Myeolchi-jeot, Fermented Anchovy, during Fermentation

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To investigate microbial communities of *myeolchi-jeot*, made by the fermentation of highly salted [approximately 25% (w/v)] anchovy in Korea, three sets of *myeolchi-jeot* samples were prepared using anchovy (*Engraulis japonicas*) with different sizes and their bacterial abundances, pH, and bacterial communities were analyzed during 280 days. The pH profiles were significantly different depending on *myeolchi-jeot* samples. Bacterial community analysis using pyrosequencing revealed that *Photobacterium*, *Vibrio*, and *Psychrobacter* were dominant at the beginning of the fermentation and the bacterial communities were significantly different depending on *myeolchi-jeot* samples during the early fermentation period, but eventually members of *Tetragenococcus*, halophilic lactic acid bacteria, became predominant. During the early fermentation period, bacterial communities in myeolchi-jeot samples prepared by small size anchovy were dominated by different genera including *Choromohalobacter*, *Salinivibrio*, *Staphylococcus*, and *Psychrobacter* depending on samples, but the *myeolchi-jeot* samples prepared by large size anchovy were not predominated by specific genera. In conclusion, bacterial successions in *myeolchi-jeot* were different during the early fermentation period depending on anchovy size, but eventually *Tetragenococcus* became predominant in all *myeolchi-jeot* regardless of anchovy sizes during the late fermentation period.

Keywords: anchovy sauce, myeolchi-jeot, bacterial community, metabolite, *Tetragenococcus*

S27-5

HPr of the *Vibrio vulnificus* PTS Confers Resistance to H_2O_2 Stress by Stimulating Pyruvate Kinase A Activity

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The bacterial phosphoenolpyruvate:sugar phosphotransferase system (PTS) consists of two general proteins (enzyme I and HPr) and several sugar-specific enzyme IIs. In addition to the phosphorylation-coupled transport of sugars, PTS components participate in various physiological processes. Here, we have identified pyruvate kinase A (PykA) as a binding partner of HPr in the opportunistic human pathogen *Vibrio vulnificus*. The interaction between HPr and PykA was dependent on the presence of inorganic phosphate and only dephosphorylated HPr interacted with PykA. Experiments involving domain swapping between the PykAs of *Vibrio vulnificus* and *Escherichia coli* revealed the requirement for the C-terminal domain of *Vibrio vulnificus* PykA for a specific interaction with *Vibrio vulnificus* HPr. Dephosphorylated HPr decreased the K_m of PykA for PEP by approximately four fold without affecting V_{max}. A *pykA* mutant was more susceptible to H_2O_2 than wild-type *Vibrio vulnificus* and this sensitivity was rescued by the addition of pyruvate to the culture medium. Based on these data, we suggest that *Vibrio vulnificus* HPr increases the affinity of PykA for PEP to confer resistance to H_2O_2 stress in the presence of glucose.

Keywords: H_2O_2 stress, phosphotransferase system, protein-protein interaction, regulation of glycolysis, *Vibrio vulnificus*

S27-6

proP P Promoter Derived Transcript is Posttranscriptionally Regulated by RNase III activity in *Escherichia coli*

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Escherichia coli RNase III regulates stability of a subgroup of mRNAs whose protein products are associated with cellular response to hyperosmotic environmental stress. Here, we report that stability of *proP* mRNA encoding a transporter of osmoprotectants is additionally controlled by RNase III in response to osmotic stress. *Escherichia coli proP* gene encodes a transporter of osmoprotectants such as proline and glycine betaine. We observed that steady-state levels of *proP* mRNA as well as ProP protein levels were inversely correlated with cellular RNase III activity, which, in turn, uptake capacity of proline into cell is dependent on cellular concentrations of RNase III under hyper osmotic condition. *In vitro* and *in vivo* cleavage analyses of *proP* mRNA indicated that RNase III cleavage sites were located in 5'-untranslated region of *proP* mRNA transcribed from P promoter. Introduction of nucleotide substitutions at the identified cleavage site abolished the ribonucleolytic activity of RNase III on *proP* mRNA, resulting in increased in both the steady-state levels and half-lives of the *proP* mRNA. *In vivo* crosslinking and immunoprecipitation analyses were performed to understand the relationships between decreased RNase III activity and increased *proP* mRNA stability under hyper osmotic condition, indicating that RNA binding capacity of RNase III was decreased. These findings suggest the the existence of an RNase III-mediated osmoregulatory network that rapidly balances out expression levels of factors associated with cellular response upon osmotic stress in *E. coli.*

Keywords: *proP*, mRNA degradation, osmotic stress, RNase III, transporter

S27-7

Functional Characterization of *Cryptococcus neoformans KRE2/MNT* and *OCH* Gene Family Encoding Novel Mannosyltransferases Involved in *O*-linked Glycans Biosynthesis

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Cryptococcus neoformans is an encapsulated basidiomycete that causes cryptococcosis in immunocompromised humans. Although the cell surface mannoproteins of *C. neoformans* were reported to be involved in fungal pathogenicity, their *O*-glycan biosynthetic pathway has not been elucidated. We had shown that the major *O*-glycans of *C. neoformans* were short manno-oligosaccharides that were connected mostly by α,2-linkages but connected by α,6-linkages at the third mannose residue. Here, we report three novel *C. neoformans* genes encoding mannosyltransferases involved in *O*-glycan extensions in the Golgi. *C. neoformans KTR3*, the only homolog of the *Saccharomyces cerevisiae KRE2/MNT* family genes, was shown to encode an α,2-mannosyltransferase that is responsible for the addition of the second α,2-linked mannose residue to the major *O*-glycans without xylose. *C. neoformans HOC* and *HOC3*, homologs of the *S. cerevisiae OCH* family genes, were shown to encode α,6-mannosyltransferases that can transfer the third mannose residue to minor *O*-glycans containing a xylose residue and major *O*-glycans, respectively. Moreover, the *ktr3*Δ mutant strain, which displayed increased sensitivity to cell wall stress, showed attenuated virulence in a mouse model of cryptococcosis. This suggests that the extended structure of *O*-glycans is required for full pathogenicity of *C. neoformans*, thus presenting the potential of Ktr3p as an ideal target for antifungal drug development.

Keywords: *Cryptococcus neoformans*, *O*-mannosylation, glycan analysis, cell wall protein, mannosyltransferases

S27-8

Isolation and Characterization of Extended-spectrum-β-lactamase-producing Non-typhoidal *Salmonella* in Retail Chicken Meat in South Korea

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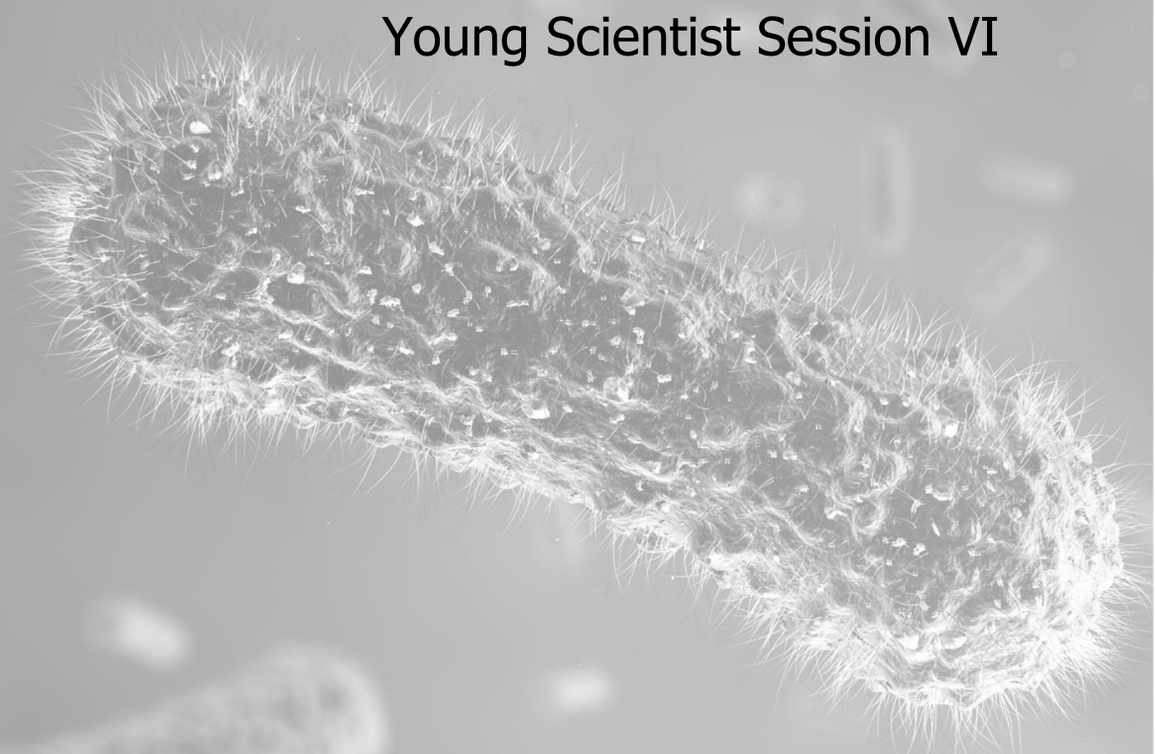
Non-typhoidal *Salmonella* (NTS) is one of the most important pathogens causing enteric infections via consumption of contaminated foods of animal origin. This pathogen is mostly prevalent in broiler chickens and eggs. A total of 100 chicken carcass samples from five different integrated broiler operation brands were analyzed for prevalence of *Salmonella* spp. Serotypes, antibiotic resistance patterns, presence of extended-spectrum-β- lactamase (ESBL) phenotype and genotype were analyzed on isolated strains. A total of 42 samples were contaminated with *Salmonella* spp.: 16 isolates (38%) of *S*. Virchow, 9 isolates (21%) of *S*. Bareilly, 8 isolates (19%) of *S*. Infantis, being the top 3 serotypes. Twenty-nine percent of the isolates portrayed multidrug resistance (MDR): resistance to 7 or more antibiotics. ESBL-producing NTS comprised 69% (29 out of 42) of the isolates, all of them displaying CTX-M-15 type (except one CTX-M-1 type). This is the first study of ESBL-producing NTS conducted on retail chicken meat in Korea. The high prevalence of MDR strains and CTX-M type ESBLs in NTS isolated from retail chicken meat raise a great concern and strategies to reduce the dissemination of hazards to human health should be taken account promptly.

Keywords: *Salmonella*, poultry, ESBLs, prevalence, antibiotic resistance

Symposium [S28]

Young Scientist Session VI



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S28-1

MicroRNA-25a Inhibits Autophagy Activation and Antimicrobial Responses during Mycobacterial Infection

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MicroRNAs (miRNAs) are small noncoding RNA molecules that regulate gene expression at the post-transcriptional level. Through binding to target messenger RNAs (mRNAs), miRNAs typically lead to modulation of diverse biological functions, including immune responses. Autophagy is a lysosome-mediated process that is important for the degradation of unwanted cytoplasmic cargos. Increasing data reveal that autophagy plays a key role in activating the antimicrobial host defense against *Mycobacterium tuberculosis* (Mtb). Although autophagy must be tightly regulated, the contribution of miRNAs to this process, including whether they specifically influence the activation of macrophage autophagy during Mtb infection, is largely unknown. In this study, we investigated whether the induction of miRNA-25a-3p (miR25a) in response to Mtb infection is involved in targeting autophagy-related genes. We thus infected RAW264.7 cells and murine bone marrow-derived macrophages (BMDMs) with Mtb, and measured the expression level of miR25a using quantitative RT-PCR analysis. We found that Mtb infection increased miR25a expression in both BMDMs and RAW264.7 cells. We further identified specific mRNA targets with miR25a-binding sites by a bioinformatic analysis. Interestingly, *UVRAG* was predicted to be a potential mRNA target of miR25a, through interaction with a complementary sequence in the 3′UTR. Using 3′UTR luciferase reporter assays, we further demonstrated that the miR25a-dependent suppression of luciferase activity in RAW264.7 cells expressing the wild-type UVRAG 3′UTR reporter. Together, these results indicate that miR25a post-transcriptionally inhibits UVRAG expression through a direct interaction with its 3′UTR binding site.

Keywords: microRNA, autophagy, mycobacteria, UVRAG, macrophages

S28-2

Evaluation of An Autotransporter Protein of *Orientia tsutsugamushi* as A Vaccine Antigen for Scrub Typhus

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Scrub typhus, caused by *Orientia tsutsugamushi* infection, is one of the main causes of febrile illness in the Asia-Pacific region, accounting for up to 20% of febrile hospital admissions in rural areas of southern Asia. It has been estimated that one billion people are at risk and one million new cases arise each year in the Asian-Pacific region. Despite aggressive attempts to develop a prophylactic vaccine against scrub typhus during the last several decades, all approaches have failed to generate effective immunity. The main issue for the development of a scrub typhus vaccine is the selection of proper antigens that cover a broad range of antigenic strains and induce long-lasting immunity. Here, we examined the potential use of ScaA protein as a vaccine antigen. Our findings demonstrate that

ScaA protein functions as a bacterial adhesion factor and an antibody against ScaA significantly inhibits bacterial infection into host cells. In addition, ScaA vaccination provides protective immunity against lethal challenges of the homologous strain, and also confers better protection against heterologous strains when combined with TSA56, the major outer membrane protein that was previously used as a potential vaccine antigen. These results indicate that ScaA proteins could be used as a novel vaccine target for scrub typhus.

Keywords: *Orientia tsutsugamushi, scrub typhus,* vaccine, autotransporter protein, heterologous strains

S28-3

Regulation of Metabolic Signaling of T cell by A Herpesviral Protein

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The herpesvirus saimiri (HVS) tyrosine kinase-interacting protein (Tip), required for the immortalization of primary T lymphocytes, targets cellular signaling molecules, including Lck tyrosine kinases and the retromer subunit Vps35. In addition we found that Tip induced sustained activation of mTORC signaling, which is dependent on the interacting with Lck and the retromer complex. These results are suggesting that the interaction between Tip and retromer complex or Lck may contribute to the T cell survival via the activation of mTORC. Physiologically, the inhibition of intracellular retromer activity by Tip is ultimately linked to the efficient in vitro immortalization of primary human T cells to interleukin-2 (IL-2)-independent permanent growth. Therefore, HVS Tip uniquely targets the retromer complex to impair the intracellular trafficking functions of infected cells, ultimately contributing to efficient T cell transformation.

Keywords: Herpesvirus saimiri, Tip, retromer, mTORC, T cell transformation

S28-4

Human Endogenous Retrovirus Envelope-Coated, Baculovirus-Based, VLP Forming DNA Vaccine for Influenza pdmHN

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Despite the advantages of DNA vaccines, overcoming their lower efficacy relative to that of conventional vaccines remains a challenge. Here, we constructed a human endogenous retrovirus (HERV) envelope-coated, nonreplicable, baculovirus-based virus like particle (VLP) forming DNA vaccine against swine influenza A/California/04/2009(HN). Previous we reported the efficacy of influenza HA DNA vaccine using a non-replicable baculoviral DNA vaccine (AcHERV-pdmHN HA). However, AcHERV-pdmHN HA vaccine only elicits an immune response against same HA antigen and limits the degree of immune response against whole viral antigen compare to the commercial killed vaccine. Here, we constructed a baculovirus carrying pdmHN HA, NA and M gene for making VLP in host cell. Comparable to monovalent HA vaccine, AcHERV-pdmHN HA-NA-M showed a strong humoral, cellular immune responses, and protected against pathogenic HN virus in challenge test. Our AcHERV-pdmHN VLP forming DNA vaccine could be a potential vaccine candidate to achieve an efficacy comparable to that of killed virus vaccines.

Keywords: influenza, recombinant baculovirus, virus-like particle, immune response, DNA vaccine

S28-5

Oral Administration of Multivalent White Spot Syndrome Virus DNA Vaccine Fused *Salmonella Typhimurium* Flagellin 2 in *Macrobrachium Nipponense*

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White spot syndrome virus (WSSV) is an infectious pathogen of penaeid shrimp and other crustaceans causing large economic losses all over the world. However, there is no efficient vaccines, adequate treatments, or approach to control this virus. Here, we report the immunogenicity of recombinant baculoviruses based multivalent WSSV nanovaccines, in which the WSSV envelope protein genes, VP28 and VP9, fused with *Salmonella typhimurium* Flagellin 2 (STF2) were inserted into a monovalent recombinant baculoviruses in *Macrobrachium nipponense*. The oral administration of multivalent WSSV nanovaccines was found to protect efficiently from the challenge with WSSV. Especially, the vaccinated shrimp with the baculovirus expressing and pseudotyped with VP28 and VP9 fused with STF2 (Ac-VP28-ieVP9+STF2) showed the highest survival rates (89.5%) compared to other vaccines groups (Ac-VP9-ieVP28+STF2: 84.2%, Ac-VP9-ieVP28: 78.9%, Ac-VP28-ieVP9: 76.3%, Ac-VP28: 7.%, and Ac-VP9: 65.8%) or non-treated group (00% mortality). These results strongly suggest that Ac-VP28-ieVP9+STF2 serves as a potential prophylactic baculoviral nanivaccine against WSSV.

Keywords: White spot syndrome virus, nanovaccine, baculovirus, immunogenicity, shrimp

S28-6

Structure of the Tripartite Multidrug Efflux Pump AcrAB-TolC Shows An Intermeshing Cogwheel Interaction between AcrA and TolC

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Escherichia coli AcrAB-TolC is a multidrug efflux pump that expels a wide range of toxic substrates. The dynamic nature of the binding or low affinity between the components has impeded elucidation of how the three components assemble in the functional state. Here, we created fusion proteins composed of AcrB, a transmembrane linker, and two copies of AcrA. The fusion protein exhibited acridine pumping activity, which suggests that the protein reflects the functional structure in vivo. To discern the assembling mode with TolC, the AcrBA fusion protein was incubated with TolC or a chimeric protein containing the TolC aperture tip region. Three-dimensional structures of the complex proteins were determined through transmission electron microscopy. The overall structure exemplifies the adaptor bridging model, wherein the funnel-like AcrA hexamer forms an intermeshing cogwheel interaction with the α-barrel tip region of TolC, and a direct interaction between AcrB and TolC is not allowed. These observations provide a structural blueprint for understanding multidrug resistance in pathogenic Gram-negative bacteria.

Keywords: AcrAB-TolC, multidrug efflux pump, multidrug resistance, X-ray crystallography, electron microscopy

S28-7

Effects of Various Culture Media on the Expression of Shiga Toxins in Enterohemorrhagic *Escherichia coli* O157:H7

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Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7, an important zoonotic foodborne pathogen, is able to cause serious diseases in humans and animals such as haemorrhagic colitis and haemolytic uremic syndrome (HUS). Previously, it is well known that the production of Shiga toxins (Stxs) by EHEC is one of the major virulence factors, which is responsible for HUS in human. In this study, we examined the production of Stxs by EHEC O157:H7 EDL933 strain grown in the five different culture media including Luria-Bertani broth (LB), Trypticase soy broth (TSB), Brain heart infusion broth (BHI), Buffered peptone water (BPW), and EC broth (EC). The results showed that the expression of Stxs was significantly increased when EDL933 was grown in EC and the number of bacteria was greatly reduced in EC compared to the others. These data imply that EC broth contains certain environmental signals able to induce the expression of Stxs in EHEC O157:H7.

Keywords: EHEC, O157:H7, Stxs, culture media

Poster Sessions

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A001

Aneurinibacillus soli sp. nov., Isolated from Mountain Soil

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A novel bacterial strain designated CB4^T was isolated from soil from the Hallasan, Jeju, Korea. Strain CB4^T was found to be strictly aerobic, Gram-positive and to form motile rods and creamy greyish colonies on nutrient agar. The major fatty acids were identified as iso-$C_{15:0}$ and iso-$C_{16:0}$, and the predominant isoprenoid quinone as MK-7, glycine and alanine as the diagnostic amino acid in the cell-wall peptidoglycan, and phosphatidyl-*N*-methylethanolamine (PME), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG) and an unidentified aminophospholipid (APL) as the polar lipids. The DNA G+C content of strain CB4^T is 46.5 mol%. Phylogenetic analysis, based on 16S rRNA gene sequence similarities, showed that strain CB4^T forms a deep branch within the genus *Aneurinibacillus*, sharing the highest level of sequence homology with *Aneurinibacillus aneurinilyticus* DSM 5562^T (96.5 %). On the basis of the phenotypic, chemotaxonomic and phylogenetic characteristics, strain CB4^T is considered to represent a novel species, for which the name *Aneurinibacillus soli* sp. nov. is proposed. The type strain is CB4^T (= KCTC 33505^T = CECT 8566^T).This work was supported by Mid-career Researcher Program through NRF grant funded by the Ministry of Science, ICT and Future Planning (MSIFP) of the Republic of Korea and a grant from the KRIBB Research Initiative Program.
Keywords : *Aneurinibacillus*, taxonomy, Hallasan, bacterial diversity

A002

Sphingobium subterraneum sp. nov., Isolated from Ground Water

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A Gram-staining-negative, strictly aerobic, non-motile, non-spore-forming, yellow colored and rod-shaped bacterium, designated S-II-13^T, was isolated from ground water at Daejeon in Korea. Strain S-II-13^T grew between 15 and 30 °C (optimal growth at 28 °C), between pH 6.0 and 9.0 (optimal growth at pH 7.5) and at salinities of 0–1.5 % (w/v) NaCl, growing optimally at 0.5 % (w/v) NaCl. On the basis of 16S rRNA gene sequence analysis, strain S-II-13^T was shown to belong to the genus *Sphingobium* showed closest phylogenetic similarity to *Rhizorhapis suberifaciens* CA1 (97.0%), *Sphingobium sufflavum* HL-25^T (96.9%) and *Sphingobium vulgare* BHU1-GD12^T (96.6%). The major polar lipids were phosphatidylglycerol, diphosphatidyl-glycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidyl coline and sphingoglycolipid. The predominant ubiquinone was Q-10. The major fatty acids were $C_{18:1}$ *ω7c* (23.1%), $C_{14:0}$ 2-OH (16.3%), $C_{16:0}$ (15.5%) and $C_{16:1}$ *ω7c* and/or $C_{15:0}$ iso 2-OH (12.4%). The DNA G+C content of this novel isolate was 63.5 mol%. DNA-DNA relatedness between strain S-II-13^T and *Rhizorhapis suberifaciens* LMG 17323^T, *Sphingobium sufflavum* KCTC 23953^T and *Sphingobium vulgare* KCTC 22289^T was 24, 52 and 55%, respectively. On the basis of polyphasic evidence from this study, strain S-II-13^T represents a novel species of the genus *Sphingobium* for which the name *Sphingobium subterraneum* sp. nov. is proposed. The type strain is S-II-13^T (=KACC 17606^T=NBRC 109814^T).
Keywords : Sphingobium, taxonomy, identification, ground water

A003

Mycological Characteristics of Nine Unrecorded Yeasts from Flowers in the Orchard of Yesan-gun, Chungcheongnam-do and Hanbat Arboretum in Daejeon City, Korea

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Six unrecorded yeasts, Cryptococcus festucosus 41-3, Cryptococcus heveanensis 56-4, Debaryomyces nepalensis 95-4, Issatchenkia occidentalis 142-1, Dioszegia zsoltii 39-1 and Kwoniella europala 47-2 were screened from one hundered eight yeasts which were isolated from flowers and fruits in orchards of Yesan-gun, Chungcheongnam-do, Korea. The morphological and cultural characteristics of these unrecorded yeasts were investigated. They were various shape such as ellipsoidal, globose and oval and also had budding mode in vegetable reproduction except I. occidentalis 142-1 (fission mode). K. europaea 47-2 only formed pseudomycelium. D. zsoliti 39-1 were not grew in yeast extract-malt extract medium, potatoes dextrose medium and vitamin-free medium. C. festucosus 41-3 grew well in 5% NaCl- containing yeast extract-peptone-dextrose medium and also had growth pH range of 7.0-10.0. Three kinds of unrecorded yeasts, Ogataea polymorpha HB45-1, Rhodotonula hinnulla HB62-2 and Cryptococcus rajasthanensis HB80-4 were screened from fifty one yeasts which were isolated from flowers in Hanbat arboretum in Daejeon city, Korea. They were globose in shape and did not formed pseudomycelium. Furthermore, O. polymorpha HB45-1 and C. rajasthanensis HB80-4 had budding mode in the vegetable reproduction. All of them grew well in vitamin-free medium and also C. rajasthanesis HB80-4 grew in 50% glucose and 5% NaCl-containg YPD medium.

A004

Paenibacillus hemerocallicola sp. nov., Isolated from the Root of *Hemerocallis fulva*

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A novel Gram-staining positive, aerobic, motile and rod-shaped bacterium designated strain DLE-12^T was isolated from the root of day lily (*Hemerocallis fulva*). Oval-shaped endospores were formed in swollen sporangia. Colonies on 5× R2A were white, round and convex. The strain grew at 15-°C (optimum = 37 °C), at pH 6-7, and also in the presence of 0-3 % (w/v) NaCl (optimum = 0 %). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain DLE-12^T was mostly related to *Paenibacillus ginsengarvi* Gsoil 139^T (96.6 % sequence similarity) and *Paenibacillus hodogayensis* SG^T (96.3 % sequence similarity). The major cellular fatty acid of strain DLE-12^T was anteiso- $C_{15:0}$ (43.9 %). The cellular polar lipids were composed of DPG, PE, PG and unidentified polar lipids. The major menaquinone was MK-7. The diamino-acid in the cell wall peptidoglycan was *meso*-diaminopimelic acid. The DNA G+C content of strain DLE-12^T was 55.2±0.5 mol%. The chemotaxonomic properties of the strain was consistent with those of *Paenibacillus*. However, the biochemical and physiological analyses distinguished the strain from related species. Based on the results of the polyphasic taxonomic analysis, strain DLE-12^T should be classified into genus *Paenibacillus* as a member of a novel species, for which the name *Paenibacillus hemerocallicola* sp. nov. is proposed. The type strain is DLE-12^T (= KCTC 33185^T = JCM 19572^T).
Keywords : *Paenibacillus, Hemerocallis fulva*

A005

Kiloniella spongiae* sp. nov., isolated from a Marine Sponge and Emended Description of the Genus *Kiloniella* Wiese *et al.* 2009 and *Kiloniella laminariae

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A gram-negative, aerobic, rod-shaped (1.3-4.7 mm 0.4-0.5 mm) and non-motile marine bacterium, designated as MEBiC09566^T was isolated from a sponge collected at Uljin in the East Sea (129°25'E, 36°55'N), Korea. The 16S rRNA gene sequence analysis revealed that strain MEBiC09566^T showed high similarity with the *Kiloniella laminariae* LD81^T (96.7%). Growth was observed at 11-31 °C (optimum 25 °C), at pH 6.0-8.5 (optimum pH 7.0) and with 0-6 % (optimum 2.5%) NaCl. The predominant cellular fatty acids were summed feature 3 (comprised of $C_{15:0}$ 2-OH and/or $C_{16:1}$ ω7c; 18.2%) and Summed feature 8 (comprised of $C_{18:1}$ ω7c / $C_{18:1}$ ω6c; 63.1%). The DNA G+C contents is 44.6 mol%. The major respiratory quinone is Q-9. Phosphatidylethanolamine, phosphatidylglycerol, two unidentified lipids, two unidentified amino-phospholipids and one unidentified aminolipid were detected as major polar lipids. On the basis of this polyphasic taxonomic data, strain MEBiC09566^T should be classified as a novel species in the genus *Kiloniella* and it is proposed as *Kiloniella spongiae* sp. nov. The type strain is MEBiC09566^T (=KCCM 43040^T =JCM 19930^T). Emended descriptions of the genus *Kiloniella* Wiese *et al.* 2009 and *Kiloniella laminariae* are also given.
Keywords : *Proteobacteria*

A006

New records of three agarics: *Clitocybe subditopoda*, *C. vibecina*, and *Galerina stylifera* from Odaesan National Park in South Korea

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In line with the effectuation of Nagoya Protocol, knowledge about biodiversity is becoming essential. In Korea, it is estimated that unrecorded fungi are much more than the recorded fungi. The studies about diversity of indigenous fungi have been carried out in Odaesan National Park in 2012-2013. During the studies, potentially unrecorded agarics were collected. They were examined morphologically and phylogenetic analysis was also performed. They were identified as *Clitocybe subditopoda*, *C. vibecina* and *Galerina stylifera*. These fungi have never been reported in South Korea. Here, we report them with the detailed descriptions and figures.

A007

***Taibaiella soli* sp. nov., isolated from *Pinus* soil**

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A Gram-staining-negative, motile by gliding, non-spore-forming and rod-shaped bacterial strain designated T1-10^T was isolated from *Pinus* soil, and its taxonomic position was investigated using a polyphasic approach. Growth occurred at 10-30°C (optimum = 30°C) and at pH 6-7 on 1/10 trypticase soy broth, and in the presence of 0-2% (w/v) (optimum = 0%) NaCl on R2A broth. Flexirubin-type pigments were produced. On the basis of 16S rRNA gene sequence similarity, strain T1-10^T was assigned to the genus *Taibaiella* of the phylum *Bacteroidetes*, and the closest species was *Taibaiella koreensis* THG-DT86^T (sequence similarity = 97.08%). The only isoprenoid quinone detected in strain T1-10^T was MK-7, and the major polyamine was *sym*-homospermidine. The major polar lipid was phosphatidylethanolamine. Phenotypic data and phylogenetic inference supported the affiliation of strain T1-10^T to the genus *Taibaiella*, but a combination of biochemical tests differentiated strain T1-10^T from other recognized species of *Taibaiella*. Therefore, the novel isolate evidently represents a novel species, for which the name *Taibaiella soli* sp. nov. is proposed (type strain = T1-10^T).
Keywords : *Taibaiella, Pinus*

A008

Characteristics of the Bacterial Strains Isolated from Human Hands I.

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As basal step for research to screen the antimicrobial plant materials, eighteen bacterial strains were isolated from human hands. Ten biochemical and morphological tests were conducted. Twelve strains are Gram-positive, two Gram-negative, and five not tested. Seventeen strains are positive to catalase test and fourteen strains are negative to urea test. One stains are positive to lactose test. In addition to this experiments more tests are needed to identification.
Keywords : characteristis, human hands

A009

Characteristics of the Bacterial Strains Isolated from Human Feet I.

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As basal research to screen the antimicrobial plant materials, sixteen bacterial strains were isolated from human feet. Ten biochemical and morphological tests were conducted. Twelve strains are Gram-positive, one Gram-negative, and five not tested. All sixteen strains are positive to catalase test and are negative to urea test. Twelve stains are negative to mannitol test. In addition to this experiments more tests are needed to identification

Keywords : characteristics, human feet

A010

Genetic Diversity and Phylogenetic Analysis of Muju virus Harbored by *Myodes regulus* in Republic of Korea

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About 5% of cases of hemorrhagic fever with renal syndrome (HFRS) occurring annually in Korea have been found to show a four fold or higher antibody titer to Puumala virus (PUUV) than to Hantaan virus (HTNV) by double-sandwich IgM ELISA, suggesting the existence of a PUUV-related hantavirus. Muju virus (MUJV), a genetically distinct hantavirus, was found in lung tissue of royal voles (*Myodes regulus*) captured in widely separated geographical regions in Korea, 1996. 101 royal voles were collected in six sites of the Republic of Korea during 2008-2013 and taxonomically verified by mitochondrial DNA (mtDNA) analysis. Among them, 3 strains of MUJV were fully sequenced to ascertain if it represented a genetically distinct hantavirus species. Entire genome sequence analysis of the1,831-nucleotide small (S), 3,652-nucleotide medium (M) and 6,544-nucleotide large (L) segments of MUJV, as well as the amino acid sequences of each segments, demonstrated that MUJV strains from different capture sites in the Korea were genetic variants of PUUV harbored by the bank vole (*My. glareolus*). Collectively, distinct geographic-specific clustering of MUJV was found in different provinces in the Korea, and phylogenetic analyses revealed that MUJV and PUUV shared a common ancestry.

Keywords : Genetic diversity, Hantavirus, Muju virus, R

A011

Endophytic Diversity of *Pinus densiflora* and *Juniperus rigida* in Mt. Baekryeon, Korea

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We investigated an endophytic fungal biodiversity of two Pinaceae and Cupressaceae species (*Pinus densiflora*, *Juniperus rigida*) in Mt. Beakryeon, Korea. Totally 25 isolates were discovered from 8 host plants and identified by ITS region and then they were checked into 7 taxa. Of them, 48.7% isolates were belongs to Leotiomycetes, 43.6% isolates Sordariomyetes, and 7.7% isolates Dothideomycetes. This result showed a similar diversity pattern with previous studies. Particularly taxon of *Lophodermium* in Leotiomycetes is a major component of endophytes' biodiversity of host plants so need to more critical research of the taxon of Korea.

Keywords : Species diversity

A012

Taxanomical Characteristics of Halophilic Bacterium *Roseivivax* sp. HJS6 from Solar Salt Pond

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Fifty halophilic bacterial strains were isolated from the five-stage (reservoir, 1st evaporation site, 2nd evaporation site, seawater storage and crystallization site) of solar salt pond by marine agar with 20% NaCl (w/v). Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolates belonged to three major taxa: *Firmicutes* (25 isolates), *α-proteobacteria* (22 isolates) and *γ-proteobacteria* (3 isolates). *Firmicutes* were categorizes as *Halobacillus*, *Thalassobacillus*, *Virgibacillus, Pontibacillus* and *Bacillus*. The *α-proteobacteria* were distributed as *Halomonas* and *Marinobacteria, γ-proteobacteria* including *Roseivivax*. In the taxonomical characterization of a strain HJS6 was most closely related to *Roseivivax roseus* BH87090^T (99.9%). Growth occurred at pH 5.0–11.0 and in the presence of 0.5-20 % (w/v) NaCl in marine broth 2216. The temperature growth at 10-37°C. The major respiratory quinine was ubiquinone-10. The major fatty acids (>10%) were $C_{16:0}$, $C_{18:1}$ ω7c, $C_{19:0}$ cyclo ω8c and 11-methyl $C_{18:1}$ ω7c, which is the same as that of *Roseivivax roseus* BH87090^T (zhang *et al.*2014). However, some biochemical characteristics such as activity of esterase, hydrolysis of citrate, esculin and urea were clearly different from *Roseivivax roseus* BH87090^T. It is considered that the strain HJS6 could be regarded as potential type strain for novel species by the result of further DNA hybridization studies.

Keywords : halophilic, *Roseivivax*, Solar salt pond

A013

Halobacillus ovalis sp. nov., a Moderately Halophilic Bacterium Isolated from Sediment of Solar Salt Pond

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Ten halophilic bacteria were isolated from the sediment of solar salt pond. Based on the phenotypic and genotypic analysis from the three isolates growing of 20% (w/v) NaCl, a moderately halophilic strain NGS2^T represented a novel species of the genus *Halobacillus*. Cells were Gram-staining-positive, motile, sharp pointed and oval shaped rods. Strain NGS2^T is catalase- and oxydase- positive and grew between 1 and 20% (w/v) NaCl (optimal growth at 8-9%), between 10-37°C(optimal growth at 28°C), between pH 5 and 10 (optimal growth at pH 8-9). On the basis of 16S rRNA gene sequence analysis, strain NGS2^T was shown to belong to the genus *Halobacillus* and showed closest phylogenetic similarity to *Halobacillus litoralis* KCTC 3687^T (98.9%), *Halobacillus locisalis* KCTC 3788^T (98.3%), *Halobacillus seohaensis* DSM 13145^T (98.4%), *Halobacillus naozhouensis* DSM 21183^T (98.2%) and *Halobacillus salinus* KCTC 3842^T (98.2%). The predominant menaquinone was MK-7. The major fatty acids were anteiso-$C_{15:0}$ (57.6%) and anteiso-$C_{17:0}$ (13.8%). On the basis of polyphasic analysis from this study, strain NGS2^T represents a novel species of the genus *Halobacillus* in the family *Bacillaceae* 2 within the phylum *Firmicutes* for which the name *Halobacillus ovalis* sp. nov. is proposed. The type strain is NGS2^T. ***Halobacillus ovalis*** **(o.val'is. L. fem. adj., egg-shaped).**

Keywords : *Halobacillus*, halophilic, solar salt pond

A014

Marinobacter salinaria sp. nov., a Halophilic Bacterium Isolated from the Crystallizing Pond of Solar Saltern

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Twenty halophilic bacteria were isolated from the crystallizing pond of a solar saltern on marine agar supplemented with 10% (w/v) NaCl. Three isolates growing at 20% (w/v) NaCl were belonged to the genera *Halomonas* and *Marinobacter*. Based on the phenotypic and genotypic analysis from the three isolates, strain CP12^T represented a novel species of the genus *Marinobacter*. Cells were Gram-staining-negative, motile, strictly aerobic and non-spore-forming rods. Strain CP12^T grew between 10 and 37¡É, between pH range of 6.0-9.0, between 0-20% NaCl, growing optimally with 3.0% (w/v) NaCl. On the basis of 16S rRNA gene sequence analysis, strainCP12^T showed closest phylogentic similarity to *Marinobacter algicola* DSM 16394^T (98.5%), *Marinobacter adhaerens* DSM 23420^T (98.5%) and *Marinobacter salsuginis* DSM 18347^T (98.1%). DNA–DNA relatedness between strain CP12^T and any of these species showed values lower than 18.6%. The predominant respiratory quinone was Q-9 and the major fatty acids were $C_{12:0}$(14.4%), $C_{16:0}$(13.8%) and $C_{12:0}$ 3OH (14.2%). On the basis of polyphasic analysis from this study, strain CP12^T represents a novel species of the genus *Marinobacter* in the family *Alteromonadaceae* within the phylum *Proteobacteria* for which the name *Marinobacter salinaria*sp.nov. is proposed. The type strain is CP12^T. ***Marinobacter salinaria*** **(sa.li.na«ria.L. adj. *salinaria*pertaining to *salinae*salterns).**

Keywords : halophilic, *Marinobacter salinaria*, solar saltern

A015

Collimonas violacea sp. nov., a Pigment Producing Bacterium Isolated from the Green-tea Upland Soil

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Pigment producing bacteria were collected from various samples, such as red beet, carrot, cherry, ginger, burdock and upland soils. The colony colors from isolates were divided into five groups; yellow (140 isolates), orange (30 isolates), red (15 isolates), brown (4 isolates) and a purple colors. In this study, we reported a bacterial violet pigment, designated DEC-B5^T, which was isolated from the green-tea upland soil in Boseong. Cells were Gram-staining-negative, strictly aerobic and motile rods. Strain DEC-B5^T grew between 4 and 28°C (optimum growth at 28°C), between pH 4.0 and 12.0 (optimum growth at pH 7.0). The major fatty acids of strain DEC-B5^T were summed feature 3 (42.46%), $C_{16:0}$ (31.14%) and $C_{18:1}$ w7c (12.1%). On the basis of 16s rRNA gene sequence analysis, strain DEC-B5^T was shown to belong to the genus Collimonas and showed closest phylogenetic similarity to *Collimonas fungivorans* LMG 21973^T (98.6%), *Collimonas arenae* LMG 23964^T (98.4%) and *Collimonas pratensis* LMG 23965^T (98.4%). DNA–DNA relatedness between strain DEC-B5^T and *C. fungivorans* LMG 21973^T, *C. arenae* LMG 23964^T and *C. pratensis* LMG 23965^T was 43.4%, 39.7% and 46.2%, respectively. On basis of phenotypic, chemotaxonomic and molecular analyses, strain DEC-B5^T represents a novel species of the genus Collimonas for which the name *Collimonas violacea* sp. nov. is proposed.***Collimonas violacea*** **(vi.o.la.ce«a. L. adj. *violaceus* violet ; M.L. adj. *violacea* violet-coloured).**

Keywords : *Collimonas violacea*, pigment

A016

New Species and New Records of *Buellia* from Jeju Island

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Two new records and one new species of lichen genus *Buellia* were discovered from Jeju Island during a recent floristic survey: *Buellia halonia* (Ach.) Tuck., *Buellia mamillana* (Tuck.) W.A. Weber and *Buellia* sp. nov. Together with previously recorded species, ten species were confirmed from Jeju Island. Among these species, three of them growing in the exposed rocky area contain xanthone (lichen thallus yellowish, UV + orange), indicating that xanthone production might be a strategy to defense lichen thalli against harmful UV light in this genus. The genus *Buellia* has been thoroughly studied in Korea before, but still, novel species continue to be discovered, and large species diversity were found within one genus, even from a tiny rocky Island. This study showed that coastal islands in Korea harbor a huge number of lichen species, and there is a great potential to discover unknown lichens in the coastal rocky area in Korea.

Keywords : Buellia, Jeju Island, lichens, new species, new records

A017

Some More Pyrenocarpous Lichen Records of South Korea

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The area of Eastern Asia is very lichenologically interesting and was overlooked in the past. Lichenological research of the wide regions in South Korea is still uncompleted mainly in lichens of pyrenocarpous group (*Verrucariales, Pyrenulales, Porinales* and some genera of *Ostropales*). In June 2014 were explored several seashore and inland areas of Jeju and Chuja Islands focusing on lichen family *Verrucariaceae*. The field trip has brought new records of 24 species of *Verrucariaceae*, of which 12 are reported for the first time from South Korea (*Agonimia flabelliformis, Anisomeridium polypori, Hydropunctaria amphibia, Hydropunctaria maura, Verrucaria aethiobola, V. halizoa, V. latebrosa, V. marinomuralis, V. minuscula, V. miyagiensis, V. praeviella, V. takagoensis* and *Wahlenbergiella striatula*) and 14 pyrenocarpous lichens for the first time from Jeju Island (*Agonimia koreana, Endocarpon pallidulum, E. pallidum, E. simplicatum, Placopyrenium fuscellum, Porina leptalea, Strigula nipponica, Verrucaria latebrosa, V. marinomuralis, V. miyagiensis, V. praeviella* and *V. takagoensis*). Some of collected species were known from Europe (*Agonimia flabelliformis*, *A. opuntiella*) or Japan only (*Verrucaria miyagiensis, V. praeviella* and *V. takagoensis*) and some of them with very rare distribution and pure known (*A. pacifica*). The total number of pyrenocarpous lichens of South Korea has increased to 76 species.
Keywords : floristic survey, lichen, new records, pyrenocarpous, coastal area

A018

Five New Species in the Family Sphaerophoraceae, with a Key to All Species Reported in China

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To be an obvious group in the temperate rainforests of the southwest China, species in the Sphaerophoraceae have a great morphological variation. Morphological, anatomical, chemical and molecular phylogenic analyses had been carried out in order to clarify the taxonomy of Sphaerophoraceae. Two genera *Sphaerophorus* and *Bunodophoron* were recognized in China, five new species belonging to *Bunodophoron* were described: *B. isidiosum* Dong Liu & Lisong Wang, *sp. nov., B. sinensis* Dong Liu & Lisong Wang, *sp. nov., B. longissimum* Dong Liu, *sp. nov., B. irpicium* Dong Liu & Yanyun Zhang, *sp. nov., B. floribundum* Dong Liu, *sp. nov.*,; *S. fragilis* and *S. globosus* were newly reported from China. Morphology suggests that *Bunodophoron* can be divided into two subgroups based on the medium solid or hole, and phylogenic analysis strongly supports the concept. Key is provided for the two genera and 13 species currently recognized in the genera *Sphaerophorus* and *Bunodophoron*.
Keywords : *Bunodophoron*, China, Lichen taxonomy, Phylogeny, *Sphaerophorus*

A019

Isolation of Strictly Anaerobic Iron-Reducing Bacterium, *Anaerosolibacter carbophilum* gen. nov., sp. nov., from a Coal-Contaminated Soil

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An iron-reducing bacterial strain IRF19^T obtained from coal-contaminated soil in the Republic of Korea was strictly anaerobic and mesophilic. Cells of IRF19^T were gram-negative, straight, rod-shaped and motile by flagella. Growth of strain IRF19^T occurred at 20-45 °C (optimal temperature is 40 °C) and at 6.5-10.0 (optimal pH is 7.5-8.0). NaCl was not required for the growth of strain IRF19^T. Growth was observed with compound as follows: yeast extract, glucose, fructose, ribose, mannitol, mannose, serine, alanine and isoleucine. Strain IRF19^T utilized Fe(III), elemental sulfur, thiosulfate, and sulfate as electron acceptor. Phylogenetic analysis based on the 16S rRNA gene sequences represented that the strain IRF19T is related to the family *Clostridiaceae* and is most closely affiliated with *Salimesophilobacter vulgaris* Zn2^T(93.5% sequence identity), *Geosporobacter subterraneus* VNs68^T (93.2%), and *Thermotalea metallivorans* B2-1^T (92.3%). The major cellular fatty acids of strain IRF19^T were $C_{14:0}$, iso-$C_{14:0}$3-OH, iso-$C_{15:1}$, iso-$C_{15:0}$, and $C_{16:0}$, and their profiles were distinct from those of the closely affiliated species. The G+C content of the genomic DNA of strains IRF19^T was showed to be 37.4 mol%. Based on in this studies, strain IRF19^T is considered to represent a novel species of a novel genus of the family *Clostridiaceae.* Therefore, we propose the name *Anaerosolibacter carbophilum* gen. nov., sp. nov., and the type strain is IRF19^T (KCTC 15396^T).
Keywords : Iron-reducing bacterium, *Anaerosolibacter carbophilum*, coal-contaminated soil

A020

Marmoricola solisilvae* sp. nov. and *Marmoricola terrae* sp. nov., isolated from soil and Emended Description of the Genus *Marmoricola

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Two *Marmoricola* strains, designated KIS18-7^T and JOS5-1^T, were isolated from soil samples in Korea. Both strains are Gram-stain positive, aerobic, rod or short-rod shaped. The 16S rRNA gene sequence of strain KIS18-7^T showed the highest similarities with *Marmoricola scoriae* Sco-D01^T (97.8%) and strain JOS5-1^T revealed the highest sequence similarities with *Marmoricola aequoreus* SST-45^T (97.5%). The sequence similarity between KIS18-7^T and JOS5-1^T was 98.1%. Neighbor-joining phylogenetic tree clearly showed that those strains grouped into the genus *Marmoricola*. DNA-DNA relatedness between strains KIS18-7^T, JOS5-1^T and the closely related species was less than 70%. The peptidoglycan of both strains contains LL-diaminopimelic acid as diagnostic diamino acid and a single glycine residue as interpeptide bridge (type A3γ). The major menaquinones of both strains were MK-8(H_4). The G+C contents of strains KIS18-7^T and JOS5-1^T were 68.0 mol% and 62.9 mol%, respectively. Combined data from phenotypic, chemotaxonomic and genotypic studies demonstrated that strains KIS18-7^T and JOS5-1^T are representatives of two novel species of the genus *Marmoricola*, for which the names *Marmoricola solisilvae* sp. nov. (type strain KIS18-7^T = KACC 17307^T=DSM 27140^T = NBRC 109601^T) and *Marmoricola terrae* sp. nov. (type strain JOS5-1^T =KACC 17308^T= DSM 27141^T = NBRC 109602^T) are proposed. An emended description of the genus *Marmoricola* is also presented.
Keywords : *Marmoricola solisilvae*, *Marmoricola terrae*, new species

A021

Parasegetibacter terrae sp. nov., isolated from paddy soil and emended* Description of the Genus *Parasegetibacter

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A Gram-stain-negative, variable-shaped, non-flagellated, yellow-pigmented, aerobic bacterium designated SGM2-10^T was isolated from a paddy soil sample from Suwon region, South Korea. Phylogenetic analysis based on 16S rRNA sequences indicated that the isolate was most closely related with *Parasegetibacter luojiensis* RHYL-37^T. The 16S rRNA gene sequence of strain SGM2-10^T showed the highest sequence similarities with *Parasegetibacter luojiensis* RHYL^T (95.1% sequence similarity), *Flavitalea populi* HY-50R^T (95.0) and *Flavitalea gansuensis* JCN-23^T (94.4%). No other species in the family *Chitinophagaceae* exceeded 94.1% 16S rRNA gene sequence similarity with strain SGM2-10^T. The major fatty acids of strain SGM2-10^T were iso-$C_{15:0}$ (28.6%), iso-$C_{15:1}$ G (21.5%) and iso-$C_{17:0}$ 3-OH (14.2%). The only menaquinone was MK-7. The polar lipids were composed of phosphatidylethanolamine (PE), seven unknown lipids and ten unknown aminolipids. The G+C content of the DNA of strain SGM2-10^T was 46.7 mol%. On the basis of the results of the polyphasic characterization presented in this study, it is concluded that strain SGM2-10^T represents a novel species of the genus *Parasegetibacter*, for which the name *Parasegetibacter terrae* is proposed. The type strain is SGM2-10^T (= KACC 17341^T = *JCM 19942*T*)*. The description of the genus *Parasegetibacter* has also been emended.

Keywords : *Parasegetibacter terrae*, *soil*, new species

A022

Phylogeographic Analysis of Imjin Virus, a Distinct Hantavirus isolated from the Ussuri white-toothed shrew (*Crocidura lasiura*), in the Republic of Korea during 2004-2014

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Imjin virus (MJNV), a genetically distinct hantavirus, was isolated from lung tissues of the Ussuri white-toothed shrew (*Crocidura lasiura*) collected near the demilitarized zone (DMZ) in the Republic of Korea. To demonstrate the genetic diversity of MJNV, partial M- and L-segment sequences were amplified from lung tissues of 12 of 38 (31.6%) anti-MJNV IgG antibody-positive Ussuri white-toothed shrews captured between 2004 and 2014. Additionally, the partial M- and L-segment sequences were obtained by RT-PCR from the lung tissues of 4 of 48 (8.3%) anti-MJMV IgG antibody-negative shrews collected between 2012 and 2014. A 531-nucleotide region of the M segment (coordinates 2,255 to 2,785) showed that the 14 MJNV strains differed by 0-12.6% and 0-2.7% at the nucleotide and amino acid levels, respectively. A similar degree of nucleotide (0.2-11.9%) and amino acid (0-3.8%) difference was found in a 632-nucleotide length of the L segment (coordinates 962 to 1,593) of nine MJNV strains. In conclusion, Phylogenetic analyses, based on the partial M and L segments of MJNV strains generated by the neighbor-joining and maximum likelihood methods, revealed geographic-specific clustering, akin to the phylogeography of rodent-borne hantaviruses.

Keywords :

A023

A new Virulence Genes-Deleted Live *Salmonella* Enteritidis Vaccine Candidate to Reduce Internal Egg Contamination

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To evaluate the efficacy of a novel attenuated *Salmonella* Enteritidis (*¡âlon¡âcpxR*) vaccine candidate (JOL919), chickens were immunized through oral and intramuscular routes to reduce egg contamination against *Salmonella* Enteritidis challenge. Birds were orally immunized with JOL919 on the first day of life and were subsequently boosted in the 6th and 16th week through oral (Group B) or intramuscular (Group C) route, while control birds were unimmunized (Group A). The chickens of all groups were challenged intravenously with the virulent *Salmonella* Enteritidis strain in the 24th week. The immunized groups B and C showed significantly higher plasma IgG and intestinal secretory IgA levels as compared to those of the control group. The lymphocyte proliferation response and $CD45^+CD3^+$ T cell number in the peripheral blood of the B and C groups were significantly increased. In addition, the egg contamination rates were significantly lower in the group B (0%, 10.7% and 0%), and the group C (3.6%, 14.3% and 3.6%) as compared to the group A (28.6%, 42.8% and 28.6%) in the 1st, 2nd and 3rd week post challenge. All animals in the groups B and C showed lower organ lesion scores in the liver and spleen, and lower bacterial counts in the liver, spleen and ovary at the 3rd week post-challenge. These results indicate that this vaccine candidate can be an efficient tool for prevention of *Salmonella* infections by inducing protective humoral and cellular immune responses.

Keywords : Salmonella, Egg contamination, Vaccine

A024

A biosafety and Immunogenecity-Enhanced *Salmonella* Enteritidis Inactivated Vaccine

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The new lysis plasmid was constructed by utilizing the approach of balanced-lethal systems based on auxotrophic gene Aspartate semialdehyde dehydrogenase (*asd*). The PhiX174 lysis gene *E* andλP_R37-cI857 temperature-sensitive regulatory system was cloned in the *asd* gene positive plasmid and this novel approach allowed the production of antibiotic resistance marker free *S.* Enteritidis ghost. The immunogenic potential of the biosafety enhanced antibiotic resistance gene free *S.* Enteritidis ghost was evaluated in chickens by employing the prime-boost vaccination strategy using a combination of oral and intramuscular routes. A total of 75 two-week-old chickens were equally divided into five groups: group A (non-immunized control), group B (intramuscularly primed and boosted), group C (primed intramuscularly and boosted orally), group D (primed and boosted orally), and group E (primed orally and boosted intramuscularly). Chickens from all immunized groups demonstrated significant increases in plasma IgG, intestinal secretory IgA levels, and antigen-specific lymphocyte proliferative response. After a virulent *S.* Enteritidis challenge, all immunized groups showed fewer gross lesions and decreased bacterial recovery from organs in comparison with the non-immunized control group. Among the immunized chickens, groups B and D chickens showed optimized protection, indicating that the prime-booster immunization with the ghost via intramuscular or oral route is efficient.

Keywords : Inactivated vaccines, Ghost, Salmonella

A025

The isolation of Kiloniella Antarctica from a Polynya of Amunsen Sea in Western Antarctic Sea

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A strain SOJ2014^T is isolated from surface water of a polynya in the Antarctic Sea. The 16S rRNA gene sequence similarity between SOJ2014^T and the closest strain, *Kiloniella laminariae* LD81^T is 96.2%. The G+C content of the genomic DNA of strain SOJ2014^T was 45.5 mol%. Major fatty acids were summed feature 8 (composed of $C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*), summed feature 3 (composed of $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) and $C_{18:0}$. It was a Gram-negative, slightly curved and spiral-shaped isolate with a single polar flagellum marine bacterium. The strain grew at 0-30°C (optimum, 25°C) with 1.5-5.1% (w/v) NaCl (optimum, 2.1-2.4%), and pH 5.5-9.5 (optimum, 7.5-8.0). The growth rate of strain SOJ2014^T was lower than that of *K. laminariae* DSM19542^T. Colonies grown on MA for 4 days at 25 °C were smaller than those of *K. laminariae* DSM 19542^T. It was microaerophilic and had different carbohydrate utilization traits compared with *K. laminariae* LD81^T. Based on phenotypic, chemotaxonomic and phylogenetic analyses, strain SOJ2014^T is proposed as a novel species, *Kiloniella antarctica*. The type strain is SOJ2014-1^T (= KCTC 42185^T = JCM 30387^T).

Keywords : Kiloniella, antarctica, microaerophillic, marine bacteria

A026

Isolation of Strictly Anaerobic Iron-reducing Bacterium, *Geosporobacter ferrireducens sp. nov.*, from a Hydrocarbon Contaminated Soil

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An alkaliphilic and heterotrophic iron-reducing bacterial strain IRF9^T was obtained from hydrocarbon-contaminated soil in the Republic of Korea. Based on 16S rRNA gene sequences, Phylogenetic analysis represented that strain IRF9^T belonged to the genus *Geosporobacter* in the family *Clostridiaceae* and was most closely affiliated to *Geosporobacter subterraneus* VNs68^T (96.9%). Cells of strain IRF9^T were straight or curved motile, rod-shaped and gram-negative. Optimal growth of strain IRF9^T occurred at pH 9.0–9.5 and 40¨¬C. The strain was observed to grow within pH and temperature range of 6.5–10.0 and 25–45¡ÆC, respectively. Sodium chloride was not required for growth. Fe(III) or elemental sulfur, but not sulfate and thiosulfate, was used as an electron acceptor. A limited number of carbohydrates and amino acids supported the growth of strain IRF9^T: glucose, fructose, mannitol, ribose and arginine. The main fatty acids (>10%) of strain IRF9^T were of $C_{14:0}$ (14.6%), iso-$C_{15:0}$ (21.0%) and $C_{16:0}$ (13.3%). The DNA G+C content of strain IRF9^T was determined to be 37.2 mol%, which was lower than that of *G. subterraneus* VNs68^T (42.2 mol%). Stand on this studies, we propose strain IRF9^T (KCTC 15395^T) as a new species of the genus *Geosporobacter*, and we propose the name *Geosporobacter ferrireducens* sp. nov.

Keywords : Iron-reducing bacterium, hydrocarbon, *Geosporobacter ferrireducens*

A027

Taxonomic Study of the Genus *Roseomonas*

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The genus *Roseomonas*, a member of the family *Acetobacteraceae* of the class *Alphaproteobacteria*, was first proposed by Rihs *et al*. (1993). Since the description of the three novel species in 1993, the number of novel species with valid names has increased. At the time of writing, the genus *Roseomonas* comprised of 20 species with validly published names. In this study, comprehensive taxonomic study was performed on the 20 species of the genus *Roseomonas*. *Roseomonas* strains were isolated from clinical specimens and environmental samples such as freshwater, water-cooling system, drinking water, air samples and soil samples. Common characteristics of the genus *Roseomonas* are gram-negative, non-spore-forming and coccoid-shape. Most of them are pink pigmented, but some species are white, orange and yellow. Most of them are catalase-positive. The major polar lipids were phosphatidylglycerol (PG), Phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylcholine (PC) and unknown aminolipids (AL). The predominant fatty acids are $C_{16:0}$, $C_{18:1}$ 2-OH and $C_{18:1}$ *ω7c*. The major quinone is Q-10 and DNA G+C content are 66.2-73 mol%. [This research was supported by the project on survey and excavationof Korean indigenous species of the National Institute of BiologicalResources (NIBR) under the Ministry of Environment, Republic ofKorea.]

Keywords : *Roseomonas*

A028

Korean Indigenous Bacterial Species

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Korean indigenous bacterial species registered at “List of prokaryotic names with standing in nomenclature (LPSN; http://www.bacterio.net/)” was investigated for the isolation place, habitat property and taxonomic position. A total of 1113 bacterial strains with valid names were listed at LPSN. Two-hundred and eighty-two strains were isolated from soil, 187 were from tidal flat, 177 were from seawater, 56 were from wastewater, and 41 were from food. Four-hundred and sixty-two species were affiliated with the phylum *Proteobacteria* (42 %), 262 were with *Bacteroidetes* (24 %), 228 were with *Actinobacteria* (20 %), and 148 were with *Firmicutes* (13 %). The most number of species were isolated from Jeju island with 113 species, followed by Daejeon (83 species), Dokdo island (51) and Pocheon (47), and so on. Nineteen species were isolated from Antarctica. [This research was supported by the project on survey and excavationof Korean indigenous species of the National Institute of BiologicalResources (NIBR) under the Ministry of Environment, Republic ofKorea.]

Keywords : indigenous bacterial

A029

***Burkholderia jirisanensis sp. nov. isolated from forest soil* sp. nov. Isolated From Forest Soil**

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A novel bacterial strain JRM2-1^T isolated from forest soil of Jirisan Mountain, Republic of Korea was identified and characterized based on the polyphasic taxonomy. The strain JRM2-1^T showed optimal growth in the range of pH 5.0-7.0 at 25°C. The antibiotic susceptibility test of strain JRM2-1^T showed that chloramphenicol, gentamicin, kanamycin, nalidixic acid, rifampicin, streptomycin, and tetracycline inhibited growth of the strain. On the basis of 16S rRNA gene sequence analysis, the closest neighbor of strain JRM2-1^T was *Burkholderia terrae* KMY02^T (97.2%) and DNA-DNA hybridization value between JRM2-1^T and *Burkholderia terrae* KCTC 12388^T was 14.4%. The major cellular fatty acids were $C_{16:0}$, cyclo-$C_{17:0}$ and cyclo-$C_{19:0}$ ω8c. Polar lipid analysis showed that the polar lipids profile of strain JRM2-1^T was consisted with diphosphatidylglycerol, phosphatidylglycerol, phosphatidylenthanolamine, several unidentified amino lipids and unidentified amino-phospholipid. Q-8 was a major isoprenoid quinone of the strain. The G+C content of strain JRM2-1^T was 63.7 mol%. Low DNA-DNA hybridization value indicated that JRM2-1^T does not belong to *Burkholderia terrae* KCTC 12388^T.On the basis of polyphasic taxonomical investigation, strain JRM2-1^T was proposed to be classified as a novel species in the genus *Burkholderia* for which the name *Burkholderia jirisanensis* sp. nov is proposed.
Keywords : 16S rRNA, Taxonomy, Novel species, Forest soil

A030

***Caulobacter Juamensis* sp. nov., Isolated from Lake Water**

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A Motile, rod-shaped, orange-colored and aerobic bacterium, designated strain JM6^T, was isolated from freshwater sample collected Juam lake (Republic of Korea). Cells were Gram stain negative, catalase-positive and oxidase-negative. The temperature and NaCl ranges for the growth of strain JM6^T were 10-37 °C and 0-1 %, respectively. A phylogenetic tree based on 16S rRNA gene sequences showed that strain JM6^T forms a lineage within the genus *Caulobacter* (94.8~95.2 %, sequence similarity) and formed a distinct branch with the clade comprising *Caulobacter fusiformis* KCTC 23687^T(95.24%), *Caulobacter mirabilis*(94.88%) and *Caulobacter daechungensis* KCTC 32211^T(94.8%). The major cellular fatty acids of strain JM6^T were summed feature 8 (consisting of $C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω6*c*; 64.4 %), $C_{18:0}$ (10.1 %), $C_{17:0}$ (7.2 %) and summed feature 7 (consisting of $C_{19:0}$ ω10*c* cyclo and/or $C_{19:1}$ ω6*c* and/or unknown 18.846; 3.8 %). The phenotypic characteristics indicate that strain JM6^T should be distinguished from the members of the genus *Caulobacter*. On the basis of the data presented in this study, strain JM6^T represents a novel species, for which the name *Caulobacter juamensis* sp. nov. is proposed. The type strain is JM6^T (=KCTC 23641^T = JCM 18259^T). [This research was supported by the project on survey and excavationof Korean indigenous species of the National Institute of BiologicalResources (NIBR) under the Ministry of Environment, Republic of Korea.]
Keywords : *Caulobacter*

A031

***Calculibacillus koreensis* gen. nov., sp. nov., A Mesophilic Anaerobic, Fe(III)-reducing Bacterium from Sediment of Abandoned Coal Mine**

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A mesophilic bacterium, strain b5^T, was isolated from sediment of abandoned coal mine in Taebaek, Republic of Korea. Cells of strain b5^T were spore-forming straight rods, 0.5 μm in diameter and 2.0-4.0 μm in length and Gram-stain-positive. Brown colonies were formed. The optimum pH and temperature for growth were pH 7.0 and 30°C, respectively, while it was able to grow within pH and temperature ranges of 5.5-7.5 and 20-45°C, respectively. Growth of strain b5^T was observed at NaCl concentrations ranging from 0 to 6.0% (w/v) with an optimum at 3.0-4.0% (w/v). Strain b5^T grew anaerobically by reducing nitrate, Fe(III)-citrate, S^0 and AQS in the presence of proteinaceous compounds, organic acids and carbohydrates as electron donors. The isolate was not able to grow with fermentation. Strain b5 did not grow under aerobic conditions during incubation with atmospheric concentration of oxygen. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain b5^T was most closely related to the genus *Tepidibacillus*: *Tepidibacillus fermentans* STGH^T (95.64%) and *Vulcanibacillus: Vulcanibacillus modesticaldus* BR^T (93.92%). Based on phenotypic, chemotaxonomic and phylogenetic properties, we describe a new species of a novel genus *Calculibacillus*, represented by strain b5^T (= KCTC 15397^T), for which we propose the name *Calculibacillus koreensis* gen. nov., sp. nov.
Keywords : abandonded coal mine, Fe(III)-reducing, mesophile, *Calculibacillus koreensis*

A032

Draft Genome Sequence of *Lactococcus chungangensis* CAU28^T

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Lactococcus chungangensis CAU28^T was isolated from activated sludge foam. It is a Gram positive, oval shape bacteria which utilizes sugars such as D-glucose, D-fructose, D-mannose, and D-mannitol as sole carbon source. Carbohydrate metabolism includes genes for lactose utilization and enzymes such as L- lactate oxidase and glucokinase, PTS system and the mannose-specific IID component were found to take up sugar monomers for metabolism and acid formation. Interestingly, methionine biosynthetic enzymes, the transport regulator MtaR, an amidohydrolase and cystathionine gamma-synthase the main metabolic pathway that to convert methionine to methanethiol a compound leading to cheese flavor generation, are present (an up regulated compared to *L. lactis*) . These new finding suggest *L. chungangensis* strain CAU28^T may lead to an innovative cheese making process. Stress response mechanisms such as heat shock protein 60 family chaperone GroEL and cold-shock DEAD-box protein A that are important in protecting the cell under stress conditions found in cheese making are also found in *L. chungangensis* CAU28^T. So whole genome sequencing was performed to classify the important functions and metabolism of genes in *L. chumgangensis* CAU28^T and to generate useful data for further applications in dairy fermented products such as cheese, yogurt and other milk derived product.
Keywords : Draft Genome Sequence, *Lactococcus*

A033

New Found Adenovirus from Chinstrap penguins (*Pygoscelis antarctica*) in Antarctica

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Adenoviruses (family *Adenoviridae*) infect various sites, and cause diseases in many species. Adenoviruses have been identified from various hosts: mammals, birds, ruminants, reptilians, marsupials, frogs and fish. In this study, we tested detection of adenovirus from Chinstrap Penguins (*Pygoscelis antarctica*), because no previous reports have identified the detection of adenovirus in Antarctic Penguins. Adenovirus detection was performed by PCR in various organ samples of ten Chinstrap Penguins collected in Antarctica during 2009 and early 2010. The hexon gene of 855 bp among the PCR product was selected for phylogentic analysis. The hexon nucleotide sequence of Chinstrap Penguin adenoviruses (CSPAdVs) showed similarity with South polar skua A (SPSAdV-A) 71.8%, raptor adenovirus 1 (RAdV-1) 71% and Turkey adenovirus 3 (TAdV-3) 71.4%. Based on the genetic analysis, we classified CSPAdVs as novel adenovirus to genus *Siadenovirus*. In conclusion, this study provides the first detection of new adenovirus species from Antarctic penguins.
Keywords : Adenovirus, Siadenovirus, Penguin, Antarctica

A034

Streptomyces Kyonggiensis sp. nov., an Antibiotic and Fungicide Producer, Isolated from Forest Soil

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Strain T258^T was isolated from forest soil collected near Bongrae Falls, lleung-Gun, Gyeongbuk, South Korea. The 16S rRNA gene sequence of the strain showed closely related to *S. diastatochromogenes* ATCC12309^T (98.5%). This aerobic, Gram-positive actinobacterium has spiral and spiny spore chain morphology. Growth occurs at 10-40^{o}C (optimum: 28-30^{o}C), at pH 5-10 (optimum: 6.8-7) and with 2% (w/v) NaCl. The aerial mycelium is white and the substrate mycelium is ivory. Melanin pigment is negative. Degrades xylan and starch but not milk. Utilizes L-arabinose, D-xylose, D-fructose, Rhammose, D-glucose, D-myo-inositol and D-manitol and raffinose as sole carbon sources. Utilizes L-ornithine and L-tyrosine as sole nitrogen sources but not L-argine and L-arginine. Nitrate reduction, H_2S and indole production were negative. The strain was resistant to ampicilin, rifampicin, streptomycin and chloramphenicol but sensitive to tetracycline. The strain shows antimicrobial activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphyloccus epidermidis*, *Paenibacillus larvae*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*. The cell wall peptidoglycan contains *LL*-diaminopimelic acid, glutamic acid, alanine and glycine. Whole-cell hydrolysates contains glucose, galactose and ribose. Based on all gained results, strain T258^T was regarded as a novel member of genus *Streptomyces* with a proposed name, *Streptomyces kyonggiensis.*
Keywords : *Streptomyces kyonggiensis*

A035

Re-evaluating the *Lactarius* and *Lactifluus* Inventory in Korea using DNA Barcode Markers

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Lactarius and *Lactifluus* are two highly diverse genera in the fungal class Agaricomycetes. Previous studies have relied on morphology to classify species, which may be difficult for *Lactarius* and *Lactifluus* because of highly variable morphologies. Approximately 400 *Lactarius* and 100 *Lactifluus* species are found worldwide, with 40 and 12 species reported in Korea, respectively. In this study, we re-evaluate the Korean inventory of the genus *Lactarius* and *Lactifluus* through DNA barcoding. Three barcoding regions (ITS, LSU, and RPB2) were sequenced for 355 specimens obtained from Seoul National University Fungus Collection (SFC) and the National Academy of Agricultural Sciences (HCCN). Phylogenetic analyses recovered 42 *Lactarius* and 12 *Lactifluus* MOTUs, where only 8 and 3 corresponded to the previously known Korean species, respectively. Some MOTUs are new records to Korea, while others are undescribed species. Our data show that the application of American, European, and Japanese species names to Korean taxa is incorrect due to morphological misclassification and cryptic diversity. Of the three markers, ITS and RPB2 performed the best in resolving *Lactarius* and *Lactifluus* species in Korea.
Keywords : *Lactarius*, *Lactifluus*, DNA barcode, inventory, ITS

A036

***Lactarius cucurbatinus* (Russulales, Basidiomycota), a New Species from South Korea Supported by Molecular and Morphological Data**

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A new species belonging to *L.* subg. *Plinthogalus* was discovered during a long-term project on the diversity of Korean *Lactarius*. This species is proposed here as *Lactarius cucurbatinus*. The status of *L. cucurbatinus* as a separate species is supported by molecular data and morphological features. Phylogenetic analysis based on internal transcribed spacer (ITS) sequences shows that *L. cucurbatinus* is closely related to *L. subplinthogalus*, *L. friabilis* and *L. oomsisiensis*, with pairwise distances of 2.8~4.3%. Distinctive morphological characters of *L. cucurbatinus* that distinguish it from these closely related species are a pale yellow to pale orange color of the pileus and non-discoloration of white latex. The new species is described and illustrated in this study.
Keywords : ectomycorrhizal fungi, Korea barcode of life, taxonomy, white latex

A037

A Contribution to Lichen Genus *Graphis* (*Graphidaceae*, Ostropales, Ascomycota) of East Asian Countries

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The genus *Graphis* is an artificial assemblage of more than 300 species characterized by lirellate ascomata, carbonized proper exciple, hyaline, clear to inspersed hymenium, 1-8-spored, non-amyloid asci, mostly hyaline, I+ violet-blue, transversely septate to muriform ascospores and a thallus producing different lichen compounds or no compounds. In East Asia the taxonomy of the genus is well understood due mainly to dynamic phytogeography and extended world-wide knowledge on *Graphis* in recent years. The evergreen forests of East Asia represent significant number of graphidaceous taxa. In India diversity of the genus was recognized for the highest number of one hundred eleven species followed by China with sixty five species (Hong-Kong with *c.* 45 species in addition). Sri Lanka though, explored significantly for other elements of the family, information on *Graphis*, however recorded least (*c.* 13 species). Korean *Graphis* enumerated for thirteen species with four new additions in the recent past. Vietnamese *Graphidaceae* studied well in the modern time, and predominantly the genus *Graphis* worked out for twelve species which were the addition to previously known six species.Though, this is undoubtedly a rather low estimate of potential species number if compared with phorophytic diversity in tropics, nevertheless scope of this genus in East Asian countries can be assumed by the new reports and species that are still being discovered and published continuously.
Keywords : Asia, diversity, *Graphidaceae*, phytogeography, taxonomy

A038

Recent Taxonomic Investigations on Epiphytic Lichens of Vietnam

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During a course of lichenological expedition in Vietnam, Bidoup Nui Ba National Park was surveyed. The national park is placed in Lam Dong province situated in central highlands of Vietnam. This random collection was totally based on crustose lichens growing on trees of the national park. A substantial amount of epiphytic lichens were collected and preserved in Korean Lichen Research Institute, South Korea. Some of the specimens were deposited in the herbarium of CSIR-National Botanical Research Institute, Lucknow, India. The material was segregated into non-graphidaceous and graphidaceous taxa. Among non-graphidaceous lichens, members of Roccellaceae, Phlyctidaceae, Pertusariaceae and Arthoniaceae were dominating the national park, while taxa studied exclusively for the *Graphidaceae* included lichen genera *Graphis*, *Hemithecium*, *Pallidogramme*, *Carbacanthographis*, *Chapsa*, *Sarcographa*, *Thecographa* and *Phaeographis*. Many of the deposited specimens were identified up to the species level, and some new and notable species were extracted from them. New records of *Arthonia excipienda*, *Chiodecton leptosporum*, *Graphidastra multiformis*, *Pertusaria pycnothelia, P. thwaitesii*, *Phlyctis uncinata*, *Carbacanthographis salazinica*, *Chapsa leprocarpa*, *C. minor*, *Graphis longiramea*, *G. marginata*, *G. pertricosa*, *G. vittata*, *Pallidogramme chrysenteron*, and *Thecographa prosiliens* have so far been described from the collection in national park.
Keywords : graphidaceous, herbarium, national park, Lam Dong province

A039

Molecular Phylogeny and Current Taxonomy of the Teloschistaceae (Lecanoromycetes, Ascomycota)

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Taxonomy of the Teloschistaceae has being dramatically exchanged on the basis of the molecular phylogenetic and totally more than 66 genera were proposed for three subfamilies of Caloplacoideae, Xanthorioideae and Teloschistoideae. However only 47 genera have confirmed from multilocus molecular phylogeny based on more than 3 voucher specimens as followings; Teloschistoideae (*Brownliella, Filsoniana, Fulgogasparrea, Josefpoeltia, Kaernefia, Niorma, Teloschistes,* and *Wetmoreana*), Caloplacoideae (*Blastenia, Bryoplaca, Caloplaca, Eilifdahlia, Elenkiniana, Franwilsia, Huneckia, Leproplaca, Marchantiana, Mikhtomia, Pyrenodesmia, Variospora,* and *Yoshimuria*) and Xanthorioideae (*Athallia, Austroplaca, Cerathallia, Dufourea, Flavoplaca, Gallowayella, Golubkovia, Gondwania, Honeggeria, Jackelixia, Jesmurrayia, Igneoplaca, Langeottia, Martijahnsia, Massjukiella, Ovealmbornia, Oxneria, Parvoplaca, Rusavskia, Scythioria, Squamulea, Verrucoplaca, Xanthocarpia, Xanthokarroa, Xanthomendoza,* and *Xanthoria*). Nine genera of *Scutaria, Stellarangia, Villophora*, *Gyalolechia,Usnochroma, Polycauliona, Shackletonia, Solitaria* and *Xanthopeltis* is in need of the further confirmation because they were positioned in the phylogenetic tree of the Teloschistaceae. Furthermore, 10 genera of *Hoffmannia, Haloplaca, Sirenophila, Teloschistopsis*, *Ioplaca, Rufoplaca, Seirophora, Calogaya, Orientophila* and *Pachypeltis* are still unclear because data on some genes of the type species are so far not obtained.
Keywords : lichen, molecular phylogeny, multilocus genes, Teloschistaceae, Toxonomy

A040

New and Noteworthy Lichen-Forming and Lichenicolous Fungi from South Korea and Eastern Asia

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A number of new for science species as well as records for Korean lichen flora have been provided after publishing the latest edition of the Korean checklist. However, there are still a number of taxa which should be clarified. Descriptions of seventeen new for science species of the genera *Absconditella*, *Caloplaca*, *Fellhanera*, *Lecania*, *Lichenostigma*, *Micarea*, *Phoma*, *Protoparmeliopsis*, *Roselliniopsis*, *Seirophora* and *Topelia* found in a number of herbaria were recently provided. About 10 taxa of the genera *Agonimia, Buellia, Lecanora, Lecidella, Micarea, Pyrenopsis* and *Russavskia* are under special revision as candidates for new to science species as well as more than 10 new for South Korea species (genera *Caloplaca, Candelariella, Catillaria, Protoparmeliopsis, Pyxine, Stigmidium, Thelotrema, Trapelia* and *Voauxiomyces*) are found during cameral treatment of the KoLRI collection of 2014 year. More than 10 new and rare for Russian Far East species of the lichen-forming and lichenicolous fungi of the genera *Bactrospora, Biatoridium, Collemopsidium, Dactylospora, Megalospora, Opegrapha, Phacopsis* and *Vouauxiomyces* are found during treatment of the KoLRI collections from 2013 in 2014 too. A number of new for China species of the genera *Caloplaca* and *Seirophora* are also found during cameral treatment of the KoLRI collections of 2014. Special publication with notes on new and noteworthy taxa of lichen-forming and lichenicolous fungi of South Korea is in preparation at the moment.
Keywords : East Asia, lichen-forming fungi, lichenicolous fungi, new species, South Korea

A041

Penicillium jejuense sp. nov., Isolated from the Marine Environments Of Jeju Island, Korea

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Three strains of an unknown *Penicillium* species were discovered during a fungal diversity survey of marine environments in Korea. These strains are described here as a new species using a multigene phylogenetic analyses of four loci (internal transcribed spacer, β-tubulin, calmodulin, and RNA polymerase II) and macro- and micromorphological characteristics. Phylogenetic analyses showed that the three strains of this unknown *Penicillium* species formed a strongly supported monophyletic group that was distinct from previously reported species within the section *Aspergilloides*. Morphologically, these strains can be distinguished from its sister species, *Penicillium crociola*, by the reverse colour on Czapek yeast autolysate agar, abundant production of sclerotia on malt extract agar, and colony features on yeast extract sucrose agar. We name this new species *Penicillium jejuense*, after the locality where it was discovered

Keywords : New species *Penicillium*, Section *Aspergilloides*, Phylogenetic analyses

A042

Pedobacter rivuli sp. nov., Isolated from a Freshwater Stream

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A Gram-staining-negative, rod-shaped and red-pigmented strain HME8457^T, was isolated from a freshwater stream located in Republic of Korea. Phylogenetic tree based on 16S rRNA gene sequences showed that strain HME8457^T formed a lineage within the genus *Pedobacter*. The strain HME8457^T was most closely related *Pedobacter* species were *P. daechungensis* Dae 13^T (96.4 % sequence similarity), *P. lentus* DS-40^T (95.3 %), *P. terricola* DS-45^T (94.9 %), *P. glucosidilyticus* 1-2^T (94.2 %) and *P. soyangensis* HME6451^T (93.6%). The major fatty acids were iso-$C_{15:0}$ (28.8 %), summed feature 3 (comprising $C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c*; 21.7 %), iso-$C_{17:0}$ 3-OH (7.7 %) and anteiso-$C_{15:0}$ (6.2 %). The only respiratory quinone was MK-7. Polar lipid analysis revealed the presence of phosphatidylethanolamine, one unidentified aminolipid and two unidentified polar lipids. Sphingolipid is present. The DNA G+C content was 33.3 mol%. On the basis of the evidence presented in this study, strain HME8457^T represents a novel species of the genus *Pedobacter*, for which the name *Pedobacter rivuli* sp. nov., is proposed the type strain HME8457^T (= KACC 17312^T = CECT 8291^T).

Keywords : *Pedobacter rivuli* sp. nov, 16S rRNA gene, freshwater

A043

Oil degrading strain *Psychrobacillus mongoliensis* sp. nov., Isolated from Oil-Contaminated Soil In Mongol

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Strain NHI-2^T is aerobic, psychrotolerant, Gram-positive, endospore forming, motile and rod-shaped. Growth occurs at wide range of temperature from 0 to 40°C, optimal at around 28°C to 37°C. It can be tolerant at the quite high salt concentration, up to 7% after 5-day incubation. In comparison of 16S rRNA gene sequence analysis, strain NHI-2^T belongs to genus *Psychrobacillus*. This value ranged from 97.83-98.18% between NHI-2^T and validated as members of *Psychrobacillus*. The DNA-DNA relatedness was exhibited below 70%. The G+C mol content of the genomic DNA was 35.8%. This strain contained MK-8 as a predominant isoprenoid menaquinone. As the similar peptidoglycan types of three references, NHI-2^T had A4β type of ornithine as diamino acid at position 3 of the peptide subunit. Furthermore, its cell wall also consisted of diminopimelic acid with iso and meso positions whereas neither the reference strains have both. The major fatty acids were $C_{15:0}$ anteiso (50.21%), $C_{17:0}$ anteiso (8.62%), $C_{15:0}$ iso (8.36%). The polar lipid profile contained phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) as the main components. These data showed the different features of strain NHI-2^T with related species based on phenotypic, including chemotaxonomic and genotype. Therefore, this strain was proposed as the new species, named *Psychrobacillus mongoliensis* NHI-2^T (KACC 18243^T) with accession number KJ956929.

Keywords : *Psychrobacillus mongoliensis*, oil degradation, Mongol, psychrotolerant

A044

Winogradskyella Marina sp. nov., Isolated from Seawater

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A novel bacterium, designated HME9613^T, isolated from seawater in jeungdo, Korea. Cells of strain HME9613^T were yellow-pigmented, aerobic, motile by gliding, Gram-negative, rod-shaped and oxidase positive. Strain HME9613^T grew optimally at 25°C, at pH 8 and in the presence of 4 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain HME9613^T belonged to a distinct lineage in the genus *Winogradskyella* of the family Flavobacteriaceae, and showed 93.7-95.8% similarity with recognized members of the genus. The predominant fatty acids were iso-$C_{15:1}$ G (29.1 %), iso-$C_{15:0}$ (13.9 %), summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c* ; 17.3 %) . The major polar lipids of strain HME9613^T were of phosphatidylethanolamine, three unidentified aminolipids, one unidentified phosphatidylcholine, one unidentified glycolipid and three unidentified lipids. The major respiratory quinone was menaquinone-6 and the DNA G+C content of the strain was 38.6 mol%. On the basis of phenotypic, chemotaxonomic, phylogenetic and genotypic data, strain HME9613^T represents a novel species within the genus *Winogradskyella*, for which the name *Winogradskyella marina* sp. nov. is proposed. The type strain is HME9613^T (=KCTC 42189^T = CECT-ing).

Keywords : *Winogradskyella marina* sp. nov, 16S rRNA gene, seawater

A045

Mucilaginibacter aquatilis sp. nov., Isolated from a Mesotrophic Artificial Lake

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A Gram-staining-negative, rod-shaped and pale-pink-pigmented bacterium, designated HME9299^T was isolated from a mesotrophic artificial lake located within the campus of Hankuk University of Foreign Studies, Yongin, Korea, and characterized by using a polyphasic taxonomic approach. The predominant fatty acids of strain HME9299^T were iso-$C_{15:0}$ (30.0%) and summed feature 3 (comprising $C_{16:1}$ $\omega6c$ and/or $C_{16:1}$ $\omega7c$; 36.2%). Strain HME9299^T contained MK-7 as the dominant menaquinone and the G+C content of its genomic DNA was 35.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain HME9299^T was affiliated to species of the genus *Mucilaginibacter*, and its closest relatives were *Mucilaginibacter daejeonensis* Jip 10^T (96.9% sequence similarity), *Mucilaginibacter polytrichastri* RG4-7^T(96.3%) and *Mucilaginibacter lappiensis* ANJLI2^T(95.7%).. Based on phylogenetic inference and phenotypic data, strain HME9299^T is considered to represent a novel species of the genus *Mucilaginibacter*, for which the name *Mucilaginibacter aquatilis* sp. nov. is proposed. The type strain is HME9299^T (=KCTC 42122^T. =DSM -ing).
Keywords : *Mucilaginibacter aquatilis*, 16S rRNA gene, mesotrophic artificial lake

A046

Paenibacillus acervicinus KUDC4121 sp. nov., Isolated from Ulleungdo Island

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Strain KUDC4121 (=KCTC 13870T =DSMZ 24950T) was isolated from the rhizosphere of Acer okamotoanum, collected from Ulleungdo Island, Republic of Korea. Acer okamotoanum is native plant in Ulleungdo Island a kind of maple tree. The predominant menaquinone was MK-7. The major fatty acids were anteiso-$C_{15:0}$ (63.78%) and iso-$C_{16:0}$ (10.73%). The polar lipids of strain KUDC4121 were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and an unidentified four phospholipids. The DNA G+C content was 48.28 mol%. The DNA-DNA relatedness between strain KUDC4121 and the reference strain *Paenibacillus chondroitinus* DSM 5051^T was 30.1%, followed by *P. pocheonesis* Gsoil 1138^T (20.3%), *P. pectinilyticus* RCB-08^T (14.2%), *P. alginolyticus* DSM5050^T (12.2%), and *P. aestuarii* CJ25^T (11.9%). All relatedness ratios were lower than 70%. The closest type strain in 16s rRNA sequence analysis was *P. chondroitinus* DSM 5051^T with 97.8% similarity followed by *P. alginolyticus* DSM5050^T (97.6%), *P. pocheonesis* Gsoil 1138^T (97.5%), *P. frigoriresistens* YIM 016^T(97.5%) and *P. pectinilyticus* RCB-08^T (97.2%).

A047

A New Species and Record of *Xylaria* Species from Korea

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We discovered some *Xylaria* species during a survey of the Korean mushrooms flora from 2011 to 2014. Among them, we found two *Xylaria* species which are new and record from Korea. To confirm the phylogenetic placement of these species, we conducted the phylogenetic investigation based on ITS sequences. In addition, we observed the morphological characteristics including macro- and micro-scopic characters. Specimens of KA11-0060-1 and KA11-0060-2 are described as a new species, for the first time collected from a natural habitat on the beach. In addition, *X. tentaculata* is formally reported as a new to Korea.
Keywords : ascomycota, morphology, phylogeny, taxonomy, Xylaria

A048

Emticicia Cheonanensis sp. nov.

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A novel bacterial strain, designated JBR12^T, was isolated from the sediment of a shallow stream in Cheonan, Korea. Phylogenetic analysis based on 16S rRNA gene sequences showed that the strain JBR12^T belonged to the genus *Emticicia* of family *Flexibacteraceae* and indicated that the closest relate type species were *E. oligotrophica* DSMZ 17448^T (97.8% sequence similarity) and *E. gensengisoli* KCTC 12588^T (94.3%). The DNA-DNA hybridization experiment revealed less than 70% of genomic relatedness between strain JBR12^T and *E. oligotrophica* DSMZ 17448^T. The major fatty acids (>5% of the total fatty acids) are iso-$C_{15:0}$, summed feature 3 ($C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$), iso-$C_{15:0}$ 3-OH, anteiso-$C_{15:0}$ and iso-$C_{15:0}$ 3-OH. The DNA G+C content of the type strain is 37.7 mol%. On the basis of polyphasic taxonomic approach, strain JBR12^T is considered to be a novel species of the genus *Emticicia*, for which the name *Emticicia cheonanensis* sp. nov. is proposed. The type strain is JBR12^T (=KACC 17466^T = JCM 19321^T). punctuation and start all major words with small letters
Keywords : Emticicia cheonanensis

A049

Actinomycetes: as an Important Source of Secondary Metabolite

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Actinomycetes produce diverse secondary metabolites which have the primary importance in medicine, agriculture and food production. Until now, almost 60% of well-known 10,000 different kinds of antibiotics are from actinomycetes and recently they are potential candidates for treatment of cancers or diseases which caused by viruses. In this research, actinomycetes as the useful resource were isolated from multiple soil and were analysed 16S rRNA sequences, enzymes(protease, amylase, lipase, cellulase) and antimicrobial activities, culture conditions depend on temperature, pH, NaCl and LC/MS profiles. Ultimately, we gather and supply not only actinomycetes secondary metabolites, but also their informations of valuable characteristics and new functions, so that the researches can speed up the development of bio-R&D, strengthen the competitive power of bio-industry. This research was supported by a grant(NRF-2013M3A9A5076601) from the Ministry of Science, ICT and Future Planning of the Korea Government
Keywords : actonomycetes, antimicrobial activites

A050

Phylogenetic Relationship within *Flammulina velutipes* Strains Based on Genome-Wide SNPs

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Next generation sequencing data was generated from 25 strains of *Flammulina velutipes* with different phenotypes using Illumina Hiseq platform. Filtered short reads were initially aligned to the reference genome (KACC42780) to construct a SNP matrix. And then we built a phylogenetic tree based on the validated SNPs. The inferred tree represented that white- and brown- fruitbody forming strains were generally separated although three brown strains, 4103, 4028, and 4195, were grouped with white ones. This topological relationship was consistently reappeared even when we used randomly selected SNPs. Group I containing 4062, 4148, and 4195 strains and group II containing 4188, 4190, and 4194 strains formed early-divergent lineages with robust nodal supports, suggesting that they are independent groups from the members in main clades. To elucidate the distinction between white-fruitbody forming strains isolated from Korea and Japan, phylogenetic analysis was performed using their SNP data with group I members as outgroup. However, no significant genetic variation was noticed in this study.

A051

Types of CagA 3'-Region of *Helicobacter pylori* Korean Isolates Recovered from Patients with Chronic Gastritis, Peptic ulcer, and Gastric Cancer

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H. pylori has infected to a half of worldwide population causing various gastro-duodenal disorders of mankind like chronic active gastritis, gastric atrophy, and peptic ulcer as well as gastric cancers. Several studies have reported an increased prevalence of CagA-positive *H. pylori* in gastric cancer. However, most of Korean isolates have shown are *cagA* positive irrespective of clinical symptoms. The structure of CagA gene reveals a 5'highly conserved region and a 3'variable region. The 3' region of the CagA gene shows a number of repeat sequence containing EPIYA motifs which are tyrosine phosphorylated by Src and Abl family kinases resulting in the impairment of a variety of intracellular signaling systems. One hundred and thirty-one Korean isolates of *H. pylori*, which were recovered from patients with chronic gastritis, peptic ulcer, and gastric cancer, were subjected to PCR-sequencing to analyze the repeat patterns of the CagA 3'-region. As a result, five types (designated types 1, 2, 3, 4, and 5) could be identified depending on the type and number of repeats. Although most strains were classified into type 1, type 4 and 5 were significantly found in the gastric cancer than other disorders. The results demonstrated that the repeat pattern of CagA 3'-region might be one of the feasible markers for virulence among of *H. pylori* Korean isolates.
Keywords : Genotyping, *Helicobacter pylori*, CagA, Gastric diseases

A052

Characterization of Human Pathogens Unidentified Using Automated Systems

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To characterization of human pathogens unidentified using automated systems, a total of 437 strains were received from regional branch of National Culture Collection of Pathogens. The sources were divided into 10 categories, and pathogens from circulatory systems were most abundant, 119 strains. Based on the 16S rRNA gene analysis, the strains were assigned to Proteobacteria (51%), Firmicutes (37%), Actinobacteria (12%) and Bacteroidetes (<0.1%). At genus level, *Bacillus* (11.9%), *Acinetobacter* (8.0%), *Streptococcus* (7.8%), *Corynebacterium* (7.6%), *Enterococcus* (7.3%), *Pseudomonas* (7.3%) and *Enterobacter* (6.6%) were the main genera. 1) the results between VITEK and 16S rRNA analysis were not consistent for 184 strains, 2) the VITEK ID results were not clear for 161 strains, 3) mixed cultures were observed for 24 strains, and 4) no VITEK data were provided for 68 strains. For mixed strains, pure cultures were obtained and analyzed. A total of 145 strains, including 86 strains of case 1) and 59 strains of case 2), were identified to species that are absent in the VITEK database. Eight strains exhibiting less than 98.5% 16S rRNA gene similarity with known species were selected as the primary candidates for new species, and 24 strains exhibiting 98.6~99.0% similarity were also selected as possible candidates. For 8 strains, growth at different temperature, pH and salinity as well as 20 enzyme activities were tested. Phylogenetic trees were inferred for all 32 candidates.
Keywords : Human pathogens, Automated ID systems, 16S rRNA analysis

A053

Novel Lactic Acid Bacteria Isolated from the Gut of the Asian Honey Bee *Apis cerana*

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Bacteria inhabiting the gut of the Asian honey bee, *Apis cerana*, were isolated using various media under aerobic and anaerobic conditions. Among the isolates, two bacterial strains C1^T and C4^T showed the highest 16S rRNA gene similarity to *Lactobacillus apis* R4B^T (97.1% and 97.8%, respectively) among type strains. DNA-DNA relatedness between strains C1^T and C4^T was 40.7% and those of R4B^T to C1^T and C4^T were 51.5% and 51.4%, respectively. Strains C1^T and C4^T were strictly anaerobic, Gram-staining-positive, non-spore-forming, non-motile, and rod-shaped bacteria. The strains grew at 20-45°C (optimum, 35°C) and at pH 4.0-6.0 (optimum, pH 6.0). They produced D-lactic acid from MRS broth. The genomic DNA G+C contents of strains C1^T and C4^T were 35.7 and 41.6 mol%, respectively. The predominant fatty acids (> 10%) were $C_{16:0}$ and $C_{18:1}$ ω9*c*. The strains also showed an inhibitory effect against the causal agent of American foulbrood, *Paenibacillus larvae* subsp. *larvae*. On the basis of evidence from our polyphasic taxonomic study, it was concluded that strains C1^T and C4^T should be classified as novel species of the genus *Lactobacillus*, for which, the name *Lactobacillus orientis* sp. nov. and *Lactobacillus ceranae* sp. nov., respectively, are proposed.
Keywords : honey bee gut, lactic acid bacteria, Lactobacillus, taxonomy

A054

***Halomonas garumicola* sp. nov., Isolated from Myeolchi-Jeot**

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A Gram-staining negative, moderately halophilic aerobic bacterium, designated strain SL-MJ3^T, was isolated from myeolchi-aekjeot, Korean traditional fermented fish sauce, which was made by the fermentation of anchovy (*Engraulis japonicus*) in Korea. Cells of the strain were short rods showing catalase-positive and oxidase-negative reactions. Growth of strain SL-MJ3^T was observed at 15-40°(optimum, 37°), at pH 6.0-9.0 (optimum, pH 7.0-7.5), and in the presence of 4-15 % (w/v) NaCl (optimum, 6 %). The G+C content of the genomic DNA was 63.4 mol % and the predominant ubiquinone was Q-9. Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain SL-MJ3^T formed a tight phyletic lineage with members of the genus *Halomonas* and was most closely related to *Halomonas ilicicola* SP8^T with 95.4 % of 16S rRNA sequence similarity. On the basis of phenotypic, chemotaxonomic and molecular features, strain SL-MJ3^T represents a novel species of the genus *Halomonas*, for which the name *Halomonas garumicola* sp. nov. is proposed. The type strain is SL-MJ3^T (=KACC 17856^T = JCM 30152^T).
Keywords : *Halomonas garumicola* sp. nov., myeolchi-aekjeot, fish sauce, taxonomy

A055

Re-study on the *Amanita virgineoides* Recorded in Korea

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Study on the Phylogenetics of molecular genetics to make sure 16 specimens recorded as *Amanita virgineoides* which were preserved in the Herbarium Conservation Center of NAAS in Wanju, were carried out by using the sequence data sets of ITS region and nLSU. Among them, 5 specimens were different from *Amanita virgineoides* and were close to *A. japonica* belonging in section *Lepidella* of subgenus *Lepidella* in phylogenetic relationship but did not exactly coined with *A. japonica.* Outhors propose this species as new scientific species, *A. pseudovirgineoides* belonging in section *Lepidella* of subgenus *Lepidella.*
Keywords : *Amanita virgineoides*, ITS, nLSU, phylogenetic relationship, section *Lepidella*

A056

***Absidia pseudocylindrospora*, a Newly Recorded Species from Dokdo Island, Korea**

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A fungal isolate EML-FSDY6 was isolated from a soil sample collected from Dokdo Island, Korea. The colonies of the isolate exhibited rapid growth on potato dextrose agar (PDA) at 27°C. The initial color of the colonies was grayish white, which later changed to grayish brown. The colony reverse was grayish white, with wavy zonation. The sporangiophores were 2.05-3.69 µm wide, erect, and arising from the stolon, a septum was always present 14.38-19.80 µm below the apophysis. The sporangia were 26.90-46.63×26.19-46.95 µm, were globose, deep gray, and multispored. The sporangiospores were discharged after the sporangial wall was deliquesced at maturity. The sporangiospores were cylindrical, measured 1.44-1.73×4.24-5.09 µm. Successfully amplified rDNA products were sequenced after PCR amplification and compared with the sequences of related Absidia species available in the GenBank database by using BLAST search. The analysis result indicated that the isolate belongs to pseudocylindrospora clade including *A. pseudocylindrospora* FSU 5893 (GenBank Accession numbers EF030525) with 96 % homology. The species presented here has not been reported in Korea

A057

A New Fungal Species of *Annulohypoxylon* From Rice Seed in Korea

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A fungal isolate (EML-HPR1-17) was isolated from indigenous rice seed using blotter method. Based on the morphological characteristics and rDNA ITS (internal transcribed spacer) and LSU sequence analysis, the isolate was identified as an *Annulohypoxylon* species which belongs to Xylariacea. The percent sequence identity (the number of matches/the complete alignment length) values of EML-HPR1-17 represented 98.15% (585/596) with *Annulohypoxylon* sp. (GenBank accession no. DQ840057). The fungus was characterized by abundant hyaline conidia (asexual spores) borne on branching tree-like conidiophores on PDA medium. The conidial shape appeared to be rugby ball and the size was 2.46–4.26 (avg. 3.50) µm wide x 5.36–7.30 (avg. 6.33) µm long. The present study revealed that the EML-HPR1-17 isolate is a new *Annulohypoxylon* species as a seed-borne fungus from indigenous rice seed in Korea.

A058

New Record of Endophytic *Paraconiothyrium brasiliense* from Chinese Maple Leaf in Korea

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The fungal endophyte EML-CM25 was isolated from surface sterilized leaf tissues of Chinese maple plant in Korea. Internal transcribed spacer (ITS) sequence analysis indicated that the isolate was closest to *Paraconiothyrium brasiliense* (Genbank accession no. JF502455) with 99% sequence similarity. The EML-CM25 isolate was cultured on PDA for 7 days at 25ºC. Aerial hyphae were absent on colony and conidiomata grown after 1 week of incubation. Rust like structure was formed on center and visible scattered black dots were present at the colony. The fungal species has been found in diverse environments-eg. *Coffee arabica* plants in Brazil, surface water in Japan, discolored wood in Italy etc. This is the first record of *P. brasiliense* in Korea.

A059

New Record of *Talaromyces amestolkiae* as Endophyte from *Ficus* sp. Tree in Korea

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The endophytic fungus EML-NCP50 was isolated from surface sterilized tissues of *Ficus* sp. tree leaves in Korea. Based on the morphological characteristics and internal transcribed spacer (ITS) sequence analysis the isolate was identified as *Talaromyces amestolkiae* (GenBank accession no. JX965214) with 99% sequence similarity. The colony of EML-NCP50 grown on yeast extract sucrose agar (YES) at 25°C were greenish with white round, reverse color was deep red. After 7 days of incubation on MEA, CYA and YES at 25°C, the colony size was 34-44, 21-24 and 34-37 mm in diameter, respectively. The fungus on MEA medium produced floccose and funiculose texture which is known as a distinguishable character of the species. *T. amestolkiae* is potentially pathogenic to immuno-compromised persons. This is the first record of *T. amestolkiae* in Korea.

A060

***Penicillium malusis* sp. nov. from fruit of *Malnus pumila* in Korea**

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Post-harvest disease caused by *Penicillium* species was observed on apple. A storage fungal isolate, EML-MP80 was purely isolated from post-harvested apple (*Malnus pumila*) fruit . Based on the morphological characteristics and rDNA ITS (internal transcribed spacer), β-tubulin and calmodulin genes sequence data, the fungus was identified as new *Penicillium* species. ITS sequence analysis indicated that the EML-MP80 isolate was closest to *Penicillium* sp. 3 (accession number, AJ004820) with 99% identity value. However, β-tubulin and calmodulin sequence similarity represented 97% with *P. hirsutum* (AY674328) and *P. allii* (AY678564)The colony of the fungus on CYA was velutinous, green or dull green and cream to yellow cream on reverse side. The penicillus of the fungus was composed of two-stage branched (terverticillate). The results of molecular phylogenetic analysis of multi loci and morphology study showed that the isolate formed each disctinct lineage, showing that it is a new *Penicillium* species.

A061

First Report of Post-harvest Fruit Rot of *Aronia melanocarpa* caused by *Geotrichum candidum* in Korea

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A fungal isolate (EML-CCB3) of *Geotrichum* was purely isolated from decaying fruit of black chokeberry (*Aronia melanocarpa* (Michx.) Elliott). Based on the morphological characteristics and rDNA ITS sequence analysis, the isolate was identified as *G. candidum*. The colony EML-CCB3 isolate on PDA was white, smooth (not shiny), with fruity odor. The diameter of arthrospores were 3.62–5.06 (avg. 4.30) µm wide x 5.12–7.15 (avg. 6.45) µm long. The sequence identity (the number of matches/the complete alignment length) value of EML-CCB3 isolate represented 98.18% (325/331) with *G. candidum* (GenBank accession no. KJ755081). Phylogenetically, EML-CCB3 isolate was placed within a clade corresponding to *G. candidum* with strong bootstrap support. So far, fruit rot by *G. candidum* has been reported on oriental melon, tomato, cucumber, potato, pumpkin and carrot in Korea. To our knowledge, this is the first report of post-harvest fruit rot of black chokeberry caused by *G. candidum* in Korea.

A062

A New Fungal Species of *Paraconiothyrium* From Rice Seed in Korea

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Paraconiothyrium is a recently established genus within the order *Pleosporales*. A fungal isolate EML-HPR1-12 was isolated from indigenous rice seed using blotter method. Based on the morphological characteristics and rDNA ITS (internal transcribed spacer) sequence analysis, the isolate was identified as a *Paraconiothyrium* species which belongs to *Montagnulaceae*. The percent sequence identity (the number of matches/the complete alignment length) values of the isolate represented 98.79% (492/498) with *Paraconiothyrium* sp. (GenBank accession no. JF502423). Typical conidiomata and pycnidia were not produced on PDA medium but black-walled hyphae were observed. The present study revealed that the EML-HPR1-12 isolate is assigned to a new *Paraconiothyrium* species as a seed-borne fungus from rice seed in Korea.

A063

Recent Applications of Phylogeny to the Systematics of Genus *Cordyceps* Fr.

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Cordyceps Fr. (Hypocreales, Ascomycota) in broad sense includes pyrenomycetous species that produce club-shaped or cylindrical stromata on insect cadavers or hypogeal/sclerotial fungi. Entomogenous species of *Cordyceps* show a lot of variation in hosts and ecological ranges. Lepidoptera and Coleoptera are the most common hosts followed by Hymenoptera, Hemiptera, Orthoptera, Diptera, Araneae etc. Ecologically, *Cordyceps* species grow on grounds or subterranean regions as well as in aerial regions on tree trunks or leaves. Among the morphological characters, *Cordyceps* species show variations in texture and color of stromata, position, arrangement and location of perithecium, and shape and size of ascospores, part-spores etc. *Cordyceps* has been recently shown as polyphyletic and consequently has been separated into different phylogenetic genera such as *Ophiocordyceps*, *Metacordyceps*, *Elaphocordyceps*, *Polycephalomyces* etc. Recent changes in the International Code of Nomenclature for algae, fungi, and plants (ICN) have replaced binomial system of fungal nomenclature with One Fungus = One Name concept. This has led to protection of a single genus name against the allied genus names in a monophyletic group. Recent nomenclatural changes of *Cordyceps* are here presented with special reference to economically important *Cordyceps* species.

Keywords : Elaphocordyceps, Metacordyceps, Metarhizium, Ophiocordyceps, Polycephalomyces

B001

Pyrosequencing Analysis of Animal Carcass Leachate from Lysimeter to Detect Bacteria Responsible for Pathogenicity

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A serious outbreak of foot and mouth disease (FMD) led to the burial of millions of animal carcass in South Korea from late 2010 to April, 2011 and potential public health impacts by leachate of carcass burial have been issued. Quantitative detection of pathogenic microorganisms such as *Salmonella*, *Shigella*, *Clostridium*, and *Campylobacter*, requires accurate enumeration of microbe in hazard-containing leachates as part of the risk characterization process. Because of these requirements, the PCR became a powerful tool in microbiological diagnostics the last decade. The view of a risk management-oriented study of leachate from animal carcass burial, we investigated the utility of the quantitative real-time PCR (qPCR) for surveying the *Salmonella* strains using specific primers of leachate from animal carcass burial. In this study, the leachate samples were monthly corrected and extracted genomic DNA from lysimeters (unit-1 and unit-2) which were constructed to compare the effects of calcium oxide (CaO) on decomposition of animal carcass burial. This methodology can contribute to meeting the increasing demand of rapid, sensitive, and specific assurance laboratories for the standard pathogenic detection method.
Keywords : Pathogen, Salmonella, Real-time PCR, Leachate, Carcass burial

B002

A New Paradigm in *Bdellovibrio bacteriovorus* Life and Ecology: Attack Phase Cells Secrete Proteases that Disperse Gram-Positive Biofilms

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Bdellovibrio bacteriovorus is a predatory bacterium which lives by invading the periplasm of Gram-negative bacteria. We found that wild type *B. bacteriovorus* is also capable of removing Gram-positive biofilms, *e.g.*, *Staphylococcus aureus*, in both 96-well plates and on silica chips and that the addition of only 10% of a cell-free predatory culture supernatant reduced the biofilms by about 70%. This activity was mainly due to the secretion of serine proteases. Attack phase *B. bacteriovorus* cells also produced and secreted proteases in the absence of prey and in a nutrient dependent manner. This impact on Gram-positive biofilms was not limited only to staphylococci but was seen with other Gram-positive bacteria from various genera. Interestingly, the addition of the cell-free predatory supernatant didn't cause a reduction in the biofilms formed by any of the Gram-negative bacterial strains tested. *B. bacteriovorus* cultures, however, were able to remove biofilms composed of both *S. enterica* and *S. aureus* by more than 80%. Consequently, the results suggest that predatory bacteria may use the secreted proteases to remove Gram-positive, non-prey bacteria from mixed biofilms so as to have better access to their prey. They also imply that the ecological impact of *B. bacteriovorus* on microbial communities and biofilms is much greater than previously thought, as they don't only mitigate Gram-negative biofilms but Gram positive ones as well
Keywords : Bdellovibrio, Staphylococcus, Biofilm, Proteases, Salmonella

B003

Population Changes and Physiological Characteristics of Halophilic Bacteria during each Evaporation Steps in Solar Salterns of Jeungdo

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This research is on halophilic bacteria inhibiting in salt pond, conducted by measuring the population density of bacterial halophiles with different concentration of diluted salt solution, and composition of cultivating ground in each step of salt production, targeting solar salterns in Jeungdo, Shinangun, JeollaNam-do, Korea. Examining the population densities after using diluted solutions of 3% and 15% (of NaCl) to Marine agar 2216, each changed in the range of $1.5 \pm 0.3 \times 103 \sim 1.1 \pm 0.1 \times 106$ cfu ml-1 and $1.9 \pm 0.8 \times 103 \sim 1.6 \pm 0.2 \times 106$ cfu ml-1, respectively. In addition, after examining the population densities using the diluted solution of 3% and 15% on the culture ground produced by adding 15% of NaCl to Marine agar 2216, the number of the cells varied in the range of $2.8 \pm 1.2 \times 102 \sim 1.5 \pm 0.3 \times 105$ cfu ml-1 and $2.3 \pm 0.5 \times 10 \sim 3.2 \pm 0.7 \times 105$ cfu ml-1, respectively. The decomposition rate of protein, lipid, cellulose, and starch, has been experimented with separately identifying 33 strain kinds that appears as dominants in salt pond. Overall, the bacteria with decomposition capability of all 4 components were Micrococcus luteus, Nocardioides daejeonensis in Actinobacteria group, Halobacillus trueperi in Firmicutes group, and Aurantimonas coralicida, Lutibacterium anuloederans, Nesiotobacter exalbescens in Alphaproteobacteria group.
Keywords : Halophilic bacteria, Solarsaltern, Jeungdo

B004

Isolation and Characterization of *Sphingobium* sp. EP60845, a Ethoprophos-degrading Bacterium

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A noble bacterium able to rapidly degrade the organophosphate ethoprophos pesticide was isolated from forest soils. A phylogenetic analysis based on the 16S rRNA sequence showed that the strain belong to the genus *Sphingobium*, and might be a novel speci. The strain could utilize ethoprophos as its sole source of carbon. One hundred mg/l could be degraded to a nondetectable level in 5h by EP60845 in soil extract broth culture. Also, the inoculation of strain EP60845 (10^6 cells g^{-1}) to soil treated with 250mg ethoprophos kg^{-1} resulted in a higher degradation rate than in noninoculated soils. Inoculumn densities as low as (10^6 cells g^{-1}) were sufficient to degrade a fresh addition of ethoprophos within 7 days. The results indicate that the ethoprophos-degrading *Sphingobium* sp. EP60845 was efficient as a bioremediation agent in a range of environmental and soil conditions.
Keywords : Bioremediation, Ethoprophos-degrading, Sphingobium

B005

Biosorption of Anionic Dye and Gold by the Novel Biosorbent *Bacillus* sp. JB-007 Strain

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Biosorption is a promising technology to remove ionic pollutants such as heavy metals, anionic/cationic dyes, and to recover precious metals. The main aim of this work was to evaluate the biosorption capacity of *Bacillus* sp. JB-007 (JB-007) biomass for the removal of anionic dye Reactive Red 4 (RR 4) and recovery of precious metal gold (Au (I)), as the industrial materials. In the pH edge experiments, the adsorption of RR 4 and Au (I) increased in acidic pH. The equilibrium isotherm experiments indicated that JB-007 biomass exhibited the maximum uptake for RR 4, i.e. 192.03 mg/g at pH 2.0 and 140.40 mg/g for Au (I) at pH 2.5. Of the two isotherm models considered, the Langmuir model provided a better description of the experimental isotherms. Kinetic experiments revealed the RR 4 and Au (I) sorption processes were found to be very rapid, and the equilibrium of the sorption processes could be reached within approximately 10 min. To confirm the surface morphology and functional groups, FE-SEM, EDX, XRD and FTIR analyses were carried out, and the results revealed that the biomass of JB-007 has surface functional groups capable of binding to anionic pollutants. These results indicate that *Bacillus* sp. JB-007 biomass has good properties as an industrial biosorbent for the removal of anionic dye and recovery of precious metal from wastewater (This subject is supported by Korea Ministry of Environment as "The Eco-Innovation project").
Keywords : Biosorption, Biomass, Ionic Dye, Precious Metal, Gold

B006

Removal of Cationic Dye and Heavy Metal by *Bacillus* sp. JB-017 Biomass

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Microbial biomass has great potential for the removal of ionic pollutants and heavy metals from aqueous solution. This study evaluated the biosorption capacity of *Bacillus* sp. JB-017 biomass for the removal of cationic dye Basic Blue 3 (BB 3) and heavy metal cadmium (Cd(Ⅱ)) from the aqueous solution. In the pH edge experiments, the adsorption of BB 3 and Cd (Ⅱ) increased with increasing pH. The equilibrium isotherm experiments showed that the maximum adsorption capacity was 127.2 mg/g of BB 3 (pH 9.0) and 46.85 mg/g of Cd (Ⅱ) (pH 6.0). The Kinetic data in the presence of BB 3 and Cd (Ⅱ) revealed the equilibrium could be reached within approximately 5 min and 50 min, respectively. FT-IR analysis showed the presents of major functional group on the biomass surface, i.e. carboxyl and phosphate groups. From all of our data, the *Bacillus* sp. JB-017 biomass has good properties as a biosorbent for the removal of cationic dyes and heavy metals from wastewater.
[This subject is supported by Korea Ministry of Environment as “The Eco-Innovation project”]

B007

Macrofungi in the Coastal Sand Dune of Taean peninsula in Korea

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Macrofungi distributions in coastal sand dune were investigated primarily at six sites in Taean peninsula. Plant community was mainly composed of herbs (Gramineae and Cyperaceae) and trees (*Pinus thunbergii* and *Robinia pseudoacacia*) in the study areas. A total of 44 fungal species were observed on the ground of the coastal sand dune in this study. The 35 genera and 44 species were identified by field examination and lab examination on the mushroom samples collected from the sites. As a result of the identification study, *Lysurus periphragmoides, Marasmiellus mesosporus, Mattirolomyces terfezioides, Peziza ammophila, Psathyrella ammophila*, and *Tulostoma brumale* have recognized as unrecorded species in Korea. For the unidentified mushroom samples collected from Taean peninsula, it is concerned that there is a need to study more for the future work.
Keywords : Coastal sand dune, Macrofungi, Taean peninsula

B008

The Effect of Bacterial Membrane Vesicles against BALO Predation

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The bacterial membrane vesicles were reported as playing important roles in bacterial community. As ecological view, they were involved in biofilm formation and pathogenesis. In this study, the new aspects of these bacterial membrane vesicles were suggested with showing the delay of predation by Bdellovibrio bacteriovorous HD100. To illustrate the impacts of the bacterial membrane vesicles between the predator and prey, we applied mutants of prey which produced more membrane vesicles in different levels. The mutant of E. coli, ΔDegP and ΔNlpI were selected and predation was compared with wild type host strain, E. coli BW25113. Confirmation could be accomplished by CFU and bioluminescence of prey, and results suggest short term of predation delay for 2 hour. It suggested that these bacterial membrane vesicles potentially were acting like decoys and blocking the predator to bind to the prey. These findings will provide ecological interactions between the bacterial membrane vesicles and Bdellovibrio bacteriovorous HD100 predation in nature.
Keywords : Bdellovibrio bacteriovorus, Bacterial membrane vesicles, Prey resistance, Predation, Bacterial interactions

B009

Assessment of Airborne Bacterial and Fungal Concentrations in Public Facilities

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This report examined the concentration of bacteria and fungi distributed in the indoor air, orienting four public facilities (library, hospital, theaters) located in Gunsan, Jeollabuk-Do, Korea, from August of 2013 to June of 2014. The collection of microorganisms, by using the impaction-type air sampler (Merck MAS 100Eco, Germany), and measured by indoor air quality testing methods of Ministry of Environment (test from the Ministry of environment 2010-24). Tryptic soy agar and Sabouraud dextrose agar were used for sampling and measuring the number of bacteria and fungi. The distribution of bacteria in the library were in a range of 2.0 ± 0.1 × 10 ~ 6.4 ± 0.1 × 102 CFU/m3, that of hospital were in a range of 3.4 ± 0.3 ~ 6.8 ± 0.1 × 102 CFU/m3, that of theaters were in a range of 7.0 ± 0.1 × 10 ~ 9.9 ± 0.3 × 102 CFU/m3. The distribution of fungi in the library were in a range of 2.0 ± 0.1 × 10 ~ 9.4 ± 0.3 × 102 CFU/m3, that of hospital were in a range of 1.0 ± 0.1 ~ 6.8 ± 0.4 × 102 CFU/m3, that of theaters were in a range of 3.0 ± 0.1 × 10 ~ 7.3 ± 0.2 × 102 CFU/m3. When we examined this by season, overall tendency did not coincide since they were the place separated into independent spaces. However, we could check that the number of germs were shown relatively higher in autumn, and it is judged that more systematic research regarding the correlation analysis of environmental factors in indoor environment is needed in the future.
Keywords : airborne bacterial, airborne fungal, indoor air, public facilities

B010

Isolation of PHA(Polycyclic Aromatic Hydrocarbons) degrading Bacteria from Chinese Desert Lichens

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Endolichenic bacteria have been recently studied for their diversity, but very little has been known on their functions in terms of lichen symbiosis. In the present study, we obtained 106 bacteria strains from Chinese desert lichens by enrichment culture method using polycyclic aromatic hydrocarbons (PAH) as a carbon source without nitrogen source. We increased the concentration of PAH chemicals to 1000mg/L to isolate endolichenic bacterial strains having higher PAH-degrading activity, and then 12 bacterial strains were obtained. Most of these bacteria were identified as *Burkholderiales* sp. by 16s rDNA sequences, which belong to *Betaproteobacteria*. The bacterial strains grew very well in the enriched medium and reached stationary growth phase within 4 days. The bacteria also grew well on the medium amended with lichen extracts as a single nutrient source, indicating that the bacteria can survive in extreme environments such as deserts and very arid areas by harboring inside of lichen thalli as a shelter and utilizing lichen substances as a nutrient. This result also suggests the bacteria are capable of fixing nitrogen which can be used by lichen symbionts. The successful culture of endolichenic bacteria is important not only to study lichenism of endolichenic microbial community, but also to provide new biological resources to clear up non degradable compounds such as PAHs in the contaminated environment.
Keywords : endolichenic bacteria, lichen, PHA-degrading bacteria, nitorgen fixation

B011

Combined Application of Cyanobacteria with Sand Fixing Chemicals for Artificial Induction of Biological Soil Crust under Laboratory Conditions

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Biological soil crusts (BSCs) have a lot of positive effects on desert ecosystems. For restoring the degraded desert soils, artificial induction of biological soil crusts is one way of solutions. This study was carried out to accelerate of artificial BSCs and improve the aggregate stability of initial stage of artificial BSCs. The combinations of cyanobacteria and sand fixing agents were applied on the sand surface under laboratory conditions. Cyanobacterial consortium (*Phormidium* sp., *Nostoc* sp., and *Scytonema* sp.) were applied on the sand surface at 10 mg FW/cm^2, SAP (superabsorbent polymer) were applied at 0.05 mg/cm^2 and T7 (fixing agent) were applied 0.1 (T1), 0.2 (T2), 0.3 (T3), 0.4 (T4), 0.5 (T5) and 0.6 (T6) mg/cm^2 then incubated at 25°C under 100 μmol photons m^{-2} s $^{-1}$ for 12 hrs per day for 3 months. Single use of Sand (C), SAP sprayed sand (S), T7 sprayed sand (T), SAP and T7 sprayed sand (ST), cyanobacteria sprayed sand (A), cyanobacteria and SAP sprayed sand (AS), cyanobacteria and T7 sprayed sand (AT) and cyanobacteria, SAP and T7 sprayed sand (AST), respectively. In the surface hardness test and aggregate stability test, the values of AT6 was significantly higher than any other treatments but similar with AST treatments. In conclusion, combined application of T7, SAP and cyanobacteria improve early settlement of cyanobacteria and strong resistance against rainfall event compared with single application of cyanobacteria in the destroyed arid regions.
Keywords : biological soil crust(BSC), cyanobacteria, arid environment, sand fixing chemicals, restoration

B012

Effects of UV Exposure Time on Biodegradation of Poly(L-lactide)(PLA) by a Mesophilic Bacterium

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Poly(L-lactide) (PLA) is one of the most economically competitive and environmentally benign polymers. Therefore, the use of PLA is expected to increase greatly in the future. Although PLA is a biodegradable polymer, its degradation should be studied comprehensively to cope with potential widespread use of this polymer, which may lead to environment contamination due to discharge of a huge amount of waste. PLA lost molecular weight and tensile properties quickly when exposed to UV irradiation. Biodegradation of PLA was examined in the modified Sturm test apparatus set up according to ASTM D 5209-91 by measuring the net amount of CO_2 evolved from mineral medium loaded with PLA by *Stenotrophomonas maltophilia* LB2-3. The apparent biodegradability of PLA reached a maximum at 8 h of UV exposure and then decreased with further increase in UV exposure. It was not generally expected that the longer the exposure, the lower the molecular weight of PLA and, thus, the higher the biodegradability. The same behavior was also observed when PLA degradation was carried out in compost. UV irradiation may transform PLA into a brittle white solid which may be poorly assimilated by microorganisms when PLA exposed to UV for longer than 8 h.
Keywords : Biodegradation, Poly(L-lactide), UV irradiation

B013

Concentration-dependent Effect of Red Clay on Change of Soil Microbial Community and Enzyme Activity

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Red clay is reported to promote plant growth and diesel biodegradation in soil by stimulating bacterial growth. To assess the concentration-dependent effect of red clay, bacterial communities of soil microcosms amended with different concentrationof red clay were monitored via PCR-DGGE, which indicated that low concentration of red clay (0.1%) supported the highest bacterial diversity while high concentration of red clay (5%) was toxic. This concentration-dependent effect was also confirmed by the growth of a soil bacterium *Acinetobacteroleivorans* DR1 on hexadecane, whose growth was enhanced and inhibited by 0.1% and 5% red clay, respectively. Recovery of DR1 growth under 5% red clay could be possible by adding glutathione, which implicated that the oxidative stress might be caused by 5% red clay. Reduction of soil enzyme activities such as FDA hydrolase and urease supported our observation under 5% red clay condition, not low concentration. Long-term (1 year) monitoring of bacterial community in arable field soil suggested that 0.5% red clay stabilized and maintained the community composition and diversity. Importantly, the proliferation of N-fixing bacteria *Bradyrhizobium* in red clay-amended soil provided the possible mechanism of red clay on plant growth promotion. Correlation between plant growth and concentration of red clay will be monitored.
Keywords : bacterial community analysis, pyrosequencing, PCR-DGGE, soil enzyme activity

B014

Incidence and Molecular Characterization of Hepatitis A Viruses in Korean Surface Water

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Several bodies of surface water in the Republic of Korea were surveyed for the presence of hepatitis A virus (HAV), between 2007 and 2010. Out of 265 surface water samples, 9 (3.4%) were HAV-positive. HAVs were mainly detected in the summer (3/62, 4.8%) and spring (4/96, 4.2%) seasons. Comparing different water sources, the highest prevalence (6.6%) of positive samples was seen in lake water, with four HAV-positive samples from lakes. Comparing prevalence rates across the four representative Korean basin systems, no HAVs were found in the Han or Nakdong river basins, the highest HAV prevalence was seen in the Yeongsan river and other basins (6.3%), and the Geum/Seom river also showed a high HAV prevalence (5.7%). HAVs from the nine positive samples were then sequenced and analyzed phylogenetically. Two of the HAVs belonged to genotype IA and fell within the same cluster as HAVs 6-3(ASAN4) (EU049548), KANSAN-PS1 (EU049554), and ASAN-KM (EU049563), which were collected from the stool of patients with gastroenteritis in Korea. The seven other HAV nucleotide sequences belonged to the genotype IB cluster. This is the first nationwide surveillance of HAV in major Korean water sources.
Keywords : fecal-oral route transmission, hepatitis A viruses, Korean basin systems, source water

B015

Biodegradation of Endocrine Disruptor by Laccase from *Pycnoporus coccineus*

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Laccase has shown efficient ability to degrade phenolic compounds and this ability makes it recognize to key-enzyme for bioremediation. We isolated and purified laccase from 7 different strains of *Pycnoporus coccineus*. We measured activity of the purified enzyme by ABTS, and confirmed the specific activity of laccase through it. The purified laccase was reacted with 3 substrates bisphenol A, biphenyl, and bis(2-ethylhexyl) phthalate known as endocrine disruptor. To confirm the level of degradation of endocrine disruptors, The UV-vis spectra were analyzed and confirmed various degrees of degradation. Through this data, the *Pycnoporus coccineus* laccase could degrade endocrine disruptors and was expected to have great effects on bioremediation of polluted environments by bisphenolA, biphenyl, bis(2-ethylhexyl) phthalate.
Keywords : Laccase, Bioremediation, Biodegradation, Endocrine disruptor

B016

Monitoring of Fecal Pollution in the Partly Restored Urban Stream, Cheonggye-cheon

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Cheonggye-cheon is a partly restored stream located in the center of a big city, Seoul, Korea. It is beloved by citizens, and used as a place of recreation. The precise monitoring of water quality for the sake of human safety is of great concern both in terms of its management and its use. In order to monitor fecal pollution, Total Coliforms (TC), Fecal Coliforms (FC), and *E. coli* were enumerated using the Colilert-18 system by the most probable number (MPN) method. Some of the presumptive TC, FC and *E. coli* colonies were identified using their 16S rRNA sequences in order to assess the validity of TC, FC and *E. coli* quantification. As a result, the total *E. coli* count provided a better reflection of the fecal polluted state of the stream better than TC and FC. The main pollutants were the inflow of fecal polluted ground water and two fecal polluted branch streams. In addition, the fecal polluted state of the shallow stream became worse on days with heavy rain because untreated sewage from its collecting facility ran over into the stream. Furthermore, the detachment of fecal indicators attached to green algae (*Spirogyra* sp.) or on plant stems (*Phragmites* sp.), and the re-suspension from sediment into the water body might deteriorate the water quality. Therefore, for proper control of the water quality, the above mentioned main pollutants should be removed, and it is suggested that an appropriate standard based on *E. coli* rather than TC and FC should be established
Keywords : fecal indicators, false positive, microbial water quality, rainfall

B017

Analysis of Rhizosphere Soil Bacterial Communities on Seonginbong, Ulleungdo Island

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The study of microbial diversity in soil samples as a volcanic island located in the east of South Korea Ulleungdo. The soil bacterial communities on Ulleungdo were analyzed using pyrosequencing method based on 16S rRNA gene. There were 1,613 operational taxonomic units (OUT) form soil sample. From results of a BLASTN search against the EzTaxon-e database, the validated reads (obtained after sequence preprocessing) were almost all classified at the phylum level. Proteobacteria was the most dominant phylum with 48.28%, followed by acidobacteria (26.30%) and actionbacteria(6.89%). Alphaproteobacteria was the most dominant class with 36.07%. Bradyrhizobiaceae was the most dominant family with 22.83%.
Keywords : Ulleungdo, pyrosequencing, 16s rRNA, bacterial community

B018

Fungal Diversity From the Root of Plants Inhabiting Goraebul Sand Dune and the Analysis of Biochemical Test

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Six plant species were collected from Goraebul sand dune in the east coast of Korea to identify culturable endophytes present in their roots. The fungal internal transcribe spacer (ITS) region (ITS1-5.8SrRNA-ITS2) was used as a DNA barcode for identification of fungi. A total of 119 fungal strains were identified and categorized into 21 genera. The genus *Fusarium* accounted for the largest number of strains, followed by the genus *Penicillium*. Furthermore, using 5 statistical methods, the diversity indices of the fungi were calculated at the genus level. After comprehensive evaluation, the endophytic fungal group from *Carex kobomugi* ranked highest in diversity analyses. Additionally, the nutrients availability of fungal strains were tested with GEN III FF microplate (Biolog, Inc.). This study provides basic data on the sheds light on the symbiotic relationship between sand dune plants and fungi.
Keywords : Endophytic fungi, Coastal sand dune, Fungal diversity

B019

Proteomic Analysis of Diesel-degrading *Acinetobacter oleivorans* DR1 Revealed Important Proteins Involved in Mature and Dispersal Biofilms

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Acinetobacter oleivorans DR1, a diesel-degrading soil-derived bacterium forms biofilm that contributes to stress defense under harsh environment. The aim of this study was to identify differentially expressed proteins between biofilm and planktonic cells in both mature (24 h) and dispersal biofilms (48 h). Here, two-dimensional gel electrophoresis with MALDI-TOF mass spectrometry in duplicate experiments was conducted. Up-regulated 12 and 16 proteins among 49 and 60 differentially expressed proteins were chosen for further study in matured and dispersed biofilms, respectively, because levels of difference were higher than 1.5 fold. Functional of 12 proteins expressed in matured biofilm are linked to outer membrane (CirA, FepA), transport and metabolism (UbiE, GuaB, HutI), energy production and conversion (AcoB, FumC). Sixteen proteins in dispersed biofilm were related to energy production and conversion (EtfA, HutU, FumC), oxidative stress (AhpC), ion transport and metabolism (FabG). Three proteins were up-regulated in common: fumarate hydratase (FumC), putative alcohol dehydrogenase, TonB-dependent receptor like protein, which indicated that those 3 proteins could be maintained throughout biofilm. Our data showed that most upregulated outer membrane-related proteins and oxidative stress related proteins could contributed to biofilm maturation and dispersal, respectively. This study will improve our understanding of biofilm formation of environmental *Acinetobacter* strains.
Keywords : Biofilm, Proteme, oxidative stress, outer membrane

B020

Analysis of Archaeal Diversity in Dalseong Wetlands Using 454 Pyrosequencing

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Wetlands which are defined as a half-way world between terrestrial and aquatic ecosystem and they play important roles in water reservoir, purification efficiencies of pollutants including floating matter and metal. In this study, we investigated diversity of archaeal community in two different sample sites in wetland which one is near the factory area (DS-C) and the other is located in wetland (DS-N). The soil samples were collected from Dalseong wetland in Daegu, South Korea. and analyzed by 454 pyrosequencing methods. A total of 21,909 sequencing reads were obtained and 1,907 and 2,762 operational taxonomic units (OTUs) were observed in the sampling sites. In site DS-N, the number of OTUs and taxonomical analysis were confirmed that higher than the site DS-C. The phylum Thaumarchaeota, Euryarchaeota, and Crenarchaeota were detected, The phylum Thaumarchaeota was comprised that higest proportion in DS-N, in contrast the phylum Euryarchaeota was highest in DS-C. The results of metagenomic analysis based on the pyrosequencing indicated that a major portion of archaea are different between the two sites.
Keywords : Wetlands, Diversity, Archaea communities, Metagenomic analysis

B021

Genetic Diversity of Endophytic Fungi Associated with Halophytic Plants from the West Coast of Korea

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This study was conducted to explore the fungal endophyte communities and their genetic diversity, isolated from the roots of plants from the west coast of Korea. Ten halophytic plant species were collected for the isolation and identification of culturable, root-associated endophytic fungi from Gochang and Buan tidal flats of Korea. To evaluate taxonomic identifications based on comparative analysis, the fungal internal transcribe spacer ITS region (ITS1), 5.8SrRNA, and ITS2 were used as a DNA barcode for the identification of fungi. Then the result of the identification of 344 fungal strains were categorized into 39 genera. The genus Alternaria was the most abundant among the strains isolated. The analysis showed that main phylum was Ascomycota, majority of class was Dothideomycetes and Eurotiomycetes.The diversity indices were calculated at the genus level using statistical methods including Menhinik's index (Dmn), Margalef's index (Dmg), Shannon diversity index (H' and Simpson' index of diversity (1-D). The highest diversity index was obtained from the endophytic fungal group associated with the plant *Suaeda glauca Bunge*.
Keywords :

B022

Isolation of Endophytic Fungi to Promote Plant-growth from Dalseong Wetland Plants

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Total 6 hygrophytes, 3 species of plant were collected from Dalseong wetland to study association with endophytic fungi. A total 84 fungal strains were isolated from each plant roots using a root surface sterilization procedure. All of endophytic fungi were identified by using fungal ITS regions(ITS1, 5.8, ITS2) of large-subunit rRNA, fungi DNA barcode marker, and by using a BLAST search tool to compare with GenBank database of NCBI nucleotide blast. All fungal strains were taxonomically analyzed into 20 genera. The obtained sequences were used for multiple sequence alignment using a ClustalX program and aligned by using a bioedit program. Phylogenetic tree was generated using Mega program, neighbor-joining, 1000 bootstrap replicates to analyse isolated endophytic fungi phylogenetic relationship. Diversity of endophytic fungi was investigated at the genus level. Some of the strains of endophytic fungi produce phytohormones as secondary metabolites, especially gibbellin. All endophytic fungi culture filtrate, effectively be related to active of plant growth promoting, were applicated to waito-c rice for screening of plant growth promoting.
Keywords : Wetland, ITS, Endophytic Fungi, Waito-c rice, Plant Growth Promoting

B023

Quantitative-PCR Analysis on the Soil Colonization Ability of *Bacillus amyloliquefaciens* GR4-5

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The soil-based microcosm systems were used to investigate the soil colonization ability of *Bacillus amyloliquefaciens* GR4-5 at indoor, outdoor environment, and a field experiment, which was conducted at Gangwon Agricutlural Research and Extension Services, Cheolwon. Each sample of three experimental designs was collected at 3~4-day intervals over a four-week period and used for measurement of the number of 16S rRNA gene copies by quantitative-PCR. At outdoor condition, the amount of *Bacillus* spp. 16S rRNA gene copies number right after GR4-5 treatment is 5.6×10^5 copies/μl. Two weeks later, 16S rRNA gene copies number (4.9×10^3 copies/μl) is similar to the control figure (4.7×10^3 copies/μl). At indoor condition, concentration of *Bacillus* spp. 16S rRNA gene maintained certain levels for a longer time than both outdoor and field. The result of field experiment showed similar pattern compared with outdoor condition. Our result showed that *B. amyloliquefaciens* GR4-5 can colonize bulk soil for 2 weeks indicating that *B. amyloliquefaciens* as biocontrol agent should be used every 2 weeks for effective application against plant disease. It suggested that outdoor microcosm system designed in this study would be good method to assess the colonization ability of valuable microorganisms.
Keywords : quantitative-PCR, microcosm, colonization, *Bacillus amyloliquefaciens*

B024

Investigation of Norovirus Occurrence in Groundwater

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Groundwater is one of the main sources of drinking water. Its cleanliness is directly related to public health. Thus this study aimed to investigate norovirus contamination of groundwater. A total of 60 groundwater samples were collected. For the analysis of water quality, the temperature, pH, turbidity, and residual chlorine content were assessed. According to the standard procedure by National Institute of Environmental Research, 500-1500L of groundwater were filtered through an electropositive filter (NanoCeram¢ç) and used for detection of Norovirus and enterovirus. After filtration with a Nanoceram filter, approximately 20mL of water eluted, and viral RNA was extracted from the eluate using the QiAamp viral RNA minikit according to the manufacturer`s instructions. For Norovirus and enterovirus detection, RT-PCR and semi-nested PCR were performed. The temperature of groundwater were 15.78 (range, 12-22.3°C) in October. The average turbidity was 0 nephelometric turbidity unit (NTU) in October. The average pH was 7.81 (range, 6.7-8.64). Total coliform, fecal coliform and E.coli detected in 21 (35%), 5 (8%) and 5 (8%) of the 60 samples, respectively. With respect to norovirus, only the GII type was detected from 14 groundwater samples, and enterovirus was not detected. It is necessary to periodically monitor waterborne viruses that frequently cause epidemic food poisoning for better public health and sanitary conditions.
Keywords : groundwater, norovirus, enterovirus, total coliform, fecal coliform

B025

Effect of The Diffusible Signaling Factor (DSF) On *Bdellovibrio bacteriovorus* Predation

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The diffusible Signaling Factor (DSF, cis-11-methyl-2-dodecenoic acid) represents a relatively newly discovered group of α, β--unsaturated fatty acids which serve as extracellular signals for bacterial communications. DSF was found to regulate various behaviors in bacteria including motility, biofilm formation and virulence. In this study, we evaluated the effect of DSF on bacterial predation by *Bdellovibrio bacteriovorus* and its behavior. *B. bacteriovorus* is an obligate predatory bacterium which attacks a wide range of gram negative bacterial pathogens and is proposed to be a potential probiotic or living antibiotic in the future. The results showed that DSF negatively impacts the predation in liquid cultures as adding it in a concentration of 50 μM delayed the predation on an *E. coli* prey for about 10 h. Microscopic analyses of the predator behavior showed that DSF caused marked reduction in *B. bacteriovorus* motility. Predation in agar plates, however, was not affected by this signaling molecule. This indicated that, at the concentration tested (50 μM), DSF delays the predation through slowing down the predator without significant interference with its prey invasion machinery.
Keywords : DSF, Bdellovibrio, Signal, Predation, Motility

B026

Isolation and Characterization of Some Actinomycetes Inhibiting Several Plant-pathogenic Fungi

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Recently there is an increasing interest in the biological control which can replace the chemical control for prevention of plant-pathogenic fungi. In this study, several actinomycetes were isolated and their antifungal activities against some important phytopathogenic fungi were examined. The growth inhibition zone on PDA medium by *Streptomycetes vellosus* HR29 against *Fusarium oxysporum* f. sp. *niveum, F. oxysporum* f. sp. *lycopersici, F. oxysporum* f. sp. *raphani* and *Rhizoctonia solani* were 16, 20, 15 and 10 mm, respectively compared to those in the control plate fully covered by each of those fungi. Isolates HR54, HR207, HR220 and HR229 also inhibited the growth of three or more of those fungi. All the isolates were tested for beneficial attributes like growth promotion, production of growth hormones such as indole acetic acid, indole butyric acid and abscisic acid, siderophore production and antagonistic activity against pathogens including chitinase, β-1,3 glucanase, rhamnolipid and antibiotics. Strains HR29, HR54 and HR229 showed 4.20, 2.51 and 4.31 μmol/min/mg of chitinase activity and 21.28, 47.01 and 5.29 μmol/min/mg of β-1,3 glucanase activity, respectively. In addition, the stability test between isolated strains and other beneficial microorganisms conducted and the isolates did not inhibit other beneficial microorganisms that grow together.
Keywords : actinomycetes, antifungal activity, plant-pathogenic fungi, chitinase, glucanase

B027

Improvement of Tomato Growth under Copper Stress by Copper-resistant Rhizobacteria andExpression of Stress-related Genes in Tomato

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Copper is an essential element for living organisms, but the high concentration of copper is toxic to many organisms including plants and bacteria. This study aimed to isolate plant growth promoting rhizobacteria that can tolerate copper stress given to plants and study the interaction of rhizobacteria and tomato plant. Isolated strains *Pseudomonas veronii* MS1 and *Pseudomonas migulae* MS2 showed 7.13 and 6.43 μmol α-ketobutyrate/mg/h of ACC deaminase activity, respectively, that can reduce the level of stress hormone ethylene in plants. They also produced 0.13 mM and 0.26 mM of siderophore, respectively, that is iron-chelating agent and also inhibits fungal growth. A pot test for tomato growth was conducted for 4 weeks with 100, 350 and 700 ppm of copper. At 700 ppm of copper conc., the root lengths of the tomato plants treated with strain MS1 and MS2 significantly increased by 186.5% and 198.8%, respectively than those of the uninoculated control. The dry weights of tomato plants also significantly increased by 255.6 and 231.1%, respectively than the control. The expressions of ACC synthase genes, *ACS4* and *ACS6* genes of tomato plants under copper stress conditions determined by a real-time PCR decreased by treatment with rhizobacteria. Oxidative stress marker MDA production was lower in inoculated tomato than the control, which indicates that the tomato inoculated with rhizobacteria shows copper resistance.
Keywords : Plant growth, Copper stress, PGPR, ACC deaminase, ACC synthase

B028

Distinct Bacterial Communities within the Rhizosphere and Endosphere of Ginseng Roots

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We analyzed bacterial community composition in bulk soil (BS), rhizosphere soil (RS) and endosphere (ES) of ginseng using pyrosequencing method based on the 16S rRNA genes. It was shown that the species richness and diversity indices were higher in BS than RS and the species richness and diversity indices of ES were much lower than BS and RS. At the phylum level, *Proteobacteria* was a predominant phylum in BS and RS (30.5 and 55.7%, respectively) and occupied 99% in ES. The most dominant classe of the phylum *Proteobacteria* was *Alphaproteobacteria* for RS but *Gammaproteobacteria* for ES. At the genus level, *Flavobacterium* (8.3%) and sphingomonads (10.2%) were dominated in RS, while almost all bacteria within ES were belonged to the genus *Pseudomonas* (97.5%). It is expected that those bacteria could be used to biotransformation of medicinal materials such as ginsenosides. This study suggests that the genus *Pseudomonas* is the candidate for microbial agent to control some diseases of ginseng.

B029

Control of Harmful Algal Blooms by Algicidal Marine Bacteria

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Nowadays, harmful algal blooms found naturally in surface waters have caused many environmental problems. One of strategies that may reduce the effect of harmful algal blooms is to use algicidal bacteria. Around 500 marine bacterial strains have been isolated from seawater environments and these bacteria can be potential to be agicidal agents of various taxa. This study used a modified culture-dependent method, i.e., the double layer agar method combined with 24 transwell plate method to find algicidal bacterial activity. As a result, we found 20 isolates that had algicidal effect against 7 algal species (*Cochlodinium polykrikoides*, *Chattonella marina*, *Heterosigma akashiwo*, *Skeletonema costatum*, *Prorocentrum minimum*, *Heterocapsa triquetra*, and *Scrippsiella trochoides*). Based on 16S rRNA gene sequence analysis, major agicidal bacterial strains were most closely related to genus *Pseudomonas*, *Vibrio*, *Albirhodobacter*, and *Marinomonas*. The highest algicidal activity was 94% for *Cochlodinium*. The aim of the present study was to investigate algicidal bacteria to control harmful algal blooms and to provide basic knowledge about inhibiting their growth by algicidal bacteria.
Keywords : algicidal marine bacteria, harmful algal bloom, Cochlodinium polykrikoides

B030

Isolation and Expression of Microbial Rhodopsin Homologue from Yellow Sea of Korea

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Many organisms use proton pump to earn energy. Some proton pumps start to work by light and these proteins are called proteorhodopsin. Recent research found proteins that use not only protons but also univalent cations, divalent cations, or mono anions in pumping, which is called DDR2. The goal of this study is to find new types of pump proteins use other ion instead of protons and also new types of proteorhodopsin. Metagenome samples were collected from the beach in Taean-gun and Incheon (Kkotji beach(36°30’0’’N, 126°19’56’’E), Kkotji mud (36°30’8’’N, 126°19’60’’E), Duegi beach (36°31’6’’N, 126°19’39’’E), Sorae salt pond (37°24’25’’N, 126°44’41’’E), swamp(37°24’59’’N, 126°44’54’’E) and reservoir (37°24’39’’N, 126°45’5’’E)). Genomic DNA of each samples were isolated and subcloned with specific primer for proteorhodopsin and sodium pumping rhodopsin by polymerase chain reaction (PCR). As a result, we obtained former reported DDR2 gene and a unidentified proteorhodopsin in Duegi beach sample. Protein of unidentified proteorhodopsin was expressed with chimeric expression system and basic photophysical characterization was performed. [This research was supported by Creative Fusion Research program of undergraduate student and NRF-2013R1A1A064883]
Keywords : Rhodopsin, Proton Pump, Metagenome

B031

Endophytic Fungi of Orchid Roots in Korea

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The Orchidaceae is one of the largest families of plants closed to one-tenth of all known flowering plant species. Many of these orchids are known to interact with the fungi.. Generally, fungi associated with orchid is called mycorrhizal fungi. Orchid mycorrhizae are critically important during orchid germination, as orchid seed has virtually no energy reserve and obtains its carbon from the fungal symbion.(typically Ceratobasidium (Rhizoctonia), Sebacina, Tulasnella.)Whereas, it is endophytic fungi that microorganisms growing inside plant tissues without causing symptoms of disease. In this study, We have separated the endophytic fungi from a variety of orchids. So, we got a 10 species of fungi were identified using partial sequences of ITS of nuclear DNA. Trichoderma sp. Cephalotheca sulfurea, Coniochaeta mutabilis, Xylaria frustulosa, Nemania sp. Cryptosporiopsis ericae, Penicillium sp. Curvularia inaequalis, Umbelopsis ramanniana, Penicillium daleae
Keywords : orchid endophytes, orchid mycorrhizal fungi

B032

Characterization and Expression of o, m, p-Xylene Degradation Pathway Genes in *Rhodococcus* spp. J7

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Xylene, an aromatic hydrocarbon, can be easily found in gasoline contaminated soil and composed of three isomers [ortho (o-), meta (m-) and para (p-) xylene]. Many bacterial strains have been reported to grow on omp-xylene isomers, but few studies have been performed to degrade them simultaneously. We have isolated strain J7 from total petroleum hydrocarbon contaminated site in Korea. Strain J7 can use xylene isomers as sole carbon source but the growth rate is significantly reduced. Xylene degradation ability of strain J7 was evaluated using 90ppm of xylene mixture (30 mg/L individually) under aerobic conditions in duplicate. 165ml serum bottles containing 50ml of mineral salt medium (MSM) with 40mg/L yeast extract (YE) were used. 90 ppm of xylene mixture was completely consumed within 36 hours. Also strain J7 can degrade xylene mixture efficiently under 1 g/L cell concentration, at 25^{o}C and pH 7. The degradation rate of the xylene mixture increased with increasing amounts of YE, but high concentration (>= 2000mg/L) made degradation rate decrease. Currently, we are analyzing the characterization and expression of genes involved in the degradation pathway of omp-xylene using qPCR and sequencing. We selected two sets of primer, catechol-2,3-oxygenase and o-xylene dioxygenase for PCR study from NCBI or previously studied primers. The results obtained during this study will improve and provide clear knowledge on biodegradation of omp-xylene from the contaminated environments.
Keywords : Xylene, *Rhodococcus*, biodegradation

B033

Screening and Isolation Of Bacteria to Control Organic Compound in Marine Environment for Preventing Red-Tide

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Red tide is serious environmental problem that caused by flagellated algae, diatom, etc. It critically damages to fishes and shellfishes, so it made massive economic losses and marine environmental disturbances in Korea. For control of red-tide, we used red clay to sink algae, but this solution provoked secondary pollution. So we need new systems to prevent and control the outbreak of this phenomenon on eco-friendly way. First of all, we focused on controlling of organic compound in sea water by using bacteria. Furthermore, we observed correlation between ability of high uptaking organic compound bacteria and their TN, TP uptaking ability. Because we assumed that if the bacteria uptakes more organic compound, then they can uptake more TN and TP. We collect sea water and mud sample from 4 sites (Masan gulf waste water treatment, Dot island, Jinhae gulf, and fish raising farm). Sea water samples were filterated using whatman filter paper. The filtered paper vigorously shook and diluted with 3% NaCl(10^{-1},10^{-2},10^{-3}) to isolate bacteria. After dilution, we incubate the bacteria using 10%, 15%, 100% marine broth agar, 10%, 15%, 100% R2A broth agar and all media had 3% NaCl same as sea water. Finally, we isolated and screened 500 bacteria and found 20 isolates that has high efficiency in degradation organic compounds. We figured out its optimum degradation condition according to temperature, pH, cell concentration. And finally we will apply the high efficiency bacteria in ocean.

Keywords : Control of Red-tide, Red-tide

B034

Study of Changes in the Microbial Community Associated with Climate Change in the Laboratory Scale

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Climate change is a significant time variation in weather patterns occurring over periods ranging from decades to millions of years. From 1906 to 2005 the past, was expected to average global temperature rose to 0.74°C, 6.4°C until the end of this century up to rise. The average temperature in the Arctic has increased at a rate of almost twice the average of the Earth during the last 100 years, global warming has been promoted faster than ocean land. In the context of climate variation, anthropogenic factors are human activities which affect the climate. The scientific consensus on climate change is "that climate is changing and that these changes are in large part caused by human activities, and it is largely irreversible.Made the two reactors to confirm the change in the microbial community due to temperature changes. Each reactor filled with real-site water of 70 L and soil of 150 kg. One reactor fixed 20°C as control, another was increased 1°C per 2 month as test. The change in microbial community was confirmed trough out DGGE and pyrosequencing. As the results, it was confirmed that the change of the dominant of the microbial community according to the change of temperature. We can assume that there is likely to occur if the change of the microbial community receive the long-term change of these results, the need for a continuous research has been required.

Keywords : Climate change, microcosm, DGGE, Pyrosequencing

B035

Characterization of Ammonia Removal Bacteria for Preventing Red-Tide in Ocean

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Organic component from fish farm lead to red-tide in the ocean. Red tide is a name for a phenomenon known as an algal bloom that change sea water to red or brown color. Some red tides are associated with the production of natural toxins, depletion of dissolved oxygen. The reason why red tides effect marine and coastal species of fish, birds, marine mammals, and other organisms. For preventing red tied, we will make a new system to control organic component especially nitrogen compound in the sea water. Bacterial strains were isolated from mud collected at Tongyeong coast. Sediment was shaken vigorously in 3% NaCl and supernatant was inoculated in the 10% and 100% of marine and R2A agar, with 3% NaCl. Ammonia (4500-NH_3 F. phenate) was detected using standard methods. Based on the results, Strain of SJ8 showed up to 100% removal efficiency at pH6.8, 30°C under aerobic condition. SJ8 was identified *Bacillus aryabhattai* (100%).

Keywords : Ammonia, Red-tide

B036

Characterization of Anaerobic Denitrifying Microorganism for Preventing Red-Tide in Marine Sediments of the Tongyeong Ocean

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In marine environments, increased concentration of nitrates in estuaries can have major negative ecological implications since nitrates cause the proliferation of algal growth creating an ecological imbalance. NO_3^- is the limiting nutrient in the growth cycle of algae and excessive NO_3^- may lead to increased algal growth causing algal blooms. Therefore, it is important to remove nitrogen source in marine environments. Denitrification is a microbial process during which nitrate or nitrite is reduced under anaerobic condition to gaseous nitrogen. Also, in marine ecosystems, both nitrite-reducing bacteria and ammonium-oxidizing (ANAMMOX) bacteria contribute to the nitrogen cycle. So, this study performed screening for searching nitrate and nitrite removal bacteria in anaerobic condition by using sediment samples obtained from Tongyeon Ocean. To screen marine bacteria capable of removing nitrogen sources (nitrate and nitrite), sediment sample was inoculated in modified marine broth (1% w/v) with NO_2^--N (20 mg/L) and NO_3^--N (20 mg/L) for enrichment culture. Results from the screening test, showed that the removal efficiencies for nitrite and nitrate over 90% for 3 strains and 50% for 1 strain, respectively.

Keywords : marine ecosystem, anaerobic denitrifying, nitrite and nitrate

B037

Screening and Characterization of Total Phosphate Removal Bacteria for Preventing Red-tide in Cage Fish Farms in Ocean

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Red tide is a common name the phenomenon known as harmful algal bloom (HAB) and it caused by higher organic components in the marine water. Red tide is usually associated with the production of toxins, and it depletion of dissolved oxygen levels in the water. In order to prevent this situation, we found a new bacterial reduction of organic compound, especially phosphorus in seawater. The total phosphate in the marine water is the major source of HABs, by using high efficiency phosphorus removal bacteria, the phosphate in the polluted water will be removed, and it inhibit the growth of algae and control red tide in sea water. We are sampled from mud and sea water at Tong-yeong and isolated bacteria strains. In the screening, Check for phosphorus removal efficiency using inorganic medium, added 0.3% glucose. In order to establish a culture condition, used Marine broth(Difco) at pH3,4,5,6,7,8,9 and 5,10,15,20,25, 30,35,40¡É. Cell mass performed 0.05, 0.1, 0.2, 0.5g/L. The phosphate removal rate experiment with different 1, 3, 5, 10 ppm in Mineral solution media added 0.3% glucose. Batch test and Fermenter experiment did inorganic medium that adjusted glucose 200ppm(COD), Ammonia nitrogen 20ppm(TN), Potassium phosphate 10ppm(TP) at pH6.8 and pH8. Total phosphate analyzed to 0-3.5 ml/L PO_4^{3-} HACH kit and Ascorbic Acid Method(Standard method 4500-P E). Furthermore analysis, I will check removal efficiency in ocean condition and detect gene related to phosphorus removal pathway.
Keywords : Total phosphate, Red-tide

B038

Assessment of Biofilm Formed in Drinking Water Distribution System by Using Fluorogenic Substrate Mixture

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We developed a quantification method for biofilm assessment by using 4-methylumbelliferone (MUF)-containing fluorescent substrates. Cleavage of MUF substrates substrates (MUF-α- and MUF-β-glucopyranoside and MUF-butyrate) by exoenzyme yields the fluorescent molecule, 4-MUF, which is measured with a fluorometer (365 nm, 445 nm). Biofilm was allowed to develop on various coupons (PE, PVC, enamel-coated steel and ductile cast iron) installed in a water purification plant while continuous tap water flow at 15 m^3/h. The tap water produced by either sand filtration or ozonation-activated carbon treatments was supplied in a parallel mode. When retrieved biofilm was incubated with MUF-substrates, the intensity of fluorescence was increased with exposure time regardless of the pipe material or treatment method. The fluorescence production correlated well (r^2 = 0.94) with the corresponding heterotrophic plate counts measured in all the biofilm samples. It was evident that biofilms exposed to the sand filtered water produced a higher fluorescence indicating more substrate degradation compared to low fluorescence in those with the advanced treatment. The results could be attributed to a low AOC (assimilable organic carbon) content in the advanced treated water (41.7 μg/L) compared to the sand-filtered water (125 μg/L). Fluorescence-based method would help to control and effectively manage the biofilm formed on water distribution pipe system. [Supported by Korea MoE "GT-11-G-02-001-3"].
Keywords : Biofilm, Drinking water, Assimilable Organic Carbon, MUF

B039

Intraspecific Functional Variation of Arbuscular Mycorrhizal Fungi Originated from Same Population on Plant Growth

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Arbuscular Mycorrhizal Fungi(AMF) is widespread symbiont forming mutualistic relationship with plant root in terrestrial forest in ecosystem. They provide improved absorption of nutrient and water, and enhance the resistance against plant pathogen or polluted soil, therefore AM fungi are important for survival and maintaining of individual or community of plant. For last decade, many studies about the functional variation of AM fungi on host plant growth response were showed that different geographic isolates, even same species, have different effect on host plant. However, little was known about functional variation of AM fungal isolates originated single population, which provide important insight about intraspecific diversity of AMF and their role in forest ecosystem. In this study, four AM fungal isolates of *Rhizophagus clarus* were cultured *in vitro* using transformed carrot (*Daucus carota*) root and they showed the difference between isolates in ontogenic characteristics such as spore density and hyphal length. The plant growth response by mycorrhizas were measured also. After 20 weeks from inoculation of these isolates to host plants, dry weight, Root:Shoot ratio, colonization rates and N, P concentration of host plant showed host plant was affected differently by AM fungal isolates. This results suggest that AM fungi have high diversity in their functionality in intraspecific level, even in same population.
Keywords : Arbuscular mycorrhizal fungi, Intraspecific variation, *in vitro* culture, *Rhizophagus clarus*

B040

Community Change of Ectomycorrhizal Fungi According to Season in the Thinning Forest

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This study was conducted to search the changes in ectomycorrhizal communities according to the thinning. Also investigated how affects the belowground microorganism changes over time. ectomycorrhizal fungal community was confirmed by the morphological characteristics of the ectomycorrhizal root tip and ITS rDNA sequence analysis. As a result, abundance of fungi species was increased in the fall. Ectomycorrhizal fungi community composition has changed by thinning and seasonal. Is an important factor affecting the belowground microorganism over time, such as thinning and disturbance.
Keywords : Ectomycorrhizal, season, thinning, community

B041

Isolation and Characterization of Fungal Diversity from Crop Field Soils of Nigeria

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In order to find indigenous beneficial fungal species from crop field soils of Nigeria, 23 soil samples were collected from various places of Nigeria in June, 2013 and fungi were isolated through serial dilution technique. Isolated fungi were purified and differentiated according to their morphological and microscopic characteristics. In total, 38 different representative isolates were recovered and the genomic DNA of each isolates was extracted using QIAGEN^{ex} Plasmid Mini Kit (QIAGEN Sciences, USA) and the identification of fungi was carried out by sequence analysis of internal transcribed spacer (ITS) region of the 18S ribosomal DNA (18S rDNA). Recovered isolates belonged to 9 fungal genera comprising *Fusarium*, *Aspergillus*, *Chaetomium*, *Coniothyrium*, *Dipodascaceae*, *Myrothecium*, *Neosartorya*, *Penicillium and Trichoderma*. *Aspergillus* spp., *Penicillium* spp. and *Trichoderma* spp. were the most dominant taxa in this study. The antagonistic potentiality of species belonged to *Trichoderma* against 10 phytopathogenic fungi (*F. oxysporum*, *C. gloesporoides*, *P. cytrophthora*, *A. alternata*, *A. solani*, *S. rolfsii*, *F. solani*, *R. solani*, *S. sclerotiorum and P. nicotiana*) was assessed *in vitro* using dual culture assay. The dual culture assay results showed varied degree of antagonism against the tested phytopathogens. The potential *Trichoderma* spp. will be further evaluated for their antagonistic and plant growth promotion potentiality under *in vivo* conditions.
Keywords : *Aspergillus* spp., Fungal diversity, *Penicillium* spp., *Trichoderma* spp.

B042

Effect of Organic Cultivation of Lettuce and Hot Pepper on Chemical and Microbial Properties of Soil

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Effect of organic cultivation on chemical property, microbial population and activity of soil of lettuce and hot pepper was studied for three years. The microbial population and microbial activity was determined from the organic cultivated soil applied with the press cake of oil seeds and the microbial fertilizer. The available phosphates of organic soil of 982 to 1,832mg/kg were significantly higher than the recommended ranges of 250 ~ 400mg/kg for cultivation (Lsd, P=0.05). The other nutrients such as exchangeable K and Ca cations, and electro-conductivity were various depending on the farm, resulting in less or far more amounts compared to the recommended ranges. In addition, organic contents of 29~66g/kg were significantly higher than the recommended range of 20~30g/kg. In lettuce soil, bacterial populations and microbial activities were significantly higher at 4 and 6 weeks after treatment compared to the no treatment. In contrast, fungal population was not significantly different between the treatments. In pepper soil, there was no significant difference in microbial population and microbial activities between treatments. The results indicated that nutrient conditions of organic soil be very different depending on farms, and available phosphate contents in organic soil be far higher than the recommended range for farming, and microbial activity be more influenced by microbial fertilizer in lettuce cultivation than pepper.
Keywords : Organic farming, Soil chemical property, Microbial population, Microbial activity

B043

Marine-derived *Penicillium* in Korea: Diversity, Enzyme Activity, and Antifungal Properties

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The diversity of marine-derived *Penicillium* from Korea was investigated using morphological and multigene phylogenetic approaches, analyzing sequences of the internal transcribed spacer region, β-tubulin gene, and RNA polymerase subunit II gene. In addition, we tested for the extracellular enzyme activity of alginase, endoglucanase, and β-glucosidase, and antifungal activity against two plant pathogens (*Colletotrichum acutatum* and *Fusarium oxysporum*). A total of 184 strains of 36 *Penicillium* species were isolated, with 27 species being identified. The most common species were *P. polonicum* (19.6%), *P. rubens* (11.4%), *P. chrysogenum* (11.4%), and *P. crustosum* (10.9%). The diversity of *Penicillium* strains isolated from soil (foreshore soil and sand) and marine macroorganisms was higher than the diversity of strains isolated from seawater. While many of the isolated strains showed alginase and β-glucosidase activity, no endoglucanase activity was found. More than half the strains (50.5%) showed antifungal activity against at least one of the plant pathogens tested. The results reported here expand our knowledge of marine-derived *Penicillium* diversity. The relatively high proportion of strains that showed antifungal and enzyme activity demonstrates that marine-derived *Penicillium* have great potential to be used in the production of natural bioactive products for pharmaceutical and/or industrial use
Keywords : Marine-derived fungi, *Penicillium*, Alginase, β-glucosidase, Antifungal activity

B044

Characterization of the Premature Lysis by Predatory Bacteria

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Bdellovibrio bacteriovorus were well known bacteria which prey upon a variety of other bacteria in a bacterial community. In our previous study, it was found that prey cells were rapidly lysed in early time of predation cycle which typically spent 3.5 hours to pop out the host. At previous study, it was defined as premature lysis by predation. This study, we tried to evaluate the downstream impact of the premature lysis onto a different prey, specifically at *Acinetobacter baumannii*. Interestingly, it was found that *Acinetobacter baumannii* were significantly lysed by the predation faster than *E. coli* which was used in previous study. To further analyze these aspects, we examined the mixed culture of *Acinetobacter baumannii* and *E. coli* then predation was investigated in different time manner. This study will provide the new ecological aspects of premature lysis in bacterial predation and further proof of prey preference.
Keywords : Premature lysis, *Bdellovibrio bacteriovorus*, Bacterial predation, Pathogen, *Acinetobacter baumannii*

B045

In vitro amplification of PrP^{Sc} from Chronic Wasting Disease(CWD) agents Contaminated Soil

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The transmissible spongiform encephalopathies are fatal neurodegenerative diseases that affect both humans and animals. Scrapie and chronic wasting disease (CWD) are particularly environmental concerns as they are horizontally transmissible and remain infectious after years in the environment. It is likely that the environment serves as a stable reservoir of infectious CWD and scrapie prions. Recent researches have suggested that the soils can differ dramatically in their capacity to absorb PrP^{Sc} due to differences in surface area and mineral composition. This difference in PrP extracting ability from soil hampered efficient PrP^{Sc} elution. Protein misfolding cyclic amplification (PMCA) is an useful technique for detecting low levels of PrP^{Sc} in biological samples. In this study we applied PMCA technique in order to improve the detection efficiency of PrP^{Sc}. The unbounded PrP^{Sc} in washing solution was detected up to 3 times washing in 1 %, 4 months spiked soil and was not detected in the rest spiked soils. PrP^{Sc} in spiked soil was not amplified in an extraction aliquot used as a PMCA seed. **T**he amount of direct-eluted PrP^{Sc} in spiked soils with low concentration of TSE infected homogenate was very low. So we has established PMCA technique to enhance the detection limit. But in vitro amplification of PrP^{Sc} did not occur in direct extraction aliquot but in washing aliquot. It is thought that unknown components were extracted in soil interfere with amplification of PrP^{Sc}.
Keywords : CWD, PMCA, Soil

B046

Characterization of Phosphate-solubilizing Bacterium *Pantoea* sp. strain 36-H-3 with Antifungal Activity

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Phosphorus is essential for plant nutrition, play an important role in the biological molecules, such as ATP, DNA and phospholipids. However, plant cannot get enough phosphate because it is insoluble easily in the soil. Due to the insoluble phosphate, cumulative inputs of P fertilizer have resulted in high accumulation of P under cultivation and a negative impact on plant growth. In this study, bacterium which was collected from forest soils selected phosphate solubilizing bacteria (PSB) from PVK agar plates. Through Nucleotide sequence analysis of the 16S rRNA, the bacterium was identified *Pantoea* sp. belong to a group of organisms of the Enterobacteriaceae. The phosphate solubilizing bacteria activity was confirmed in the tri-calcium phosphate medium. The pH of medium showed the lowest level at day 6 as the pH 4.52 and the highest levels of available phosphate is 485.4ppm at day 5. Plant pathogenic fungi and *Pantoea* sp. 36-H-3 was replaced on the PDA agar plate. As a result, *Pantoea* sp. 36-H-3 showed antifungal activity in *Rhizoctonia solani* (damping-off), *Phytophthora capsici*(Phytophthora blight), *Botrytis cinerea* (gray mold rot). Therefore, *Pantoea* sp. 36-H-3 can be to use by microbial pesticides as well as microbial fertilizer
Keywords : Agenda Program, Administration

B047

Comparative Transcriptome Analysis of an Alkylphenol Polyethoxylate Degrader *Pseudomonas nitroreducens* TX1 Grown on Succinate and Triton X-100

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Pseudomonas nitroreducens TX1 has the capability to utilize a group of nonionic surfactants such as alkylphenol polyethoxylates (APEOn) even at high concentrations (up to 20% with Triton X-100) as a sole carbon source. Because the genes in the draft genome sequence of the TX1 strain do not show similarities to known genes for APEOn degradation in other bacteria, RNA-seq-based global transcriptome analysis was performed to identify genes related to the degradation. The profile of transcriptome of TX1 strain grown on Triton X-100 and that grown on Na-succinate were compared. In total, 6253 transcripts were identified and 263 and 385 genes were up- and down regulated in Triton X-100 with a cutoff at levels of more than 5 times. Among them, several dehydrogenses were identified to be up-regulated. Currently the key genes are being specifically mutated to identify the function in APEOn degradation by TX1 strain. This study will allow a deeper understanding of molecular mechanisms underlying Triton X-100 degradation and adaptation to the toxic chemical by *P. nitroreducens* TX1 at high concentrations
Keywords : *Pseudomonas*, RNAseq, alkylphenol polyethoxylates, Triton X-100

B048

A Rolling Circle Replication Plasmid Found in *Pseudomonas nitroreducens* TX1, an Alkylphenol Polyethoxylate Degrader

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The genus *Pseudomonas* is widely distributed in the environment and plays an important role in biodegradation and biotransformation of organic and xenobiotic compounds. *Pseudomonas nitroreducens* TX1 can use a group of nonionic surfactants such as Triton X-100 at high concentrations as a sole carbon source. In this study, a small cryptic circular plasmid, pTX1, was characterized from *P. nitroreducens* TX1. The copy number of pTX1 was estimated to be about 150 in each cell. It is 2,286 bp in length with a GC content of 63.3% and harbors three open reading frames, Rep_{pTX1} and functionally unidentified ORF1 and ORF2. The predicted rep_{pTX1} gene product is homologous to Rep proteins of plasmids known to replicate by the rolling-circle mechanism. Based on the genetic fingerprints and comparison with other plasmids, it is concluded that pTX1 replicates by a rolling circle mechanism which is rarely found for *Pseudomonas* plasmids
Keywords : *Pseudomonas*, plasmid, rolling circle, biodegradation

B049

Detection of Wood Inhabiting Basidiomycetes from Soils in Odaesan National Park, South Korea

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When dead wood is newly exposed, it is colonized by a variety of fungi. Their colonization on wood surface is achieved by air-borne basidiospores. Soil-borne spores and mycelia could also colonize woods. In this study, we tried to understand which wood inhabiting basidiomycetes are present in soils. ITS2 region amplicon libraries were constructed using the soil samples in Odaesan National Park, and they were sequenced and analyzed by 454 sequencing. As results, a total of 96 OTUs of potential wood inhabiting basidiomycetes were found. *Ganoderma applanatum* was the major wood inhabiting fungus in soils. Also, this was the only species found from all the soil samples. Compared with the diversity of wood inhabiting fungi measured over the same region and time period, it was shown that 8 OTUs have been found from woods. On the other hand, the rest OTUs were not found from woods yet. These species might represent unaccounted diversity of wood inhabiting fungi and further surveys are needed to confirm their existence on wood.
Keywords : basidiomycetes, diversity, wood inhabiting fungi, soil

B050

Concentration and Diversity of Bacteria Isolated from Indoor Air of Green Houses for Shiitake Cultivation

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This study was carried out to measure temperature, humidity, and bacterial concentration and species from indoor air of a greenhouse for shiitake cultivation. The highest humidity of the greenhouse was over 91.5% and the lowest humidity was 50% during 12 months. Temperature was 5.1-30.5°C except January. These results indicated that bacteria can survive in indoor air of the greenhouse. Total bacterial concentration was over the Korean indoor air quality standard value(8.0×102 cfu/m3) in winter. A total of 13 genera and 17 species were isolated and identified from the indoor air of the greenhouse. Especially, 3 species(Kocuria rosea, Staphylococcus xylosus and Curtobacterium flaccumfaciens) were reported to affect on human health. This is first report of airborne bacteria in a greenhouse for shiitake cultivation
Keywords : Bacterial concentration, Greenhouse, Indoor air quality, Shiitake, Species

B051

Molecular Analysis of Bacterial Community Structures in Soils for Environmental Risk Assessment with Genetically Modified Soybean, Kwangan Atsiz-6

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The influences of transgenic soybeans on soil microbial communities were evaluated using cultivation and molecular methods. The soybean field plot consisted of three subplots planted with genetically modified (*Glycine max L. Merr, introduction of osmotic stress inducible gene* (*AtSIZ*)), non-genetically modified (*Glycine max L. Merr*), and wild soybean (*Glycine soja Siebold & Zucc.*). The microbial population dynamics (bacteria, actinomycetes, and fungi) measured by cultivation methods were quite similar among the three subplots throughout the experiments. Analysis with real-time PCR DGGE (denaturing gradient gel electrophoresis) method of 16S rRNA genes showed that the bacterial community structures were very similar to each other in a given month, indicating that there were no significant differences in bacterial communities between GM, non-GM, and wild soybean soils. When analyzed with quantitative PCR of soil DNAs using primers for the *AtSIZ* gene, which was inserted into GM soybean, relatively higher copies of the *AtSIZ* were detected in soils cultivated with GM soybeans, indicating that some of the inserted genes were released into the soils. The result of this study suggested that, in spite of their seasonal fluctuations, the composition and structure of the microbial communities of the experimental soybean field were not significantly affected by cultivation of GM soybeans
Keywords : transgenic plant, bacterial community, 16S rRNA, DGGE, soybean field

B052

Characterization of *Draconibacterium filum* sp. nov., isolated from sediment of East Sea

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Strain $F2^T$ was isolated from marine sediment sample collected from the coast near Yangyang. The strain was Gram-stain negative, non- motile, filamentous and facultative anaerobic. The 16S rRNA gene sequence of $F2^T$ showed closely related taxa; *Draconibacterium orientale* $FH5^T$ (97.9 %) and *Marinifilum fragile* $JC2469^T$ (89.0%). Phylogenetic analysis exposed distinct phyletic line between $F2^T$ and *Draconibacterium orientale* $FH5^T$ with single clade. The isolate was growth at 20–35 °C, pH 6.5–8.5 and sea salts 0.5–6 %. The dominant fatty acid of $F2^T$ were iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:00}$, iso-$C_{17:0}$ 3-OH and iso-$C_{16:0}$ 3-OH. The DNA G+C content was 44.7 mol%. The DNA-DNA association between strain $F2^T$ and *D. orientale* DSM 25947^T was 34.6 %. On the basis of phylogenetic distinction and differential phenotypic properties, the strain $F2^T$ should be assigned to the genus *Draconibacterium,* for which the name *Draconibacterium filum* sp. nov. is proposed. The type strain of *Draconibacterium filum* is $F2^T$ (= KCTC 32486^T = JCM 19986^T).
Keywords : Draconibacteriaceae; Draconibacterium filum; Marine sediment; Polyphasic taxonomy

B053

Effect of *Arthrobacter woluwensis* ED on Growth of *Aster koraiensis* in Barren Lakeside Land

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This study was conducted to examine the plant growth promotion by *Arthrobacter woluwensis* ED isolated from the rhizosphere of a wild plant *Isachne globosa*. Twenty five seedlings of *Aster koraiensis*, an indigenous wild plant species that can grow on barren land, were planted at each 1 x 1 m experimental plot in barren lakeside at Lake Paro, Korea. *A. woluwensis* ED was cultivated, washed with DW, harvested by centrifugation and applied to the plots at 10^6 cells/g surface soil at 4-week interval from Apr. to Sept. 2014. The inoculation of the bacterial cells suspended in 4 l of lake water for each plot increased the stem length, root length and fresh weight of the grown plants of *A. koraiensis* by 20, 8.5 and 25.9%, respectively compared to those of the uninoculated control plots after 22-weeks of experimental period. The bacteria immobilized in sodium alginate could enhance them by 36.5, 22.6 and 57.8%, respectively compared to those of the uninoculated control. In the field test at 5 x 5 m plots in which any plants or seeds had not been planted, the uninoculated plot, the inoculated plot with the bacteria suspended and the immobilized bacteria showed 15, 18 and 22 species of wild plant grown, respectively. The total populations of the grown plants increased by 30.4 and 70%, respectively compared to the uninoculated control plot. This study shows that *A. woluwensis* ED can be utilized for the revegetation of environmentally sensitive barren lands such as lakeside areas
Keywords : *Arthrobacter woluwensis* ED, plant growth promotion, PGPR, revegetation, immobilized bacteria

B054

Identification of Pseudomonas aeruginosa in Natural Environment by Physiobiochemical Characterization

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Pseudomonas aeruginosa is an opportunistic pathogen that inhabits various natural and artificial environments, such as pathogenesis, water, soil and air. They can cause serious problems, such as pathogenic infection. In this study, 220 colonies were isolated from water and soil environment that assumed to be *P. aeruginosa* using a membrane filter method based on International Organization for Standardization (ISO). Identification of the isolates was determined by physiobiochemical characteristics using ISO and Korea drinking water standard method. Only one of 220 presumed *P. aeruginosa* strains isolated from effluence water using a drain swab was determined as *P. aeruginosa*-positive. Subsequently, the resistance to 1,10 Phenanthroline test, which was newly proposed by ISO in 2014 and applied in this study, was considered as more precise and improvable method for identification of *P. aeruginosa*
Keywords : Pseudomonas aeruginosa

B055

Effects of Paenebacillus Polymyxa E681 on the Durability of Cement Paste

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This study shows that the potential use of microorganisms in the development of novel, multi-functional cement paste formulations showing calcium carbonate precipitation and antifungal activity. *Paenibacillus polymyxa* E681 which has antifungal activity against *Aspergillus niger* on concrete construction related environment was used as a new concept in the bio-cement field. This strain has combinational effects on fungal inhibition and calcium carbonate precipitation on cement mortar. In this study *P. polymyxa* E681 induced calcium carbonate crystals on B4 solid medium and the calcite sealed artificial cracks in cement paste. In addition, the E681 induced coating of calcium aggregates onto the cement paste surface, and the strength of cement-sand mortar added *P. polymyxa* E681 increased. Above all growth of *A. niger*, a well-known fungal strain with deleterious effects on cement surfaces was severely inhibited by the antifungal activity of the E681 strain.
Keywords : Biomineralization, Antifungal activity, Cement paste, Paenibacillus polymyxa E681

B056

Occurrence of Aflatoxigenic Species in Korean Cereals

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For monitoring the presence of aflatoxigenic species in Korean cereals, grains were collected nationwide from barley, wheat, corn fields or harvested soybean during 2013-2014. The grain samples were surface-sterilized and placed on potato dextrose agar plates for 5 days at 25¡É. Among fungal colonies grown out from grains, those having *Aspergillus*-like spores were isolated and identified based on β-tubulin sequence BLAST against NCBI database. A total of 64 isolates were tentatively identified as *Aspergillus* species including *A. flavus* (17), *A. oryzae* (13), *A. tritici* (9), *A. chevalieri* (5), *A. ruber* (3), *A. fumigates* (2), *A. peyronelii* (2), *A. clavatus* (1), *A. amstelodami* (1), *A. proliferans* (1), *A. pseudoglaucus* (1), *A. sclerotiorum* (1), *A. versicolor* (4), *A. wentii* (1), *A. sydowii* (1), *A. terreus* (1), *A. tubingensis* (1). Most of *Aspergillus flavus* or *A. oryzae* isolates were originated from barley (13/17) or wheat (13/14). To examine if *A. flavus* or *A. oryzae* isolates possess 4 essential biosynthetic genes for aflatoxin production, PCRs to amplify 4 *aflR*, *omt-A*, *nor-1*, and *ver-1* were performed. Unexpectedly all 4 genes were amplified from all the isolates tested. To measure aflatoxin production by these isolates, the isolates were inoculated in Czapek-dox broth to induce aflatoxin production. Toxin analysis using UPLC will be conducted.
Keywords : Aspergillus flavus, Aspergillus oryzae, aflatoxin, aflR

B057

Metagenomic Analysis of Methane Derived Carbonates Subsurface Sediment in Ulleung Basin, East Sea

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Authigenic carbonate crusts formed at subseafloor are a striking phenomenon that occurs at hydrocarbon seeping area It carried out by prokaryotic consortia composed of methanotroph and sulphate-reducer. Here we report on the microbial community and functional diversity of the authigenic carbonate crusts found on the surface sediment ('Hemire site') from the Ulleung Basin. The abundance of total prokaryotic cells (archaeal and bacterial 16S rRNA genes) and methyl coenzyme M reductase alpha subunit gene (*mcr*A) was $1.20\times10^{8}\pm6.96\times10^{6}$, $2.69\times10^{10}\pm3.02\times10^{8}$ and $2.06\times10^{7}\pm4.93\times10^{5}$ copies per wet weight, respectively. These number of gene copies were relatively higher than that of normal sediments. The phylogenic analysis of the *mcr*A gene showed that 60% (24 clones) of phylotypes affiliated with ANME-2c (*mcr*A group c and d) group with the closest relatives (99-100% similarity) originating from cold seeps of Okhotsk Sea and Eel River Basin. We obtained 3 Gb of metagenomic sequences using Illumina Miseq pared-end sequencing, which applied to unveil the taxonomic composition and functional diversity. The taxonomic composition of metagenome showed that *Deltaproteobacteria* (22.09%), *Gammaproteobacteria* (18.92%) and *Methanomicrobia* (18.78%) were the most abundant. All genes required for performing the seven steps of methanogenesis from CO_2 were found present in the metagenome. This study provided with understanding of the differentiation of ANME groups by the environmental niche

Keywords : Authigenic carbonate crusts, Anaerobic oxidation, ANME, Metagenome

B058

Relevance of Abundance of Methanogen as an Indicator for Methane Emission in Wetlands

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Methane is 25times stronger greenhouse gas than carbon dioxide on a molecular basis, which is produced under anaerobic conditions by specific class of archaea known as methanogen. Many studies have conducted to reveal either the abundance and community structure of methanogen or methane emission rate, but the relationship between the two factors (i.e. microbial abundance vs. process rates) is yet to be fully understood. We measured abundance of methanogen and methane emission rate from various soil (forest, rice paddy, permafrost, and alpine wetlands), and analyzed relationship between the two factors. When all data were combined, no significant correlation was found. However, if we classified the data set by soil organic content and season, significant correlations were appeared in specific conditions. For example, samples with soil organic matter of 30~70% (organic soil), exhibited a positive correlation, while samples with higher and lower soil organic matter (peat and miceral soil) did not show any relationship. A significant correlation was observed in winter only, while it was absent in other seasons. These results suggest that abundance of methanogen is not the critical factor controlling methane emission rate across diverse ecosystems, but other biogeochemical variables (water availability, methane oxidation, microbial community structure, and/or soil properties) may take an important role in methane emission in wetlands.

Keywords : methanogen, methane emission, wetland, organic matter content, seasonality

B059

Genetically Distinct Lineages of *Vibrio vulnificus* Revealed by Multilocus Sequence Analysis

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The genetic diversity and population structure of *Vibrio vulnificus* were investigated using MLST. rep-PCR fingerprinting resulted in a total of 52 unique genotypes out of 84 *V. vulnificus* isolates originated from environmental and clinical sources, including three genome-sequenced strains. The rep-PCR genotypes were subjected to a MLST analysis, which analyzed internal fragments of six housekeeping genes (*glnA*, *glp*, *gyrB*, *mdh*, *pyrC*, and *recA*) and one virulence-related gene (*vvh*). The mean number of alleles at each locus was 26.6, ranging from 21 (*glnA*) to 35 (*recA*). Phylogenetic analyses (neighbor joining and splits network) of the concatenated sequences from the genotypes identified 44 sequence types STs, and the STs formed two distinct monophyletic groups (lineages). Each lineage showed star-shaped topology, implying recent clonal expansions. The genetic distance between the two lineages far exceeded intra-lineage genetic distances. Sawyer's test showed evidences of inter-lineage and intra-lineage recombination events (global inner recombination) in the *glnA*, *glp*, *gyrB*, and *pyrC* ($p < 0.05$). All the pairwise comparisons of the seven MLST loci were incongruent, which was consistent with the results from Sawyer's test. This work is limited to show the evidence for the intra-species lineages within *V. vulnificus*. Further studies on the genetic and phenotypic differences between the intra-species lineages can help us understand the evolution and diversification of *V. vulnificus*

Keywords : *Vibrio vulnificus*, diversity

B060

Novel PCR Primers for the Archaeal Phylum *Thaumarchaeota* Designed Based on the Comparative Analysis of 16S rRNA Gene Sequences

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Based on comparative phylogenetic analysis of 16S rRNA gene sequences, we constructed a local database of thaumarchaeotal 16S rRNA gene sequences and developed a novel PCR primer specific for the archaeal phylum *Thaumarchaeota*. Among 9,727 quality-filtered (chimeral-checked, size > 1.2 kb) archaeal sequences, 1,549 thaumarchaeotal sequences were identified and included in our local database. In our study, *Thaumarchaeota* included archaeal groups MG-I, SAGMCG-I, SCG, FSCG, RC, and HWCG-III, forming a monophyletic group in the phylogenetic tree. A phylum-directed primer was designed from a consensus sequence of the phylotype sequences, and the primer's specificity was evaluated for coverage and tolerance both *in silico* and empirically. The phylum-directed primer, designated THAUM-494, showed >90% coverage for *Thaumarchaeota* and <1% tolerance to non-target taxa, indicating high specificity. To validate this result experimentally, PCRs were performed with THAUM-494 in combination with a universal archaeal primer and DNAs from five environmental samples to construct clone libraries. Phylogenetic analysis of 859 cloned sequences obtained from 10 clone libraries revealed that >95% of the amplified sequences belonged to *Thaumarchaeota*. To our knowledge, THAUM-494 is the first phylum-level primer for *Thaumarchaeota*. Furthermore, THAUM-494 primer will make it a potentially valuable tool in understanding the phylogenetic diversity and ecological niche of *Thaumarchaeota*.

Keywords : *Thaumarchaeota*, PCR primer, 16S rRNA gene, phylogeny

B061

A comparison of the Soil Components

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The taste of wine is simply grape seed, not only of natural elements such as soil conditions are determined by a comprehensive concept 'terroir'.Terroir the soil, the natural environment, land structure, orientation, geographical location and climatic conditions, as well as the micro-environment concept and refers all. Finally finished with the same varieties of wine grape production,but Why differences occur 'terroir' is.The study used a wine yeast and bacteria during fermentation pathway of fermentation, the wine and grape composition and the relationship between climate and pest-infected grapes and wine quality in the impact of changes in climate are focused on. Natural climate factors each of the other two countries, Germany and Greece to collect the soil of the vineyard, the soil microbes in the soil compared to the impact on soil microorganisms and the resulting degree of cultivation of wine grapes affect the taste and quality gives examined.To do this, the vineyards of the soil sample was taken in each country to separate the three different types of microorganisms in a nutrient medium (YM, MRS, PDA) to observe the culture and the Active Active Growth curve is selected by high-strain experiments and the O.D values were analyzed for Glucose values. The glucose measurements using the glucose kit.Based on this, its impact on microbial activity in the soil the grapes are grown in soil and its impact on the quality of the wine were investigated.
Keywords : Wine Field Soil component, Growth Curve, Growth-promoting, Soil microorganisms, Geohumus

B062

Effects of AMF Pelltes on Seed Germination and Growth Rates of *Sorghum bicolor*

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In this study, we have produced pellets to cover seeds and supply nourishments using moss and by-product of rice milling and studied effects of arbuscular mycorrhizal fungi (AMF) on germination rate of seeds and growth rates of the host plants, sorghum (Sorghum bicolor). Thegermination rate of sorghum seeds was the highest in the pellets made from 1:1 mixture of moss and by-product of rice milling. However, AMF did not affect the germination rates of the seeds.Sorghum inoculated with G. mosseaeshowed the highest growth rate.The results suggest that the pellets could be used to recover destroyed ecosystem.
Keywords : arbuscular mycorrhiza, pellet, sorghum, seed germination

B063

The Effect of High Salinity on the Biological Reduction of Perchlorate

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This research was done to evaluate the effect of high salinity on the biological reduction of perchlorate. Laboratory experiments were conducted in flasks containing 100mL of synthetic sewage, and sewage mixed with defined amount of perchlorate and sodium acetate as sole carbon source.During the concentration of sodium chloride rise 0% to 1.5%, the rate of the biological reduction of perchlorate is decrease significantly. And above 1.5%, the biological reduction did not occured. Sewage contain that the same concentration of sodium chloride, ammonium chloride, and sodium bicarbonate, sewage contain the sodium bicarbonate have the most slow rate of the biological reduction of perchlorate.
Keywords : salinity, biological reduction, perchlorate

B064

The Study on Comparison of Treatment Efficiency Using PFR and CSTR

Yongjae Lee, Jungwon Hwang, MoonHwee Jun, and Duri Park
The study on comparison of treatment efficiency using PFR and CSTR

1,4-dioxane is widely used as a stabilizer of organic solvent for its high solubility to water(4.31×105 mg/L) and low vapor pressure(37mmHg at 25¡É) which makes it hard to be removed by degastification or absorption using activated carbon.in this study, we compared with treatment efficiency of Plug Flow Rreactor and Continuous Stirred Tank Reactor by using kinetic parameters previously determined. by operating lab-scale reactors, we have observed 1,4-dioxane concentration of effluent reducing HRT to 40, 30 and 20 hour. in case of CSTR, even though HRT is enough, it is hard to treat 1,4-dioxane to certain concentration. it is thought that because of Smin. and in case of PFR, comparing with CSTR, when HRT is reduced, fluctuation of 1,4-dioxane concentration of effluent is more stable and treatment efficiency is also better than CSTR.
Keywords : 1,4-dioxane, biodegradation, parameter, cstr, pfr

B065

Effects on Tomato Growth and Lavender, Rosemary, Chrysanthemum using Soil Moisturizer (Geohumus) by Effective Microorganisms (EM) on Immobilized Treatment Effects

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The safety of agricultural products has been required, The importance of agriculture organic farming-friendly environment is growing. In this study, We use the Geohumus which is a soil wetting agent. Geohumus is composed of a superabsorbent polymer. It includes Trace Nutrient organic carbon, nitrogen, calcium, and magnesium oxide etc. It has a structure capable of absorbing at least about 40 times their weight of water than a sponge like. If you use to mix with the soil and Geohumus(GEO), you can see effects of enhanced moisture-holding capacity, Promotion of plant growth, increase yield, micronutrient losses inhibition, even at high temperatures, improved dry soil. In particular, it is totally harmless in nature consists of natural materials. Futhermore, we tried to immobilize the EM in GEO. EM (Effective Microorganisms) of Lactobacillus were identified based results and investigated by Candida Versatilis Yeast in YM medium and it was confirmed by photosynthetic bacteria, including Cyanobacteria with etc. It was conducted on the effects about growth of soil Microorganisms with immobilized GEO. It was consisted GEO immobilized EM, only Geohumus as control and only soil.Therefore, this study was performed September to November 2013 so that it can be utilized for agricultural and scenery areas, In the field of agriculture, used mini tomatoes seeds. In the landscaping field, used grown lavender, rosemary, chrysanthemum.
Keywords : Geohumus, EM, microorganism, immobilization, growth environment

B066

Linoleic Acid and Phenylacetic Acid-induced Expression of Defense Genes and Enzymes in Tobacco

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The plant growth-promoting rhizobacteria, *Ochrobactrum lupini* KUDC1013 induced systemic resistance (ISR) in tobacco against soft rot disease caused by *Pectobacterium carotovorum* subsp. *carotovorum* (PCC) and ISR determinants from KUDC1013 include the polyunsaturated fatty acid linoleic acid (LA) and the non-indole compound phenylacetic acid (PAA). In the present study, we examined the effect of exogenous application of different LA and PAA concentrations on growth promotion and ISR activity. The response of tobacco was evaluated based on root formation, membrane permeability and on defense related enzymes such as phenylalanine ammonia lyase (PAL), peroxidase (POD) and polyphenoloxidase (PPO) and β-glucuronidase. Moreover (RT-PCR) analysis was performed to determine the induction of defense related genes. Results revealed the modulation of both LA and PAA on plant growth and defensive mechanisms by inducing the expression of defense genes and enhancement of defense enzyme activities. Altogether, this study supports the role of LA and PAA in plant growth development and in eliciting plant defense signaling.
Keywords : linoleic acid, phenylacetic acid, induced systemic resistance, defense genes, defense enzymes

B067

Analysis of Bacterial Community Structure of Soybean

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We analysed bacterial community and diversity in soils (bulk and rhizosphere), roots and nodules of soybeans (*Glycine max* and *Glycine soja*) using pyrosequencing based on the 16S rRNA genes. At the phylum level, *Acidobacteria* (34-38%) was a predominant bacteria in bulk soil and rhizosphere of soybean. *Proteobacteria* (24%) and *Chloroflexi* (8-11%) and *Firmicutes* (3-5%) were followed. *Proteobacteria* was a predominant phylum (more than 90%) in the nodule of soybeans. Soybean root had higher portion of the phylum *Proteobacteria* than bulk and rhizosphere soils. At the genus level, *Bradyrhizobium* was the most predominant genus in the roots and nodules. We carried out clone library analysis of 16S rRNA gene from soybean nodules in order to check what species of the genus *Bradyrhizobium* is abundant in the nodule. There was no clear distinction in the rhizosphere bacterial community structure between *G. max* and *G. soja*. This study revealed that bacterial community of nodule was simple and *B. diazoefficiens* occupied the largest portion of bacterial community composition in nodule.

B068

Screening of Antagonistic Microorganism for Control the Ginger Root Rot

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Ginger(Zingiber officinale) is a root crop of fragrant exciting perennial grass belonging to the Zingiberaceae family, Scitamineae order. Ginger grows over a wide area and operated in Korea 1,018 years ago. Normally, gingers have been stored in underground tunnel. Soft rot of ginger caused by Pythium sp. is extensive damage to the production of ginger. These problems occur even to the storage. This study was performed for improve the storage quality.Antagonistic microorganisms screening from field and isolated bacteria AM25. It was showed that the antifungal activity by paper disc method(10.66~11.79mm). And experiments in vitro shows 10% storage rate. We can be expected to increased the activity by isolated bacteria optimization. So We thought that it is possible to take advantage as biological agricultural materials during storage.
Keywords : Ginger, Antagonistic bacteria, Biological control, Pythium sp.

B069

Investigation of Optimal Medium Conditions Antagonistic Microorganisms to Control for Ginger Pathogens

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Biological antagonists of ginger disease were isolated from soil in Gyeongbuk, Korea. AML 1116 which can strongly a antifungal activity and antibacterial activity against ginger pathogens. Culture conditions for the maximum production of the antagonistic substance were optimized for Davis minimal medium. As a result it is composed the best activity on Maltose, NH4Cl, Yeast extract and MnSO4 at 2%, 0.3%, 0.04% and 0.01% respectively. By time course of culture solution selected AML 1116 the culture solution after 48hrs had strongly growth inhibition rete against ginger pathogens. And culture solution of AML 1116 was stable within a pH range 5-11 and temperature range 4-70.
Keywords : Ginger pathogens, Antagonistic bacteria, Optimal medium condition

B070

Investigation of Adjuvants for Formulation toward Entomopathogenic Fungus *Beauveria bassiana* M130

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The greenhouse whitefly is an economically important pest for greenhouse crops because they cause direct damage by feeding on plant nutrients and indirect damage as transmits many virus vectors. It has recently become a serious problem because of the continuous use of insecticide resulting in resistance among greenhouse whitefly population. Enhancing effect of the microbial pesticide development adjuvant selection is needed. In this study, microbial insecticides for control of the greenhouse whitefly adjuvant for the development of research on the search was conducted. Studies dispersing agents, humectants, sunscreen and oil was performed in selection, individual spores dispersing agents, humectants, sunscreens and oil heamocytometer after treatment with a mixture of spores that were selected by checking. As a result, the dispersing agent is a DO-113 100ppm, humectants is a Gelatin 0.5%, the sunscreen is Lowilite22 100ppm, oil is corn oil 10% was selected. This results in the further development of greenhouse whitefly insecticides used for controlling microorganisms may be considered.
Keywords : *Beauveria bassiana*, Greenhouse whitefly, Formulation

B071

The Effect of Quorum Quenching on the Characteristics of Sludge Supernatant

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Recently, quorum sensing (QS) has been found to play a key role in biofilm formation on the membrane surface of submerged membrane bioreactors (MBRs) for wastewater treatment. Thus quorum quenching (QQ) which interrupts QS systems has received great attention as a fundamental solution to biofouling in MBRs. Various studies have proved that QQ could alleviate biofouling in MBRs, mostly focusing on the structure and the composition of biofilm on membrane. However, the effect of QQ on soluble microbial product (SMP), which are also an important factor in membrane fouling, has not been determined yet. The aim of this study was to investigate the characteristics of the sludge supernatant while applying QQ. A QQ microbial vessel, which is microporous hollow fiber membrane containing immobilized Rhodococcus sp. BH4 (AHL-lactonase producing bacteria), was inserted in anoxic reactor of anoxic/aerobic combined submerged MBR. When QQ microbial vessel was applied, rise of transmembrane pressure was less steep than MBR without QQ microbial vessel. In addition, the fouling resistance of the supernatant decreased in the dead-end filtration experiment. Furthermore, HPLC-SEC fluorescence analysis showed that the amount of aromatic protein-like substances in supernatant decreased, especially, those with molecular weight ranging from 100 to 1000 kDa were found to be reduced more than others.
Keywords : Membrane biofouling, Quorum sensing, Quorum quenching

B072

Microbial Responses to Functional Mortar Containing Copper-ion Beads

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Biofouling is the accumulation of living organisms on wetted surfaces which may reduce the durability and stability of the structure. Biofouling is often initiated by proliferation of bacteria and algae for which inhibition of microbial biofilm is important. Furthermore microorganism forming biofilm produces various organic acids which degrade the surface of the concrete. In this study, we developed functional mortar containing Cu-ion beads which can act as an anti-fouling agent. To test effects of the functional mortar, we analyzed algal biomass (Chl-a), bacterial abundance (16S rRNA gene copy number), and bacterial community structure (t-RFLP, NMS analysis) on the surface of the mortar after exposing it for 8 weeks in water. In freshwater, the functional mortar reduced algal biomass without any changes in bacterial abundance. In seawater, conversely, bacterial abundance was decreased on the surface of mortar containing 1.0mol% of Cu-ion without any changes in algal biomass. While the functional mortar exhibited significant anti-fouling effects the response differed for the types of organisms and sources of waters.
Keywords : Biofouling, Biofilm, Microbes, Anti-fouling, Concrete

B073

Investigation of Optimum Carbon Source on Heterotrpic Cultivation for High Lipid Productivity of *Chlorella protothecoides* B25

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Biodiesel production from the microalgae was needs several key factors such as cultivation, photobioreactor, harvest, and lipid extract. Generally almost microalgae was synthesis of carbohydrate, protein, and lipid using the light and carbon dioxide, however biomass productivity of microalgae on photosynthesis was not fast of microalgal growth. Furthermore when was cultivated of microalgae on photoautotroph, it was demanded too many factors. The other way for high biomass productivity of microalgae that can be used to carbon sources on non-light source. But microalgae cultivation on heterotrophic culture condition were supply of huge some of cost, it was because by administer carbon sources. Therefore in this study was supposed to using the waste carbon sources for *C. protothecoides* B25 cultivation on heterotrophic. Before the used to waste carbon sources for cultivation of microalgae on heterotrophic condition, it was using the commercial carbon sources (glucose, yeast extract, glycerol) for control group. Moreover investigated of lipid productivity of *C. protothecoides* B25 effect on concentration and mixed ratio of three carbon sources. For heterotrophic cultivation of *C. protothecoides* B25 was cultured in BBM at 25-26.5¡É on an orbital shaker at 110 rpm. The highest production and productivity of biomass and lipid were 0.2 M glucose, 10 g yeast extract, and 0.2 M glycerol, respectively. Also appeared of highest production and productivity at 0.2 M glucose.
Keywords : *Chlorella protothecoides*, Carbon Sources, Heterotrophic, Lipid Productivity, Biomass

B074

Bacterial Diversity and Community Structure in Upland Soil in Korea

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Soil bacteria play an essential role in nearly all biogeochemical cycles and participate in most nutrient transformations in soil. To assess how soil bacterial community and diversity are affected by environmental factors, a total of 220 upland soil samples were collected across Korea and subjected to pyrosequencing analysis. By analysis of high-quality screened 692,471 16S rRNA pyrosequences, 37,116 OTU (operational taxonomic unit) were found to exist in soils studied, estimating 76,456 and 63,187 putative species based on ACE and Chao richness estimator, respectively. The dominant phyla of soil bacteria were *Proteobacteria* (34.5%), *Acidobacteria* (20.4%), *Actinobacteria* (8.7%), *Bacteroidetes* (7.0%) and *Chloroflexi* (5.5%). Multivariate analysis showed that physical properties (soil texture, soil type and soil series) had little effect on bacterial communities of upland soils compared with chemical properties. Among chemical properties, pH were main driver for community structure change.
Keywords : upland soil, bacterial community, diversity, pyrosequencing analysis

B075

Isolation of Ampicillin- and Kanamycin-Resistant Bacteria from Playgrounds and Sewages

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The diversity of bacteria resistant to ampicillin and kanamycin were investigated through cultivation using samples from playgrounds and sewages. As expected, samples from sewages showed higher colony forming units than those from playgrounds for antibiotics-resistant bacteria. More than 60 strains were selected and 16S rRNA genes were sequenced to identify their taxonomic position. *Pseudomonas, Flavobacterium, Alcaligenes, Aeromonas* and *Sphingobacterium* are major genera identified. Some potentially pathogenic bacteria relating to *Shigella dysenteriae* and *Aeromonas salmonicida* were isolated. Several strains showed low similarity (<98%) with known species implying novel species at the species and genus levels.
Keywords : ampicillin, kanamycin, antibiotic resistant bacteria

B076

Seasonal Comparison of Bacterial Communities Associated with the Marine Sponge, *Halichondria panicea*

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The bacterial community structures of the sponge, *Halichondria panicea* collected from Jeju Yongmeori coast in August (summer) and December (winter) 2013 were compared by the PCR-DGGE based on cultivation independent method. The V3 regions of the 16S rRNA genes of two sponges were amplified to perform the DGGE. Two sponges showed different DGGE band patterns from each other. The 16S rRNA gene sequences derived from the DGGE bands showed 91-99% similarities to the known bacterial species in the public database and most of the sequences belonged to the uncultured bacteria. DGGE band patterns showed seasonal differences in two sponges, *H. panicea*. However, the bacterial diversities of *H. panicea* sponges collected in summer and winter were identically composed of 1 phylum 2 classes (*Alphaproteobacteria*, *Gammaproteobacteria*) at the phylum level.
Keywords : Sponge, bacteria, *Halichondria panicea*, DGGE

B077

Regional Difference of Bacterial Diversity in the Marine Sponge, *Hymeniacidon sinapium*

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The bacterial diversities of the marine sponge, *Hymeniacidon sinapium*, collected from Taean, Chungnam and Jeju island in 2014, were compared by the PCR-RFLP based on the cultivation dependent method. 162 bacterial strains from Taean sponge and 122 bacterial strains from the Jeju island sponge were incubated for 7 days at 26°C on Zobell and Marine agar media. For RFLP analysis, 16S rRNA genes of their genomic DNA were amplified by PCR and then PCR products were digested with restriction enzymes *Msp*I and *Hae*III. From the RFLP patterns 1 to 2 strains were selected for sequencing. The sequences showed 97-100% similarity to the known bacterial species in the public database. The bacterial diversities of two sponges were identical at the phylum level and were composed of 4 phylum, 5 classes (*Alphaproteobacteria, Gammaproteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Firmicutes*). However, Bacterial diversities at the genus and species level showed regional differences, revealing that bacterial community structure of Taean sponge was more diverse than that of Jeju island sponge.
Keywords : Sponge, Bacteria, *Hymeniacidon sinapium*, RFLP

B078

Isolation of Aerobic Thermophiles from Wastewater of Petrochemical Industry

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The purpose of this study was to isolate phenol-degrading aerobic thermophiles from sludges treating wastewater of petrochemical industry. As the first step, we established 25 pure cultures which showed growth on solid medium at 55°C. Among them, 11 isolates were confirmed as thermophilic bacteria of which optimum growth temperature was 55°C. Based on the 16S rRNA gene sequences, 21 out of 25 isolates were identified as genus *Chelatococcus*, and the other 4 isolates were identified as uncultured bacterial clone. We are currently measuring phenol-degrading activity of the 11 thermophilic isolates.
Keywords : thermophilic bacteria, wastewater treatment, phenol

B079

Isolation of Aerobic Thermophiles from High Salt Wastewater

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The purpose of this study was to isolate halophilic aerobic thermophiles from sludges treating wastewater of two chemical industries. As the first step, we established 14 pure cultures which could grow and form colonies on solid medium at 55°C. Among them, 7 isolates showed maximum growth at 55°C. Based on the 16S rRNA gene sequences, 11 out of 14 isolates were identified as genus *Bacillus*, while such genera as *Ureibacillus*, *Anoxybacillus*, and *Geobacillus* were the closest match of the other 3 isolates. Among the isolates identified as genus *Bacillus*, *B. licheniformis* was the most abundant one with 7 isolates, followed by *B. gelatini* (2 isolates), etc. We are currently analyzing the effect of NaCl concentration on the growth of the 14 isolates.
Keywords : thermophilic bacteria, wastewater treatment, NaCl

B080

Detection of Rare Species in Soil by CaO Treatment

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In this study, we hypothesized that the numbers of actively growing microorganisms might reduce to appropriate levels, with bearable level of reduction in richness of rare species, by treatment of a general purpose germicidal agent. Richness of t-RFLP fragments linearly decreased from 110 to 62 along CaO concentration gradient of 0-10%, with the slope of -4.3 fragments per 1%, indicating about 4% loss of richness per 1% CaO. Shannon-Weaver diversity for t-RFLP showed the range of 2.3-3.7, in which a steep increase of diversity culminating to the maximum at 5% CaO. Chao1 estimates of richness for pyro-reads were 639±121, 2,933±103 and 1,880±87 for 0%, 1% and 10% CaO treatments, respectively. Rarefaction analysis implied that richness of the untreated sample reached saturation while other richness values did not reach saturation. Shannon-Weaver diversity for pyro-reads were 1.8, 6.7 and 6.5 for 0%, 1% and 10% treatments, respectively. Based on these results, metagenomic analysis on the untreated soil could reveal genetic information only for about 640 species. In contrast, 1-5% CaO treatment could provide information about 3,000 species or more. Although 49 pyro-reads were found only in the untreated sample, the level of loss was within the range of statistical sampling error. Therefore, it could be concluded that CaO treatment can increase richness of genetic information in metagenomic DNA from soil, by raising evenness of species distribution with loss of only a few species.
Keywords : CaO, Evenness, t-RFLP, Pyrosequencing, Richness

B081

Isolation of Rhodovulum sp. NS6, a New Naphthalene Degrading Bacterium, Isolated from Tidal Flat Sediment

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Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds composed of two or more fused aromatic rings and they are important components of crude oil, creosotes, and coal tar. PAHs are causing great environmental concerns because of their persistence, toxicity, mutagenicity, and carcinogenicity. Many studies for bacteria capable of PAH degradations have been reported in marine environments, and many naphthalene-degrading bacteria including Pseudomonas sp. and Burkhoderia sp. have been isolated from various contaminated habitats. In this study, we constructed a slurry enrichment system using tidal flat sediment and a naphthalene degrading bacterium, designated Rhodovulum sp. NS6, was isolated from crude oil-contaminated marine sediment. Biodegradation tests showed that Rhodovulum sp. NS6 degraded naphthalene quickly in ONR7a as well as in seawater. PCR experiments for naphthalene dioxygenase gene detections of Rhodovulum sp. NS6 showed that only NDO 201 among NahAc, Phn, NDO200, NDO201, pPAH, and RISKE primers amplified successfully, which suggested that strain NS6 may use a nah catabolic pathway similar to that of Rhodococcus. In addition, the PAH degradation properties, physiologies, and PAH degradation gene structure of strain NS6 will be discussed more in the poster section.
Keywords : naphthalene, biodegradation, enrichment culture, PAHs, tidal flat

B082

Multivariate Analysis of Staphylococcus Community on Smartphones toward Determination of Risk Factors of Staphylococcal Infection

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In this study, we characterized staphylococcal community grown on BPA medium inoculated with bacteria from 52 smartphones. Incidences and relative frequencies of species were analyzed for underling factor shaping the community structure. Canonical correspondence analysis, carried out against characteristics of the phones, caregivers and children, revealed that age of children was a factor explaining variations in species incidence ($P<0.05$; permutation test). Phones of caregivers attending 1- to 2-year old toddlers were dominated by S. haemoliticus, S. hominis and S. saprophyticus while those for 5- to 6-year old children were by S. warneri. To identify physiological characteristics shaping the age-stratified community structures on the 52 phones, redundancy analysis were performed. Total variance in community structures were explained by two axes by 51% and 37%, respectively. The two axes collaborated to distinguish frequencies of S. saprophyticus and S. haemoliticus by higher usage rates of mannitol and xylitol. Based on the results, xylitol and mannitol usage appears to be the physiological feature that distinguish Staphylococcus on toddler caregiver' phone from those of others. In conclusion, smartphones built up staphylococcal community significantly differed by caregivers' activity. Thus, toddlers' caregivers should pay different types or levels of attention on hygiene of their smartphones.
Keywords : Staphylococcus Community, Multivariate Analysis, CCA, PCA, RDA

B083

Structural Analysis of Bacterial Communities in Local and Imported Sea Salts

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To explore the potential of using bacterial community composition in identifying origin of table salts circulated in the market, we analyzed variability of community composition of bacteria in sea salt samples from Sinan-gun, China and Vietnam. Bacterial colonies were enumerated after culturing on PCA medium at 37°C for 24 h. There were 10±4, 17±8 and 11±3 CFU/g for samples KS,CS, and VS, respectively. Bacterial abundance were not significantly different among the samples. Upon microscopic observation, isolates from KS and CS samples were mostly Gram-negative bacteria with 0.86-0.89 dominance values while only 33% of isolates Gram-negative for VS sample. When genetic similarity of isolates were analyzed by GTG5rep-PCR, Korean isolates and Chinese isolates were genetically highly similar. Shannon-Weaver diversity was 0.72,0.30 and 0.89 while evenness was 0.62,0.93 and 0.98 for samples KS, CS and VS, respectively. Those results indicated that the community in the Chinese salt was genetically similar to some Korean strains, but it had very low diversity and predominated by a few clonal strains. In conclusion, the number of culturable bacteria in salts are similar, but their community compositions were significantly different, rendering use of the information in tracing origin of marketed salts. Vietnamese origin may be distinguished by dominance of gram-positive bacteria. Chinese origin may be diagnosed by low diversity in spite of genetic identity of Korean and Chinese strains..
Keywords : Sea Salts, Bacterial Community, Shannon-Weaver diversity, evenness, GTG5 rep-PCR

B084

Improvement in Richness Estimation of Insect Microbiome by Use of a Germicidal Chemical

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Although study on insect pest microbiome might reveal information valuable for biotechnological applications, conventional metagenomic analysis, typically based on high-throughput DNA sequencing, produces highly redundant information, because of huge differences in the number of organisms. In this study, applied CaO, a germicidal chemical used to kill all kinds of organisms, to confirm increase evenness of the entomo-microbiome community in spite of loss in the total number of organisms. Larvae of diamondback moth (*Plutella xylostella*), at the same age were ground with a tissue grinder and treated with using CaO to the final concentration of 0-5%(w/w) for 30 minutes. After extracting metagenomic DNA, microbial community structure was assessed by 16S rRNA gene PCR and t-RFLP method. t-RFLP patterns were similar between the samples treated with 2% and 3% with similarity of 0.85, while samples with 4% and 5% CaO also showed the similarity by 0.81. While CaO conc. increased from 0% to 3%, diversity estimates and evenness estimate increased. At above 4% in CaO conc., diversity estimate decreased while evenness did not change significantly. Therefore, it could be concluded that treating diamondback moth larvae with CaO to the final conc. of 3% can increase richness of genetic information in metagenomic DNA samples. The optimized treatment condition is suggested as a pre-treatment method for estimation of richness of microbiome without use a deep-sequencing technique.
Keywords :

B085

Isolation and Characterization of Iron-reducing Lactic Acid Bacteria from Soil Mesocosms with Decomposing Meat

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It has been reported that CaO-treated livestock carcasses in landfills undergo a series of anaerobic microbial decomposition activities. Particularly, we have reported the case that lactic acid fermentation by *Leuconostoc gelidum* was succeeded by Fe(III)-reduction respiration by *Tepdimicrobium* and *Tissierella* species. In this study, iron-reducing bacteria were isolated from a mesocosm with meat-soil-CaO mixture, and the isolates were characterized. Landfill mesocosms were established as a mixture of soil, pork and quicklime(1% w/w). After incubating the mesocosm, samples were suspended and spread plated on TB or TS media (pH 9.2). The TS and TB plates were incubated at 37°C for 7 days in anaerobic jars. Isolates were identified as *Enterococcus gallinarum* by 16S rRNA gene sequencing. *E. gallinarum* has been reported to be able to reduce Fe(III) while performing lactic acid fermentation; therefore, iron-reduction could be carried out by this lactic acid bacterium at the same time with lactic acid fermentation. While further characterization is on way, it was concluded that alkaline soil might permit coupling of lactic acid fermentation and iron reduction by a single microorganism, unlike the temporally segregated case of *Leuconostoc* and *Tepdimicrobium-Tissierella* communities. Into our knowledge, this is the first report of finding the iron-reducing lactic acid bacterium *E. gallinarum* in Korean soil, although its isolation was once reported from Malaysian wetland.
Keywords : Iron-reducing bacteria, Landfills, *Enterococcus gallinarum*, Quicklime, Mesocosm

B086

Effects on Plant Growth from Nitrogen Fixing Rhizobacteria

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This study was focused on isolation and characterization of nitrogen-fixing bacteria from pepper (*Capsicum annum. L*) rhizospheric soil in Mir-Yang, South Korea, and investigate the effects on *in vivo* plant growth by them on plants. Thirty four strains were isolated using nitrogen free medium from pepper rhizospheric soil and partially identified using 16s rRNA gene sequencing.All strains were screened for plant growth-promoting characteristics. *In vitro* direct plant growth-promoting assays including phosphate solubilization, quantitative indole-3-acetic acid (IAA) production, siderophore production a colorimetric ammonia assay, cellulose production and a HCN production assay were performed. Twenty three isolates were able to solubilize phosphate, 13 produced siderophores and 10 isolates showed high IAA production ability, 18 strains produced cellulase while only two strains showed slight HCN production. As indirect PGP characteristic, these strains were assayed for antifungal activity against the fungal phytopathogen *Stemphylium lycopersici*. Five strains including strains were chosen as elicitors of plant growth promotion activity on pepper plants.
Keywords : Nitrogen fixing rhizobacteria, Rhizobacteria, Pepper, Phytopathgen

C001

Purification and Characterization of the Oligosaccharyltransferases in Bacteria and Protist

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Oligosaccharyltransferase (OTase) catalyze the transfer of a lipid-linked oligosacchride to the nascent polypeptide. Although eukaryote have a multi-complex OTase, whereas bacteria and archaea have a subunit. In addition, the kinetoplastids also might have only a Stt3p subunit in OTase. Phylogenetic tree analysis indicated that Stt3p would be split into three distinct groups; Archaea, Bacterial, and Eukaryotic Stt3p. Interestingly, most kinetoplastids protein in the eukaryotic group were clustered independently. Multiple amino acid sequence alignments of Stt3p homologues showed these enzymes have WWDYG and DK motif in the luminal domain. We purified two proteins, *Leishmania major* Stt3p (Lm_STT3D) in the kinetoplastids and *Campylobacter jejuni* PglB (Cje_PglB) (a homologue of eukaryotic OTase) in bacteria. We have detergent-solubilized, purified, and reconstituted enzymatically active His-tagged LmSTT3D from *Saccharomyces cerevisiae*, and Cje_PglB from *Escherichia coli*. Among the extensive panel of detergents that was screened, optimal solubilization and retention of Lm_Stt3p activity occurred with some of non-ionic detergents. In vitro and in vivo glycosylation experiments reveal that Lm_Stt3p can transfer the eukaryotic lipid linked oligosaccharide (LLO), $Glc_3Man_9GlcNAc_2$-PP-Dol, as donor substrate to a nascent peptide, whereas can not do a bacterial LLO, $GalNAc_2(Glc)GalNAc_3Bac$-PP-Und.
Keywords : oligosccharyltransferase, Stt3p, N-glycan, *Leishmania major*, Campylobacter jejuni

C002

Molecular and Biochemical Characterization of a Novel Class D Carbapenemase from Clinical Isolates

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Carbapenem-hydrolyzing class D β-lactamases (CHDLs) have been reported increasingly and common among *Acinetobacter baumannii* isolates. A total of 38 non-duplicate and carbapenem-resistant *A. baumannii* were recovered from a University hospital in Korea. MICs of the antimicrobial agents were determined, according to guidelines of CLSI, by an agar dilution method. Molecular characterizations of β-lactamases were performed by PCR amplification, DNA sequencing, and Southern blot analysis. The $bla_{OXA-418}$ gene was expressed by a pET-30 system and the gene product was purified by His-Bind column and Mono S column. Steady-state kinetic constants of the purified enzyme were determined by fitting the initial rates directly to the Henri-Michaelis-Menten equation using nonlinear regression with the program DYNAFIT. Among 38 isolates, one carbapenem-resistant *A. baumannii* harbored a novel variant ($bla_{OXA-418}$) of OXAs, which was encoded by the chromosome. The clinical isolate and its transformant showed resistance to carbapenems. Notable changes in MIC values were in line with the respective kinetic parameter differences. OXA-418 was most closely to OXA-228, from which it differed by five amino acid substitutions (Val25Glu, Ser192Arg, Asp201Asn, Glu227Lys, and Asn257Asp; OXA numbering system). Among these substitutions, Asp201Asn and Glu227Lys are new sites for carbapenem resistance among OXA-228-like genes.
Keywords : *Acinetobacter baumannii*, class D carbapenemase, carbapenem resistance

C003

A New Method for the Cytoplasm Extraction from Oak Pollen by Basidiomycotina

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This study was conducted to establish the optimized protocol for cytoplasm isolation of bee pollen. we measured the antioxidant activities as DPPH radical scavenging and the total polyphenol content of the pulverized and lyophilized oak pollens inoculated with fungi to confirm the husk removal effect. The total polyphenol content of oak pollen was the highest in the lyophilized pollen medium inoculated with Armillaria mellea, and was lowest in the pollen inoculated with Lentinula edodes. Total polyphenol content of the lyophilized pollen was higher than the refined pollen and the pulverized pollen in oak pollen germinated with A. mellea. The total polyphenol content of the lyophilized oak pollen germinated with A. mellea was 1.4-fold higher than that extracted with water. The antioxidant activity measured by the DPPH (2, 2 diphenyl-1-picrylhydrazyl) free radical scavenging method exhibited that the lyophilized oak pollen germinated with A. mellea had the highest and that germinated with L. edodes was lowest in antioxidant activities. The lyophilized oak pollen germinated with A. mellea was 2 to 4 times higher than that extracted with water in the antioxidant activity of DPPH free radical scavenging. Many germinated cells were formed around pore of acorn pollen inoculated with L. edodes, while those were formed at the end of hyphae derived from oak pollen inoculated with A. mellea.
Keywords : Antioxidant activity, Bee pollen, Armillaria mellea

C004

Mycelial Growth and Enzyme Activity of *Mycena* spp. Collected from Korean Forests

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Mycena is a genus with a small conical or bell-shaped cap. They are saprotrophic mushrooms commonly found on forest litter. Therefore, they play an important role in forest ecosystem as a decomposer decaying wood or litter. The pure cultures of six *Mycena* species collected from Korea forests were obtained by germinating spores of fruit body, respectively. We investigated the effect of culture media and temperature on mycelial growth of *Mycena* spp. After 21 days of incubation, 4 species showed the highest growth on potato dextrose agar (PDA). *M. amygdalina* and *M. rorida* were not grown on malt extract agar (MEA). The optimum temperature for mycelial growth of *Mycena* sp. was 20-25°C. *M. amygdalina* and *M. rorida* were not grown at high temperature (30°C), whereas *M. osmundicola* was not grown at low temperature (10°C). All species showed the carboxymethylcellulase (CM-cellulase) activity on CMC agar plates (pH6.0) after 8 days of incubation. *Mycena polygramma* among 6 species of *Mycena* showed the highest CM-cellulase activity. All species showed a laccase activity on 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) agar plates after 5 days of incubation. Two species (*M. amygdalina*, *M. viscidocruenta*) among 6 species of *Mycena* showed the highest laccase activity.
Keywords : Mycelial growth, *Mycena* spp., CM-cellulase, Laccase

C005

Disruption of CodY Expression Induces Global Changes in Transcriptome and Secretome Profiles of *Bacillus anthracis* Sterne

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A transcriptional repressor CodY plays a pivotal role in biological processes in gram positive bacteria. Previous reports reviewed its role in virulence and stringent response in Bacillus family, including *Bacillus anthracis* (*B. anthracis*). Although being crucial, the provided microarray information is limited as compared to data from the next generation sequencing. In this study we performed RNA sequencing (RNA-seq) to observe global regulation pattern of CodY during anthrax pathogenesis. Additionally, secretomes from *B. anthracis* were collected and differentially secreted proteins were identified using two dimensional electrophoresis (2DE) coupled with peptide mass fingerprinting (PMF). From RNA-seq, 139 genes were significantly affected by the *codY* disruption, with 132 genes depressed and seven genes upregulated. Major depression in secretome also could be observed from 2DE, mostly related to a virulence factor protective antigen and other metabolic proteins. Contrast with a previous work, our results showed deregulation of anthrax virulence activator AtxA by CodY disruption. We were able to detect genes previously predicted to be bound by CodY. From this work, CodY regulates genes that participate in gene transcription, translation, metabolic processes, stress response and cell survival. It can be speculated that CodY indirectly regulates virulence of *B. anthracis* by regulating virulence factor translation and enhancing survival in host cells.
Keywords : *Bacillus anthracis*, *CodY*, RNA sequencing, secretome

C006

Mycelial Growth and Cellulase activity of *Polyporus umbellatus* on Solid Media

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Polyporus umbellatus is a medicinal mushroom in East Asia. Three strains of *P. umbellatus* were isolated from sclerotium. We investigated the effect of culture media and temperatures on their mycelial growth. Interestingly, all test strains showed the highest mycelial growth on Modified Melin-Norkran's agar (MMNA) among four different culture media. This medium has been typically used to isolate or culture of ectomycorrhizal fungi not saprophytic fungi. The mycelial growth rate of *P. umbellatus* on MMNA was significantly (2- fold) higher than those on potato dextrose agar (PDA) after 60 days of incubation at 25°C. The optimum temperature for mycelial growth on PDA was dependent on strains (15-25°C). The growth of *P. umbellatus* mycelium was poor at 10 and 30°C. The cellulase activity of *P. umbellatus* mycelium was examined on carboxymethylcellulose (CMC) agar plate (pH6.0). After 8 days, all strains showed the carboxymethylcellulase (CM-cellulase) activity. One of the three *P. umbellatus* strains showed the higher enzyme activity (≥ 2.2-fold) than those of others.
Keywords : Mycelial growth, *Polyporus umbellatus*, CM-cellulase

C007

The Regulation Mechanism of the Iron-Responsive Transcription Repressor Fep1 in *Schizosaccharomyces pombe*

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The expression of iron transport genes in *Schizosaccharomyces pombe* is controlled by the Fep1 transcription factor. When iron is sufficient, Fep1 represses genes involved in iron uptake. In contrast, when iron levels are low, Fep1 becomes inactive and loses its ability to associate with chromatin. We have previously observed the Grx4 protein serves as an inhibitory partner for Fep1 in response to iron deficiency, but the mechanism by which Grx4 communicates low levels of iron to Fep1 remains unclear. In order to unravel the regulation mechanism of Fep1, biochemical characteristics of Fep1 was investigated. We have overexpressed *pombe* Fep1 in *E.coli*. During Ni-affinity purification, the wild-type Fep1 eluted as an orange-brown-colored protein. In contrast, the conserved cysteine mutants involved in iron sensing eluted as yellow-colored protein. UV-visible absorption and EPR studies of Fep1 indicate that Fep1 directly bind iron, most likely Fe-S cofactor.
Keywords : iron homeostasis

C008

Mycelial Growth And Enzyme Activity of Basidiomycetes Preserved in Low-Temperature

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Mycelial preservation has an effect on mycelial vitality according to preservation periods. Mycelial growth of strains preserved at a low temperate for one more year was measured on potato dextrose agar (PDA) or modified Melin-Norkran's agar (MMN) to investigate their vitality. Most of the tested strains were grown on agar plates. The mycelium of *Tricholoma matsutake*, *Sarcodon aspratus*, and *Suillus* spp. was slowly grown on PDA or MMN plates from 15 to 30 mm in diameter for 21 days. *Gloeostereum incarnatum, Mucidula brunneomarginata*, and *Albatrellus* sp. were fastly grown. In general, the mycelial growth of reactivated strains tends to slow as increasing preservation periods. Almost strains showed carboxymethylcellulase (CM-cellulase) activity. *Tricholoma* spp., *Amanita melleiceps*, *Suillus* spp. among tested showed high cellulase activity. The preservation periods of mycelium did not affect CM-cellulase activity. One the other hand, Laccase activity was decreased with increasing preservation period. *Inonotus obliquus*, *Amanita melleiceps*, *A. rubescens*, *G. incarnatum*, *M. brunneomarginata*, *Ganoderma lucidum*, *Hericium erinaceus*, and *Xerula pudens* showed high laccase activity by two 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) based on colorimetric assays.
Keywords : basidiomycetes, preservation, vitality, carboxymethylcellulase, laccase

C009

Biochemical Characterization of a Secreted Chitinase from *Coprinellus congregatus*

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A mushroom forming fungus, *Coprinellus congregatus*, generates several chitinases during its life cycle: chitinase 1 (Chi1) is produced throughout the whole life cycle, from hyphal stage to mushroom autolyzing stage, and chitinase 2 (Chi2) is strongly expressed at the mushroom autolysing stage. When this fungus is grown in the presence of colloidal chitin, a chitinase is secreted to the culture supernatant. We have isolated and purified this chitinase, which probably the third chitinase in this fungus. We will determine its biochemical characteristics and its possible functions in this fungus.
Keywords : chitianse, Coprinellus congregatus, mushroom forming fungus

C010

Isolation of Heterotrophic Bacteria from Polynya of Amunsen Sea of Antarctic Ocean

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Polynya has been known as sites where algae bloom repeatedly occurs during austral summer. Following high primary production by the algae blooms, heterotrophic bacteria become abundant. Polynya seawater samples were obtained from the polynya center in Amundsen Sea of Antarctic Ocean. Dominant heterotrophic bacteria were isolated using various media. Strains of five genera (*Bizionia*, *Leeuwenhoekiella*, *Pseudoalteromonas*, *Pseudomonas* and *Sulfitobacter*) could obtain. These genera were members of the bacterial community as by pyrosequencing of 16S rRNA genes. Seven strains from the genera were selected in each genus and physiological and biochemical characterizations were conducted. This study may expand the understanding of the roles of bacteria in the carbon cycles in antarctic polynyas.
Keywords : Heterotrophic bacteria, algae bloom, Antarctic Ocean

C011

Attempts to Estimate Anti-Prion Activity of Mycosporine-Like Amino Acid (MAA)

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Prion is the proteinaceous infectious particle that causes neurodegenerative diseases, such as scrapie, bovine spongiform encephalopathy and Creutzfeldt-Jakob disease in various species. Although the detail process, regarding abnormal conversion of prion protein (PrP) is still unknown, various environmental factors affect formation of misfolded PrP, termed PrP^{Sc}. Previous studies showed that UV light induces misfolding of PrP. We hypothesized that blocking of UV light results in inhibition of PrP^{Sc} formation. Because the oceanic algae contains MAAs which absorb UV spectra, we tested the inhibitory effect of various MAAs, including mycosporine-glycine, porphyra and shinorine, in prion-infected cells. When judged by the level of proteinase-resistant in western blot analysis, formation of PrP^{Sc} was not affected in spite of treatment with MAAs. The current results indicate that MAAs tested in this study are not effective in inhibiting prions.
Keywords : anti-prion, mycosporine-like amino acid

C012

The Effect of Rsd, the Anti-sigma Factor of σ^{70}, on Biofilm Formation in *Escherichia coli*

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In bacteria, s subunit of RNA polymerase (RNAP) directs transcription initiation. The regulation of s activity is important for fine tuning of gene expression. σ activity is determined by their cellular level, affinity for core RNAP, and interactions with regulatory proteins. In *Escherichia coli*, housekeeping s factor, σ^{70}, has the highest affinity for core RNAP and is the most abundant s factor. Rsd, regulator of sigma D, binds specifically to σ^{70} and it has been known as an anti-s factor of σ^{70}. In the previous study, it was shown that Rsd could affect the expression of subset of σ^{S}-dependent genes needed for the survival of *E. coli* in low-pH condition. However, an *rsd*-deficient mutant shows no apparent differences in its growth and viability in various media, compared to wild type. In this study, we found new phenotypes of the *rsd* mutant. Deletion of *rsd* affected the cell surface hydrophobicity and also mutant cells sank much faster than wild type. In spite of its increased cell surface hydrophobicity, biofilm formation decreased in the *rsd* mutant. There was an enriched protein in the *rsd* mutant when outer membrane proteins were compared with wild type and Rsd-overexpressed cell. Peptide mass fingerprinting revealed that this enriched protein is antigen 43 (Ag43). *agn43* encodes Ag43 and its transcription is σ^{70}-dependent. Therefore, we propose that Rsd influences the cell surface hydrophobicity and biofilm formation through the regulation of σ^{70} activity.
Keywords : anti-sigma factor, biofilm, outer membrane protein

C013

Genome-wide Analysis of the Extremophilic Bacterium *Fervidobacterium Islandicum* AW-1 Revealed the Degradation Mechanism of Feather Keratin

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The extremophilic bacterium *Fervidobacterium islandicum* AW-1 isolated from a geothermal hot stream in Indonesia could degrade native feathers (0.8%, w/v) completely at 70 lated from a geothermal hot stream in Indonesia could degrade native feathers (0.8%, w/v) completely at 70°C and pH 7 in the modified *Thermotoga-Fervidobacterium* (TF) medium. After 24 h of culture, feather degradation led to an increase in free amino acids such as histidine, cysteine and lysine. Moreover, nutritionally essential amino acids such as tryptophan and methionine, which are rare in feather keratin, were also produced as microbial metabolites. To better understand the mechanism of native feather-degradation, we first sequenced the 2.72-Mb genome of *F. islandicum* AW-1 that contains 2,938 protein-coding genes including 68 genes encoding proteolytic and redox-related enzymes. Genomic comparison of *F. islandicum* AW-1 with the closely-related *F. nodosum* which cannot degrade a native feather suggested that several protein-coding genes might be highly involved in keratin degradation, which was further investigated using the next-generation sequencing-aided RNA-seq. Based on the transcriptome data, we chose several putative genes for keratin degradation, overexpressed them in *Escherichia coli* and characterized the recombinant enzymes in detail. Consequently, this study provide the basis of identification of keratinolytic enzymes, which is potentially applicable for development of novel biomaterials for cosmetics as well as treatment of poultry wastes.

Keywords : *Fervidobacterium* sp., *F. islandicum* AW-1, Feather-degradation, Proteolytic enzymes, Redox-related enzymes

C014

Dephosphorylated NPr is Involved In an Envelope Stress Response of *Escherichia coli*

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Besides the canonical phosphoenolpyruvate-dependent phosphotransferase system (PTS) for carbohydrate transport, most *Proteobacteria* possess the so-called nitrogen PTS (PTS^{Ntr}) that transfers a phosphate group from PEP over enzyme I^{Ntr} (EI^{Ntr}) and NPr to enzyme IIA^{Ntr} ($EIIA^{Ntr}$). The PTS^{Ntr} lacks membrane-bound components and functions exclusively in a regulatory capacity. While $EIIA^{Ntr}$ has been implicated in a variety of cellular processes, such as potassium homeostasis, phosphate starvation, nitrogen metabolism, carbon metabolism, regulation of ABC transporters, and poly-β-hydroxybutyrate accumulation in many *Proteobacteria*, the only identified role of NPr is the regulation of biosynthesis of the lipopolysaccharide (LPS) layer by direct interaction with LpxD in *Escherichia coli*. In this study, we provide another phenotype related to NPr. Several lines of evidence demonstrate that *E. coli* strains with increased levels of dephosphorylated NPr are hypersensitive to envelope-elated stresses, such as osmotic stress, ethanol stress, and SDS stress, and these phenotype are independent of LpxD. The C-terminal region of NPr plays an important role in hypersensitivity to envelope-related stresses. Thus, our data suggest that the dephospho-form of NPr affects adaptation to an envelope-related stress through a C-terminal region-mediated unknown mechanism.

Keywords : nitrogen PTS, NPr, Escherichia coli, envelope-related stress

C015

Anthranilate has a Biofilm-Crumbling Activity and Antagonizes the Indole Singling in *Pseudomonas aeruginosa*

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Anthranilate and indole are aromatic compounds that are alternatively produced from tryptophan degradation according to bacterial species. We investigated the anthranilate effects on the *P. aeruginosa* biofilm formation. While indole enhances and accelerates the biofilm formation of *P. aeruginosa* throughout development, the anthranilate effect on biofilm formation was differentially exerted depending on the developmental stage and the presence of shear force. Anthranilate a bit accelerated the initial attachment of *P. aeruginosa* at the early stage of biofilm development and appeared to build more biofilm without shear force, but with shear force, it dampened the maturation of mushroom structure and crumbled biofilm at late stage, making flat biofilm. Anthranilate was able to crumble the pre-formed biofilm and extracellular polymeric substance (EPS) staining also showed the biofilm-crumbling effect of anthranilate. To investigate the interplay of anthranilate with indole in the biofilm formation, we co-treated anthranilate with indole and found that the addition of anthranilate abolished the biofilm-enhancing effect of indole. Interestingly, the anthranilate degradation pathway was synergistically activated by co-treatment of anthranilate and indole. HPLC analysis shows that the anthranilate accumulation in *P. aeruginosa* decreased by the indole-treatment, demonstrating that the indole-activation of the anthranilate degradation pathway reduced the anthranilate level.

Keywords : Anthranilate, indole, biofilm, Pseudomonas aeruginosa

C016

The role of *Pseudomonas aeruginosa* Quorum Sensing-Deficient Mutant in Biofilm Formation

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As sociobiology is a study based on the sociality of higher organisms, the sociomicrobiology is newly suggested field based on the social behaviors of microorganism. In order to do social behavior, organisms need to communicate each other and coordinate their group activity. A remarkable communication paradigm that enables microorganisms to do the social behaviors is quorum sensing (QS), the cell-density dependent signaling mechanism of bacteria. In this study, we tried to find out the cooperation of wild type and QS-deficient mutant in the biofilm formation, one of the representative group behavior of bacteria that is controlled by QS. Using *Pseudomonas aeruginosa*, an opportunistic human pathogen, the biofilm formation was carried out with three groups; one included only wild type, another, only QS-deficient mutant, and the other, mixed group of wild type and QS-mutant at the ratio of 1:1. The result showed the possibility that the QS-deficient mutants can have an important role in biofilm formation, enhancing the initial attachment at the early stage of biofilm formation.

Keywords : Sociomicrobiology, Quorum sensing, Biofilm formation, *Pseudomonas aeruginosa*

C017

Metabolome Analysis of Rice Leaf Tissue to Staurosporine Treatment under and Free of Bacterial Blight by *Xanthomonas oryzae* pathovar *oryzae*

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Rice bacterial blight by *Xanthomonas oryzae* pv. oryzae (Xoo) is one of the most severe diseases of rice nearly all over the world. We found that staurosporine isolated from Streptomyces sp. protect or enhance the rice leaf tissue from the disease. To evaluate the difference of rice leaf metabolites by staurosporine and understand the mechanism protecting rice leaf against Xoo, we examined the metabolites of four treatments; control (without Xoo and staurosporine), Xoo, staurosporine and staurosporine with Xoo treated rice leaf. After 4 day treatment, extracts were analyzed by LC-MS and GC-MS. In staurosporine-treated rice tissues, increase of class of flavonoids and amino acids were observed.
Keywords : staurosporine, rice, *Xanthomonas oryzae* pv. *oryzae*, metabolome

C018

Ornithine Lipid Influences the Biofilm Formation And Virulence of *Pseudomonas aeruginosa*

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OLs are widely found in outer membrane of many gram-negative bacteria, but not detected in Eukarya and Archaea. *Pseudomonas aeruginosa*, an opportunistic pathogen has *olsBA* genes that constitute an operon to function the ornithine lipid biosynthesis. *olsBA* encodes acyltransferases and works in two steps for the OL biosynthesis, in which OlsB transfers an acyl group to ornithine to make lyso-ornithine lipid and OlsA converts the lyso-ornithine lipid into ornithine lipid by another acyl-group transfer. OLs are reported to increase in phosphorus-free culture condition and *olsBA* operon of *P. aeruginosa* is induced in phosphate-limiting condition. While OLs were suggested to reduce the toxic effect of endotoxin probably functioning as an antagonist, a recently study showed that the mutation of this operon had no effect on the virulence of *P. aeruginosa*. In this study we found that the overexpression of *olsBA* operon modulated some virulence related-phenotypes of *P. aeruginosa*, including quorum sensing regulation, biofilm formation, and motility. We found that the overexpression of this operon modulates quorum sensing by reducing the quorum sensing signal production. Interestingly, the *olsBA*-overexpressing *P. aeruginosa* cells induced calcium release of animal cells, implying that it may modulate the physiology of host cells.
Keywords : ornithine lipid, biofilm, *Pseudomonas aeruginosa*, virulence, quorum sensing

C019

Structural Analysis of *Bacillus subtilis* YtqB and Its Implication in SAM-dependent Methylation

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Methyltransferases (MTases) methylate diverse acceptor molecules, including small chemicals and protein or nucleic acid macromolecules, using an S-adenosyl-L-methionine (SAM) cofactor, and contribute to a wide variety of cellular processes, such as cell signaling, metabolite synthesis, and gene regulation. The ytqB gene of Bacillus subtilis encodes a putative MTase, and its biological function and molecular structure remain to be revealed. To address the structural mechanism used by YtqB to methylate its cognate substrate, we have determined the crystal structures of YtqB alone and in association with SAM. YtqB adopts the αβα sandwich architecture in a dimeric assembly. Each YtqB monomer possesses one SAM binding site and exposes the reactive methyl group of SAM potentially to a substrate. Moreover, we will discuss the identity of YtqB's substrate and a possible substrate binding site, based on the comparative analysis.
Keywords : *Bacillus subtilis*, YtqB, Methyltransferase, S-adenosyl-L-methionine, Crystal structure

C020

Transcriptome Analysis of Rice Leaf Tissue to Staurosporine Treatment under and Free of Bacterial Blight by *Xanthomonas oryzae* pathovar *oryzae*

Jeong-Gu Kim[1]*, Hyang Yeon Kim[2], Mee Youn Lee[2], Sait Byul Park[1,2], Joo-Won Suh[3], In Ae Lee[3], Jinhua Cheng[3], and Choong Hwan Lee[2]
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Rice bacterial blight by *Xanthomonas oryzae* pv. oryzae (Xoo) is one of the most severe diseases of rice nearly all over the world. We found that staurosporine isolated from Streptomyces sp. protect or enhance the rice leaf tissue from the disease. To evaluate the difference of rice leaf transcripts by staurosporine and understand the mechanism protecting rice leaf against Xoo, we examined the metabolites of four treatments; control (without Xoo and staurosporine), Xoo, staurosporine and staurosporine with Xoo treated rice leaf. After 4 day treatment, in staurosporine-treated rice tissues, increase of class of flavonoids and amino acids were observed. Some genes such as signal transduction, resistance protein homologues, and chorismate mutase were enhanced by the treatment, where several genes involving peroxidases and proteinase inhibitors were down regulated.
Keywords : staurosporine, *Xanthomonas oryzae* pv. *oryzae*

C021

Expression, Purification, Crystallization and Preliminary X-ray Analysis of an HD-Domain Containing Protein, YpgQ, from *Bacillus subtilis*

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The histidine-aspartate (HD) domain is ubiquitously found as a stand-alone protein or as a part of multi-domain proteins, and plays a key role in the nucleotide metabolism and signaling. The HD-domain-containing protein is involved in metal-dependent hydrolysis of canonical or modified nucleotides. The genome of *Bacillus subtilis* encodes a YpgQ protein that is known to possess the HD domain on the basis of the amino acid sequence analysis. However, the physiological function and the structural architecture of YpgQ have not been revealed to date. As the first step to address the molecular structure and the biological function of YpgQ, we have performed the expression, purification, crystallization and X-ray diffraction analyses of YpgQ. The recombinant protein of YpgQ was expressed in the *Escherichia coli* cell expression system and purified to homogeneity by Ni-NTA affinity and anion exchange chromatography. YpgQ was crystallized in PEG600 solutions in space group $P2_1$. X-ray fluorescence scattering on a YpgQ crystal identified a metal ion which is characteristic of the HD domain. X-ray diffraction data of YpgQ crystals were collected to 2.3 Å resolution and would make a significant contribution to defining the enzymatic activity of YpgQ.
Keywords : X-ray diffraction, Crystallization, HD domain, YpgQ, Metal dependent hydrolysis

C022

YraA, DJ-1 Superfamily Protein, Plays a Major Role in thiol-independent Glyoxalase III system in *B. subtilis*

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Methylglyoxal (MG), a reactive carbonyl compound, is generated ubiquitously as a by-product of cellular metabolism. MG can damage cellular constituents by glycation. MG can be detoxified to form D-lactate by thiol-dependent glyoxalase (GLO) I/II system and thiol-independent GLO III system. It has recently been shown that *Bacillus subtilis* utilizes bacillithiol (BSH) for GLO I (GlxA) and II (GlxB) system. And several DJ-1 superfamily proteins (YdeA, YraA and YfkM) have been suggested as candidate GLO III enzymes which can directly detoxify MG in a BSH-independent manner. We have investigated the GLO III system in *B. subtilis* using combinations of mutations in DJ-1 superfamily proteins (YdeA, YraA, YfkM and YoaZ). In the presence of BSH-dependent GLO I/II system, no single mutation in *ydeA, yraA, yfkM,* or *yoaZ* led to a significant decrease in resistance to MG. However, triple mutant strains containing *yraA::tet* exhibited significantly decreased resistance to MG, but not triple mutant strain (*ΔydeA, yfkM, yoaZ*) which has intact *yraA*. Furthermore, without functional BSH-dependent GLO I/II system, mutation in only *yraA* significantly decreased resistance to MG. All these results indicate that YraA plays an important role in thiol-independent detoxification of MG in *B. subtilis*. Indeed, purified YraA protein exhibited thiol-independent GLO activity, and mutation in putative active site abolished GLO activity, indicating that YraA is a main enzyme in GLO III system in *B. subtilis*.
Keywords : methylglyoxal, glyoxalase, *Bacillus subtilis*, bacillithiol, *yraA*

C023

Study of Antifungal Activity of *Bacillus* sp. *in vitro* and Partial Characterization of its Antifungal Compound

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A potential antagonistic strain of *Bacillus* sp. showing biocontrol activity against *Cylindrocarpon destructans* 8005 was isolated from the rhizosphere of ginseng. *Panax Ginseng* (C.A.Meyer) is a medicinal crop with high demand all over the world. The ginseng is affected by several pathogens of fungal species. The phytopathogenic effect reduces the crop yield and results in huge loss to the economy due to the reduction in export of the high valued crop. The biocontrol application has to be integrated with a ecological and economical point of view. The bacterial species were isolated by serial dilution method, and was identified based on the results of 16s rRNA sequencing. The initial studies on antagonism was tested with dual plate assay. To identify the optimized condition for the production of antifungal metabolites the growth of bacteria was tested on different media. The optimization studies included pH and temperature. The application of culture filtrate of *Bacillus* sp. showed inhibitory effect on *Cylindrocarpon destructans* a potential phytopathogen of ginseng. The effect of culture filtrate was tested depending on the optimized conditions for the metabolite production. Radial plate assay with culture filtrate showed 50% inhibition to the *Cylindrocarpons*. To identify the metabolite responsible for the inhibitory effect, thin layer chromatography was performed. Further studies, focuses to characterize the metabolite based on various factors and condition using HPLC.
Keywords : Antifungal activity, *Cylindrocarpon destructans*

C024

Multifunctional Control of UGPase by Stress-Responsive Transcription Factors, Msn2/4, in *Saccharomyces cerevisiae*

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Ugp1, a UDP-glucose pyrophosphorylase, has been considered essential for a variety of cellular activities since its product, UDP-glucose, is a sole glycosyl donor in several metabolic pathways, including the biosynthesis of carbohydrate storage molecules such as glycogen, trehalose, and the formation of some cell wall components. Although many regulators have been expected to be involved in regulation of *UGP1*, only Pho85 kinase has been reported to inhibit the *UGP1* transcription until now. Here, we propose that the regulation of *UGP1* is conducted by the transcription factors Msn2/4 according to protein kinase A, Pho85 kinase activity, and general stresses. We also found that three stress response elements (STREs) in the promoter of *UGP1* are necessary for binding of Msn2/4. Furthermore, we show that modulation of *UGP1* expression is required for stress response to external stimuli like reactive oxygen species (ROS), chronological life span (CLS) as well as for soluble carbohydrates synthesis. These results suggest that the regulation of Ugp1 level through Msn2/4 contributes to cellular homeostasis by inducing the glucose partitioning to synthesis of carbohydrates which act as defensive metabolites.
Keywords : UGPase, PKA pathway, Trehalose, Stress response, Life span

C025

The Crystal Structure of a Novel Phosphopantothenate Synthetase from the Hyperthermophilic Archaea, *Thermococcus onnurineus* NA1

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Pantothenate is the essential precursor of coenzyme A (CoA), a fundamental cofactor in all aspects of metabolism. In bacteria and eukaryotes, pantothenate synthetase (PS) catalyzes the last step in the pantothenate biosynthetic pathway, and pantothenate kinase (PanK) phosphorylates pantothenate for its entry into the CoA biosynthetic pathway. However, genes encoding PS and PanK have not been identified in archaeal genomes. Recently, a comparative genomic analysis and the identification and characterization of two novel archaea-specific enzymes show that archaeal pantoate kinase (PoK) and phosphopantothenate synthetase (PPS) represent counterparts to the PS/PanK pathway in bacteria and eukaryotes. The TON1374 protein from *Thermococcus onnurineus* NA1 is a PPS, that shares 54% sequence identity with the first reported archaeal PPS candidate, MM2281, from *Methanosarcina mazei* and 91% sequence identity with TK1686, the PPS from *Thermococcus kodakarensis*. Here, we report the apo and ATP-complex structures of TON1374 and discuss the substrate-binding mode and reaction mechanism.
Keywords : Coenzyme A biosynthesis, Archaea, Phosphopantothenate synthetase, TON1374, TON1374/ATP complex

C026

ATP-Binding Mode Including a Carbamoylated Lysine and Two Mg(2+) ions, and Substrate-Binding Mode In Acinetobacter Baumannii MurF

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MurF adds d-Ala-d-Ala dipeptide to UDP-*N*-acetylmuramyl-l-Ala-¥ã-d-Glu-*m*-DAP (or l-Lys) in an ATP-dependent manner, which is the last step in the biosynthesis of monomeric precursor of peptidoglycan. Here we report crystal structures of two MurF–ATP complexes: the MurF–ATP complex and the MurF–ATP–UDP complex. The ATP-binding mode revealed by the crystal structure of the MurF–ATP complex confirms the previous biochemical demonstration that a carbamoylated lysine and two Mg^{2+} ions are required for enzyme activity of MurF. The UDP–MurF interactions observed in the crystal structure of the MurF–ATP–UDP complex depict the characteristic substrate-binding mode of MurF. The emergence and dissemination of multidrug-resistant *Acinetobacter baumannii* strains are great threats to public health. Therefore, the structural information on *A. baumannii* MurF as a validated target for drug discovery will provide a framework to develop antibacterial agents against multidrug-resistant *A. baumannii* infections as well as to understand the reaction mechanism of MurF.
Keywords : Crystal structure, MurF–ATP complex, MurF–ATP–UDP complex, Carbamoylated lysine

C028

α-Factor Binding Assay to the G Protein Coupled Receptor of *Saccharomices cerevisiae* by using Spectrophotometric Method

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The α-factor pheromone receptor of *S. cerevisiae*, Ste2p, belongs to the family of GPCRs associated with cell signaling system. Ste2p provides understanding about the mode of function of GPCR receptor associated with signaling transduction. To develop the readily accessible affinity assay on yeasts GPCRs, we have designed new method based on the detection of free sulfhydryl groups. This analytical method makes possible the spectrophotometric measurement of receptor affinity and efficient ligand binding assays on yeast GPCRs. The interaction of ¥á-factor detectors with Ste2p sites was characterized in terms of the equilibrium dissociation constant (K_D). The total numbers of receptors for various numbers of yeasts using this detector were determined. The affinities of various achromic analogs for the cognate GPCR of *S. cerevisiae MAT*a were also determined. This method was proved to be rapid and convenient for determining the relative affinities of achromic competing pheromone by measuring their effect on the bound of the detectors.
Keywords : α-factor, GPCR, *S. cerevisiae*, receptor affinity, detector

C029

The Enzyme IIA^{Ntr} ($EIIA^{Ntr}$) Regulates Amino Sugar Metabolism by Direct Interaction with Glucosamine-6-phosphate Synthase (GlmS) in *Salmonella enterica* serovar Typhimurium

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The nitrogen-metabolic phosphotransferase system (PTS^{Ntr}) consists of enzyme I^{Ntr} (EI^{Ntr}, encoded by *ptsP*), NPr (encoded by *ptsO*) and enzyme IIA^{Ntr} ($EIIA^{Ntr}$, encoded by *ptsN*). Due to the location of *ptsO* and *ptsN* downstream of *rpoN* in the same operon, this system is postulated to be involved in nitrogen metabolism. Since a specific substrate transferred by this PTS^{Ntr} is yet to be determined, it has also been suggested that this system functions mainly in a regulatory capacity. It has been reported that $EIIA^{Ntr}$ controls the function of various enzymes by protein-protein interaction. To further understand a function of $EIIA^{Ntr}$, we used ligand fishing and found that glucosamine-6-phosphate synthase (GlmS) directly interacted with $EIIA^{Ntr}$. GlmS, which converts D-fructose-6-phosphate into D-glucosamine-6-phosphate (GlcN6P), is a key enzyme of amino sugar metabolism in bacteria. Amino sugar is an essential structural building block for the bacterial peptidoglycan and LPS. In this study we demonstrate that $EIIA^{Ntr}$ inhibits GlmS activity by direct interaction in a phosphorylation state dependent manner. Since the phosphorylation state of PTS^{Ntr} is affected by nitrogen availability and $EIIA^{Ntr}$ protein is degraded by Lon protease under GlcN6P limited condition, we suggest that PTS^{Ntr} plays a key role in maintaining the amino sugar homeostasis in response to nitrogen availability and amino sugar concentration in bacterial cytoplasm.
Keywords : Nitrogen-PTS^{Ntr}, GlmS, Protein-protein interaction

C030

Characterization of two *Pseudomonas aeruginosa* Mutant Strains, which are Defective in Rhamnolipid Biosynthesis

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Pseudomonas aeruginosa is gram-negative, rod-shaped, aerobic, opportunistic pathogen. *P. aeruginosa* is well known to produce biosurfactant rhamnolipids and to secrete several pigments. In this study, Two *P. aeruginosa* strains, Inh3 and Inh7, were derived mutants from wild-type strain (NO4) which was isolated from Rotenone solution. Two mutant strains (Inh3 and Inh7) produced nearly half amount of pyocyanin (green pigment) and pyoverdine (fluorescent yellow pigment) than NO4. Inh3 and Inh7 strains produced little rhamnolipid, compared to NO4. In addition, their swarming motilities, which was controlled by rhamnolipid, were steeply decreased than NO4 strain. These mutants showed a little antagonistic activity against gram-positive bacteria. The N-butyryl-homoserine lactone (C4-HSL), as quorum sensing (QS) molecule, is well known to regulate the rhamnolipid

Keywords : Pseudomonas aeruginosa, mutant, rhamnolipid, quorum sensing, C4-HSL

C031

Effect of Maltodextrin Glucosidase (MalZ) with Transglycosylation Activity on glycogen/mltodextrin mtabolism in *Vibrio vulnificus*

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Vibrio vulnificus, a pathogenic marine bacterium that can cause septicemia in human, experiences the host and aquatic environments alternatively during its life cycle. Therefore, it needs to have various response systems that ensure energy supply for survival. *V. vulnificus* accumulates higher amounts of glycogen with shorter side chains than *Escherichia coli* or *Bacillus subtilis*. The *malZ* gene of *V. vulnificus* encoding a maltodextrin (mdx) glucosidase was cloned and overexpressed in *E.coli* to investigate its role in glycogen/mdx metabolism. The *malZ* mutant constructed by allelic exchange grew slower and accumulated less glycogen than wild type in LB broth or M63 minimal media supplemented with glucose, maltose, or mdx. Both wild type and the mutant accumulated more glycogen when supplemented with mdx than with glucose or maltose. Glycogen extracted from each strain had short side chains with degree of polymerization (DP) less than 10. Side chains with DP4 were most abundant in both strains. Side chains of DP3 in glycogen from wild type increased and those of DP5 or longer decreased during growth, suggesting that longer side chains were degraded into shorter ones. On the other hand, glycogen from the mutant showed an opposite pattern. The results suggested that MalZ might be directly involved in biosynthesis and degradation of glycogen by modulating side chains of the molecules and the bacterium had multiple pathways utilizing various carbon sources for glycogen biosynthesis.

Keywords : Glycogen, Maltodextrin, *Vibrio vulnificus*, MalZ, Maltodextrin glucosidase

C032

Cloning and Characterization of Oligo-1,6-Glucosidase from *Vibrio vulnificus* MO6-24/0

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Vibrio vulnificus is a rod-shaped, gram-negative halophilic marine bacterium, which can cause septicemia in human via intake of raw seafoods or wound infection. Since the bacterium experiences a life cycle between nutrient rich host and poor aquatic environment, it needs to response to the alternative environments and ensure energy sources to support the life style. *V. vulnificus* accumulates more glycogen than other bacterium and carries various genes involved in glycogen metabolism. MalL of *V. vulnificus* shared 62% and 58% of identity with the homologues of *Bacillus subtilis* 168 and *Escherichia coli*, respectively. MalL is known as a oligo-1,6-1,4-glucosidase in *B. subtilis*. A 1.6 kb DNA fragment carrying the *malL* gene of *V. vullnificus* was amplified by PCR, cloned in pET28a, and overexpressed in *E. coli* BL21(DE3) as an effort to investigate its role in glycogen metabolism. The gene encoded a protein of 586 amino acids with a predicted molecular mass of 63,210 Da. Purified MalL hydrolyzed *p*-nitrophenyl-¥á-glucopyranoside most efficiently, and then in the order of glycogen, maltodextrin, and starch but not pullulan. The optimal pH and temperature for the enzyme was 6.5and 35¡É, respectively. The activity of MalL was dependent on Mg^{2+} (400 mM).

Keywords : Glycogen metabolism, *Vibrio vulnificus*, MalL, Oligo-1,6-glucosidase

C033

Purification and Characterization of Anti-MRSA Substance from *Streptomyces* sp.

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This work focused on screening and characterizing antibiotic-producing actinobacterium to develop new antibiotics that can overcome the growing multidrug resistance of infectious disease-causing microbes, especially methicillin-resistant *Staphylococcus aureus* (MRSA). An actinobacterial strain exhibiting antagonistic activity against MRSA was isolated from a garden soil sample using selective isolation on ISP-2 medium. The isolate, designated SW-01 exhibited strong antibiotic activity against a wide range of bacteria, especially MRSA. The crude extract from SW-01 culture supernatant was used for further purification and antibacterial activity tests. Various methods was applied to purify and characterize the compounds like as column chromatography with Sephadex LH-20 and silica gel, thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and tandem mass spectrometry (MS/MS). Through the comparative analysis of 16S rRNA genes, the isolate could be assigned to the genus *Streptomyces*, as *S. zaomyceticus* was found to be the mostly related species, but the strain formed an independent phylogenetic lineage. Phenotypic analyses showed that the isolate was neutrophilic, mesophilic and oligohaline.

Keywords : antimicrobial activity, *Streptomyces*, actinobacteria, MRSA

D001

Identification and Characterization of New Antibiotic Peptide from *Bacillus* Strains Derived from Korean Traditional Fermentated Food

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Recently there is great effort to discover new type of antibiotics which can overcome current resistance issues, and one of them is to find the peptide antibiotics. To isolate and characterize a novel antimicrobial peptide, *Bacillus* strain YG1 was isolated from Korean traditional fermented soybean paste, *doenjang*. This *Bacillus* strain was identified through 16S ribosomal RNA analysis and revealed to have close similarity to *Bacillus amyloliquefaciens* sp. *plantarum* (99.93%). This strain shows remarkable antimicrobial activity against Gram positive bacteria like *Enterococcus faecalis* ATCC 29212, *Mycobacterium smegmatis* ATCC 9341 and also against several Gram negative bacteria like *Pseudomonas aeruginosa* KCTC 1637 and *Escherichia coli* KCTC 1923 by disc diffusion method. This antimicrobial peptide is produced in optimum condition of 37¡É and 160~180rpm in the 1% dextrose, 0.5% malt extract, 0.5% tryptone. The peptide was purified through ammonium sulfate precipitation and gel permeation column chromatography and general molecular weight is measured by Tricine SDS PAGE.
Keywords : Peptide, Antibiotics, *Bacillus* sp., Purification, Characterization

D002

An Effective Microbial Means for The Isolation and Characterization of Novel Antimicrobial Peptide from Fermented Food

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Antimicrobial peptides are considered as one of the candidate for the development of novel therapeutic agent alternative to conventional antibiotic therapy. They have extended range of action alike bactericidal opposing bacteriostatic having short contact time to induce killing. Resistance to antimicrobial peptides copies similar to those produced by humans which make bacteria more resistant to human own immune system rather than just antibiotics. With an aim of developing a novel antimicrobial peptide of effective therapeutic application, microbial source was selected of edible product. An antibacterial peptide produced by bacillus strain was isolated form fermented food, under optimum condition. Antibacterial peptide produced by this strain was purified to homogeneity by ammonuim sulfate precipitation and desalting by sequential amicon. Further, Sephadex G-25 column gel chromatography was performed. Active fractions were identified by antimicrobial activity and protein determination by Bradford assay. Molecular weight determination was done by Tricine SDS-PAGE which was found below 10 kDa and agar overlay bioassay against indicator organism was carried out. In addition, characteristic analysis shows no significant difference in various temperature range and pH stability in broad range.
Keywords : Antimicrobial peptides, Sephadex G-25, Bradford, Tricine SDS-PAGE

D003

Characteristics of Unrecorded Yeasts from Wild Flowers in Seonyudo of Gogunsanyeoldo, Korea and its Physiological Functionality

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Six kinds of newly recorded yeasts such as Rhodosporidium diobovatum SY4-2, Cryptococcus bestiolae SY7-1, Kazachstania unispora SY14-1, Kazachstania servazzii SY14-3, Pichia holstii SY20-2 and Cryptococcus tephrensis SY26-1 were screened in sixty one yeasts from wild flowers in Gogunsanyeoldo including Seonyudo, Jeollabuk-do, Korea. Their mycological characteristics and some physiological functionalities were investigated. All of them were oval and global in shape and Cryptococcus tephrensis SY26-1 only formed pseudomycelium. They grew well in vitamin-free yeast extract-peptone-dextrose (YPD) broth and 50% glucose-containing YPD broth. Pichia holstii SY20-2 also was halophile, growing in 20% NaCl-containing YPD broth. Cell-free extract from Kazachstania servazzii SY14-3 showed the highest 98.6% of α-glucosidase inhibitory activity and maximal production of the α-glucosidase inhibitor was obtained from 24h incubation at 30°C.

D004

Isolation and Physiological Functionality of Yeasts From Wild Flowers in Seonyudo of Gogunsanyeoldo, Jeollabuk-do, Korea

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Sixty one yeast strains of twenty one species were isolated from wild flowers in Gogunsanyeoldo including Seonyudo of Jeollabuk-do, Korea and identified by comparison of nucleotide sequences for PCR-amplified D1/D2 region of 26S rDNA using BLAST. Among them, *Cryptococcus* sp. including *C. aureus* SY1-4 were seen to be dominant, and *Metschnikowia* sp. including *M. reukaufii* SY20-1 and *Rhodotnula* sp. such as *R. ingeniosa* SY1-1 were also isolated abundantly. Some physiological functionalities of the culture broth and cell-free extracts from sixty one yeast strains were determined. Supernatant from *Metschnilowia reukaufii* SY44-6 was showed 49.6% of anti-gout xanthine oxidase inhibitory activity and 38.4% of whitening tyrosinase inhibitory activity, respectively.

D005

Cellulolytic Enzymes Produced by a Newly Isolated Soil Fungus *Penicillium* sp. TG2 with Potential for Use in Cellulosic Ethanol Production

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A newly isolated soil fungus, *Penicillium* sp. TG2, had cellulase activities that were comparable to those of *Trichoderma reesei* RUT-C30, a common commercial strain used for cellulase production. The maximal and specific activities were 1.27 U/mL and 2.28 U/mg for endoglucanase, 0.31 U/mL and 0.56 U/mg for exoglucanase, 0.54 U/mL and 1.03 U/mg for b-glucosidase, and 0.45 U/mL and 0.81 U/mg for filter paper cellulase (FPase), respectively. Optimal FPase activity was at pH 5.0 and 50°C. We used a simultaneous saccharification and fermentation (SSF) process, which employed the yeast *Kluyveromyces marxianus* and *Penicillium* sp. TG2 cellulolytic enzymes, to produce ethanol from empty palm fruit bunches (EFBs), a waste product from the palm oil industry. The present findings indicate that *Penicillium* sp. TG2 has great potential as an alternative source of enzymes for saccharification of lignocellulosic biomass.
Keywords : Cellulosic materials, empty palm fruit bunches, ethanol, *Penicillium* sp., SSF

D006

Screening of Antagonistic Bacteria with the Potential as Biological Control Agents Against Ginseng Damping-off

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Korean ginseng (*Panax ginseng*) has been recognized in the Orient as one of the most important medicinal root crops. Stable production of ginseng is limited by many factors including fungal diseases in field as well as during storage prior to consumption. Therefore, successful production of ginseng roots depends primarily on the control of these diseases. Ginseng damping-off is caused by the soil-borne plant pathogens, mainly *Rhizoctonia solani* and *Pythium* sp.. *R. solani* and *Pythium* sp. are widespread in soil and cause a seed rot or pre-emergent rot. Fungicides are commonly used to control ginseng damping-off, but their effectiveness is reduced because of the rapid evolution of fungicide resistance. There is an increasing need for new alternative antifungal agents different from those currently in use. Actinomycetes have been considered as alternative biocontrol agents of plant diseases. In our screening program for antagonistic bacteria with the potential as a biological control agent against *R. solani* and *Pythium* sp., which cause ginseng damping-off, several *Streptomyces* strains were selected. In *in vitro* test, these *Streptomyces* strains exhibited potent antifungal activity against *R. solani* and *Pythium* sp.. Among them, a *Streptomyces* sp. BS065 showed the most potent activity against *R. solani*. An antifungal substance was purified from the culture broth of *Streptomyces* sp. BS065 using chromatographic methods and identified by spectroscopic methods.
Keywords : Korean ginseng, Biological control agent

D007

Effect of a Novel Histidine Sensor Kinase Cloned from *Streptomyces acidiscabies* on Secondary Metabolite Production

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Streptomycetes are soil prokaryotic microorganisms that have a complex morphological life cycle and produce up to 70% of the known therapeutically useful antibiotics. *Streptomyces acidiscabies* produces not only an angucyclinone polyketide with antimicrobial properties designated WS5995B but also thaxtomin A that is the major species causing common scab. Two-component systems (TCSs) are an important signaling transduction pathway that adapt to changing environments. Commonly, a TCS comprises a sensor kinase that is usually an integral membrane histidine sensor kinase and a response regulator that mediates the cellular responses. Presently, however, we cloned a novel unpaired sensor kinase gene (*tcsK*) from *S. acidiscabies* and identified its functional involvement in the production of secondary metabolite. The elevated expression and disruption of the *tcsK* gene enhanced 7.1-fold and almost abolished WS5995B production in *S. acidiscabies*, respectively, but did not affect the production of thaxtomin A. In addition, the actinorhodin production of *S. lividans* TK24 was increased 5.7-fold by the high expression of *tcsK*. These results indicate that the novel unpaired *tcsK* gene may be related to the control of secondary metabolite production in Streptomycetes. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0008443)]
Keywords : *Streptomyces acidiscabies*, Two-component systems, Histidine sensor kinase, Secondary metabolite, *Streptomyces lividans*

D008

Isolation and Characterization of *Leuconostoc mesenteroides* CS-5 Isolated from Kimchi that Produced Dextran

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Strains of lactic acid bacteria were isolated from kimchi fermented at a mesophilic temperature ranged from 14°C to 30°C. Among isolates, a strain named as CS-5 showed a narrow optimal temperature that ranged from 24°C to 28°C and the strain was characterized as *Leuc. mesenteroides*. Interestingly the CS-5 was turned out to be a dextran producing strain. It could grow in the media containing 6% NaCl. From the disaccharide fermentation test, salt tolerance of the CS-5 was increased in media containing sucrose or maltose but its ability was decreased in media containing trehalose, melibiose or cellobiose. For enhancing dextran production of the CS-5, treatment of 30% sucrose (w/v) yielded higher values of viscosity (0.70 $Pa.s^n$) comparing to the control (0.01 $Pa.s^n$) that was measured by a Reometer system. When 1.5% of skim milk was added to the media, its consistency index was enhanced upto 0.99 $Pa.s^n$. Typically viscosity of fermented media was greatly enhanced by treatment of 0.01% $CaCl_2$ (1.67 $Pa.s^n$). This kind of rheological properties was also found in a DNS test for measuring reducing sugar content that revealed amount of dextran produced by *Leuc. mesenteroides* CS-5.
Keywords : Dextran, Kimchi, *Leuconostoc mesenteroides*, Viscosity

D009

Polyphenols from the Fruiting Bodies of *Inonotus obliquus* and Their Antioxidant Activities

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The medicinal fungus *Inonotus obliquus* in the family Hymenochaetaceae has been used as traditional medicine for the treatment of various diseases. During the search for natural antioxidants from medicinal fungi, we found that fruiting bodies of *I. obliquus* exhibited potent antioxidant activity. In this study, we isolated free radical scavengers from the fruiting bodies of *I. obliquus*, and their chemical structures were determined by spectroscopic methods. Antioxidant capacity of these compounds was estimated by the ABTS and DPPH radical scavenging assay methods. The ethyl acetate-soluble portion was concentrated under reduced pressure, and the concentrate was subjected to Silica gel column chromatography, followed by Sephadex LH-20 column chromatography. Finally, active fractions were separated by preparative high-performance liquid chromatography (HPLC). The structures of these compounds were determined as two new compounds, 4-(3,4-dihydroxyphenyl)but-3-en-2-one, 4-hydroxy-3,5-dimethoxybenzoic acid, 3,4-dihydroxybenzaldehyde, 4-hydroxybenzene-1,3-dioic acid, and 3,4-dihydroxybenzoic acid by spectroscopic methods.
Keywords : *Inonotus obliquus*, Antioxidant

D010

Active Constituents of Korean Oak Pollen on Neuraminidase Inhibition

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Neuraminidase plays an important role in viral proliferation and is therefore a drug target for prevention of the spread of influenza. For this reason, neuraminidase inhibitors may provide a protection against viral diseases, so neuraminidase inhibitor has received attention as a new target to control neuraminidase-related infection. In this study, we investigated neuraminidase inhibitory activity of the oak pollen. The methanolic extract of oak pollen was concentrated to eliminate methanol and partitioned consecutively between hexane, chloroform, ethyl acetate, butanol and water. The ethyl acetate-soluble portion was concentrated under reduced pressure, and the concentrate was subjected to ODS column chromatography eluted with an increasing amount of methanol in water, followed by Sephadex LH-20 column chromatography eluted with methanol. Finally, Active fractions were separated by preparative high-performance liquid chromatography. Their chemical structures were determined by spectroscopic methods including one- and two-dimensional NMR and mass. Detailed biological activity of these compounds will be presented.
Keywords : Oak pollen, Neuraminidase

D011

Comparison Analysis of ergothioneine in *Hericium erinaceum* using by LC/MS

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Ergothioneine is known for natural amino acid as the antioxidant and anti-inflammatory substance. This study was carried out to investigate comparision anaylsis of ergothioneine in *Hericium erinaceum*(21 kinds of strains). For the analysis of ergothioneine, we extracted this mushroom with 30% and 50% EtOH. Metabolic analysis was performed using by UPLC-MS spectrometry in gradient eluent condition by acetonitrile(0.1% FA) and water(0.1% FA) with flow rate (0.3ml/min). Instruments conditions were followed by detection ion mode(positive ion), curtain gas(10), collision gas(Medium), ionspray voltage(5.5kV), temperature (520°C), ion source gas 1(50), ion source gas 2(50), interface heater(on). In this study, ergothioneine contents of the *Hericium erinaceum* were ranged from 3.65 mg/100g to 483.65mg/100g (dry weight). The contents of ergothioneine was higher in three strains with (483.65 mg/100g), (246.48mg/100g), (256.78mg/100g) than in other strains.
Keywords : Mushroom, Hericium erinaceum, ergothioneine

D012

Dural Function of Fungal Secondary Metabolites Isolated from Highland and Desert Lichens on UV Absorption Activity and Melanoma Cell Cytotoxicity

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Lichens have been well known to have unique substances which have many biological activities such as antioxidant activity, antimicrobial activity, and anticancer activity. Ultraviolet radiations exist in the sunlight which is harmful for human health. UVB (280-315nm) radiation is considered to be a carcinogen, and UVA (315-400nm) may be the primary cause of sunlight-induced melanoma. Nowadays, the use of sunscreen is the most common UV protective method. We investigated the lichen species growing on exposed rocks to strong sun light in highland of Yunnan and arid area of Xinjiang province, China, for screening UV absorption active and cytotoxic compounds against mouse melanoma cell. Preliminary results showed *Cetrariopsis wallichiana* and *Flavocetraria cucullata* crude extracts which have both UVB and UVA absorption activity contain the similar compounds: usnic acid, secalonic acid A and red compound (unknown).These compounds were related to UV absorption, moreover, both secalonic acid A and red compound have absorption peak in UVA region. The cytotoxicity of these two crude extracts is higher than other lichens against mouse melanoma cell line, B16F1 and B16F10. IC_{50} of fraction secalonic acid A isolated from *F. cucullata* is the lowest against mouse melanoma cell, although it has cytotoxicity against HaCaT cell.
Keywords : cytotoxicity, lichen, natural substances, secalonic acid A, UV absorption activity

D013

Mycoflora Analysis of Korean Traditional Wheat Nuruk

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Nuruk is extensively used in the brewing of *Makgeolli*, one of Korea¡¯s most popular alcoholic beverages gaining global popularity recently. In the wake of this, the quality of traditional *nuruk*, needs to be enhanced. This study attempted to characterize the mycoflora and their temporal variations associated with traditional wheat based *nuruks* fermented at 2 different conditions (A-36°C and B-45°C) over a span of 30 days. The mycoflora load increased from 3.59 to 8.39 log CFU/g in case of *nuruk* A and 7.59 log CFU/g in case of *nuruk* B on the 3rd Day, followed by a decrease up to 7.25 and 7.34 log CFU/g respectively until 10th day and becoming almost stationary till the 30th day. 59 fungal isolates belonging to 9 genera and 14 species were identified. Prominent isolates included *Lichtheimia*, *Penicillium*, *Trametes*, *Aspergillus*, *Rhizomucor* and *Mucor*. A total of 20 different yeast isolates were characterized belonging to 6 genera and 7 species, prominent among which were *Rhodotorula*, *Pichia*, *Debaryomyces*, *Saccharomycopsis* and *Torulospora.* These results suggested a prominent mycofloral diversity associated with wheat based traditional *nuruk.* Community analysis predicted temporal variations in the different genera associated with them throughout the 30 day fermentation process. With the mycoflora of traditional wheat based *nuruk* being explored, selective enrichment of useful genera may lead to quality enhancement of Korean alcoholic beverages.

Keywords : *nuruk*, mycoflora, *Lichtheimia*, *Aspergillus*

D014

Effects of Oral Intake of Kimchi-derived *Lactobacillus plantarum* K8 Lysates on Skin Moisturizing

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Skin is the soft outer covering of vertebrates that provides protection from pathogenic infection, physical damage, or UV irradiation and controls body temperature and water content. In this study, we examined the effects of oral intake of Kimchi-derived *Lactobacillus plantarum* K8 lysates on skin moisturizing. In an *in vitro* study, we observed that hyaluronic acid content increased in HaCaT cells treated with *L. plantarum* K8 lysates. Oral administration of *L. plantarum* K8 lysates effectively attenuated the horny layer formation and decreased epidermal thickening in DNCB-treated SKH-1 hairless mice skin. The damage to barrier function was reduced after 8 weeks of oral administration of *L. plantarum* K8 lysates as compared to that in the atopic dermatitis mice. A significant increase in hydration in the experimental group as compared to control group was observed on the face after 4 and 8 weeks, and on the forearm after 4 weeks. Decrease in horny layer thickness and TEWL value were observed on the face and forearm of the experimental group. Together, the *in vitro* cell line and *in vivo* mouse studies revealed that *L. plantarum* K8 lysates have a moisturizing effect. A clinical research study with healthy volunteers also showed an improvement in barrier repair and function when volunteers took *L. plantarum* K8 lysates. Thus, our result suggest that *L. plantarum* K8 lysates may help to improve skin barrier function.

Keywords : *Lactobacillus plantarum*, *L. plantarum* K8 lysates, bacterial lysates, atopic dermatitis, aglycone isoflavone

D015

Oral Administration of *Lactobacillus plantarum* Lysates Attenuates the Development of Atopic Dermatitis Lesions in Mouse Models

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Lactobacillus plantarum is a well-documented probiotic that has been used in clinical trials for the regulation of the immune system and treatment of gastrointestinal disease. In this study, we evaluated the effects of *L. plantarum* cell lysates on the immune regulation through the *in vitro* and *in vivo* studies. *L. plantarum* lysates were prepared by sonication method, and we observed that the repetition of disruption step increased active components within the bacteria lysates. Active components might affect TNF-α production, while LPS-induced TNF-α production was dramatically inhibited in a sonication-dependent manner in THP-1 cells. Oral administration of *L. plantarum* lysates effectively attenuated the horny layer formation and decreased epidermal thickening in NC/Nga mice skin. The damage to barrier function after the 8 weeks oral administration was reduced by *L. plantarum* lysates as compared to that in the atopic dermatitis (AD) mice. Further study revealed that *L. plantarum* lysates polarized Th1 response via induction of IL-12 and IFN- α production and inhibition of IL-4 and IgE production in NC/Nga mice. Together, our results suggest that *L. plantarum* lysates are remarkable material for host homeostasis and it could be used for the treatment of inflammatory diseases.

Keywords : *Lactobacillus plantarum*, Bacterial lysates, Atopic dermatitis, Cytokine, Immune regulation

D016

Stabilization of Symbiotic Bacteria of Nematodes for the Production of Antifungal Antibiotic against Plant Pathogenic Fungi

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The objective of this study was to find ways to increase antifungal activity while maintaining strain activity so that a product could be stored over a long period if the product was manufactured by using symbiotic bacteria of nematodes. The symbiotic bacteria of nematodes spontaneously produced two colony form variants called phase variants. Phase 1 variant produced non-protein antibiotics, whereas phase 2 variant produced no such compounds. Strains for maintaining phase 1 was selected from three strains (SB1, SB2 and SB3) from entomopathogenic nematodes. The growth of the selected SB2 strain on general medium was higher than that of the other two strains. Packed cell volume of SB2 strain reduced in culture broth showed radical pH change. Phase 1 of SB2 strain was maintained in TSB media after being stored for 2 weeks at 4¡É. If main culture was prepared by preventing radical pH changes during the pre-culture of isolated symbiotic bacteria, the production of antifungal active substances was increased with bacterial activity sustained.

D017

Efficient Bioconversion Reaction for Mass Production of D-Psicose from D-Fructose Using Engineered *Corynebacterium glutamicum*

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Bioconversion refers to the use of living organisms, often microorganisms, to carry out a chemical reaction that is more costly or not feasible nonbiologically. The aim of this study is to optimize the bioconversion process from D-fructose to D-Psicose. D-Psicose is a rare sugar which is not abundant in nature, and belongs to keto-hexose. D-Psicose has 70% of the sweetness of sucrose but almost no calories. It has several health benefits, such as suppressing lipid synthesis in the liver to reduce abdominal obesity, preventing the development of diabetes and functioning as a medicine for arteriosclerosis. D-Tagatose 3-epimerase enzymes can efficiently catalyze the epimerization of free keto-sugars, which could be used to produce D-Psicose from D-fructose, with a conversion yield of approximately 30%. To achieve mass production of D-psicose, D-tagatose 3-epimerase gene from *A. tumefaciens C58* was cloned and transformed into *C. glutamicum*, which enabled in vivo bioconversion reaction in which D-fructose was epimerized to D-psicose. Furthermore, the bioconversion reaction conditions were optimized to enhance the conversion efficiency and D-psicose production yield. Under the optimized reaction condition, the maximum conversion yield of D-psicose was obtained within 3hour, which was previously taken more than 24 hours. This work was supported by a grant from the Next-Generation BioGreen 21 Program (SSAC, grant# : PJ00948601), RDA, Korea
Keywords : bioconversion, d-psicose, d-fructose, d-tagatose 3-epimerase, *corynebacterium glutamicum*

D018

Diversity, Saccharification Capacity, and Toxigenicity Analyses of Fungal Isolates in Nuruk

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Nuruk samples collected from various regions in Korea were investigated in terms of fungal contents and diversity. Measurement of colony forming unit (CFU) in the nuruk suspensions on DRBC agar revealed that the nuruk samples, MS4, MS8, and MS10, were among the highest fungal density by showing 1,278.9±21.6 ($\times10^4$), 1,868.0±27.7 ($\times10^4$), 775.1±19.2 ($\times10^4$) CFU per 20 mg nuruk, respectively. The majority of the fungal components were yeasts, including *Pichia anomala*, *P. kudriavzevii, Kluyveromyces marxianus,* and *Saccharomycopsis fibuligera,* whereas *Aspergillus oryzae* and *Rhizopus oryzae,* the representative nuruk fungi, were predominant only in the low fungal density nuruks (MS2, MS5, and MS11). Saccharification capability of the fungal isolates was assessed by the measurement of amylase activity in the culture broth. The amylase activity was the highest in *A. niger* and *A. luchuensis* followed by *S. fibuligera. A. oryzae* and *R. oryzae* showed fair amylase activities but significantly lower than the three fungal species. *R. oryzae* was suggested to play an additional role in the degradation of b-glucan in crop component of nuruk. Aflatoxigenicity of the isolated *Aspergilli* to confirm the safety of the nuruk was estimated using the DNA makers including *norB-cypA*, *aflR,* and *omtA*. All the isolates were turned out to be non-aflatoxigenic as evidenced by the deletion of gene markers, *norB-cypA* and *aflR,* and the absence of aflatoxin in the culture supernatants shown by TLC analysis.
Keywords : Aflatoxin, Fungal diversity, Nuruk, Saccharification, Yeast

D019

Condition of Solid-State Fermentation for Conidia Production of Fungal Pathogens to Control *Spodoptera exigua*

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To optimize conidia production of *Metarhizium anisopliae* FT83 and *Paecilomyces fumosoroseus* FG340 which have a virulence against beet armyworm, wheat bran was selected for two fungi as a solid substrate and sucrose were selected for *P. fumosoroseus* FG340 as additives in former study. In this study we investigated optimal temperature, inoculum concentration and inoculum volume for solid state fermentation. Conidia production of *M. anisopliae* FT83 was the most at 25°C as 3.8×10^9/g of wheat bran than 27, 30°C and *P. fumosoroseus* FG340 was the most at 30°C as 7.8×10^9/g of wheat bran. Optimal inoculation concentration of *M. anisopliae* FT83 and *P. fumosoroseus* FG340 were 2×10^6 conidia and $1x10^8$ conidia / 33g of wheat bran respectively. Inoculation volume added to solid substrate influenced on conidia production. when 25ml of *M. anisopliae* FT83 conidia suspension was inoculated on 33g of wheat bran, 6.9×10^9 conidia /g of wheat bran was produced. 20ml of volume was the best for *P. fumosoroseus* FG340. As a result best condition for *M. anisopliae* FT83 is that 2×10^6 conidia/ 25ml is added to 33g of wheat bran and incubated at 25°C. when *P. fumosoroseus* FG340 1×10^8 conidia / 20ml is added to 33g of wheat bran with 1% sucrose and incubated at 30°C, the most conidia was produced as 1×10^{10} conidia/ g of wheat bran.
Keywords : beetarmy worm, *Metarhizium anisopliae*, *Paecilomyces fumosoroseus*, mass production

D020

Isolation and Identification of Transglutaminase Gene from *Bacillus spp*. isolated from *Chungkukjangs*

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Transglutaminases (protein-glutamine:amine γ-glutamyl-transferase, E.C.2.3.2.13) catalyses an acyl-transfer reaction between the γ-carboxyamide groups of glutamine residues and the ε-amino group of lysines in proteins. They are widely present in most animal tissues and body fluids. They are used as a food processing material, specifically in gelling protein-rich foods after the formation of cross-links. Microbial transglutaminases (MTG) are generally produced from *Streptoverticillium mobaraense*, *Sv. cinnamoneum* and *Sv. Ladakanum*. In this study, high MTG producing *Bacillus spp*. are screened form the starins isolated from *chungkukjangs* and the MTG gene is isolated and identified. To isolate the TG-producing microorganisms, *chungkukjangs* were collected from Young-In, An-Seong, Kwang-Ju, Odaesan, and Jung-Sun in Korea. Among *33 Bacillus* strains isolated from *chungkukjangs*, *B. vallismortis* (1.61 ± 0.32), *B. amyloliquefaciens* (1.82 ± 0.07), *B. subtilis SCK-2* (4.86 ± 1.51) and *B. subtilis SC-8* (3.40 ± 1.71) showed the high activity of TG. With these *Bacillus* strains, TG gene is isolated by using PCR with the proper primers in conserved region and identified in progress.
Keywords : Transglutaminase, *Bacillus*, *Chungkukjangs*

D021

Isolation of Lactic Acid Bacteria as Biotransformer of Ginsenosides of Red Ginseng Extract

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Lactic acid bacteria have been known as a good biotransformer of ginsenoside as well as a probiotic. Ginseng saponins, called ginsenosides, are the principal components responsible for the pharmacological and biological activities of ginseng. Ginsenosides are specifically biotransformed to different functional forms with microbial fermentation. In this study, we isolated lactic acid bacteria from *kimchi* and analyzed biotransformation of ginsenosides of red ginseng extract.Red ginseng extract (~65 Brix°) was diluted to 15 Brix° with distilled water and incubated with the same volume of kimchi solution. 50 ml of each sample was incubated at 37°C for 9 days. Ginsenosides of Sample were analyzed every 3 days. Among the 6 type of kimchi, BWM, BWB and SI samples increased ginsenoside Rd. At the same time, Lactic Acid Bacteria with high activity of B-glucosidase were isolated with esculin agar plate. 16s rRNA sequencing is performed with comparison to sequence from the database using the EzTaxon program. The conversion pattern of ginsenoside is analyzed with HPLC in progress.
Keywords : biotransformation, lactic acid bacteria, HPLC

D022

Strain Development for the Production of Gamma-aminobutyric acid by Multiple Integration of Glutamate Decarboxylase Gene in *Corynebacterium glutamicum*

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Gamma-aminobutyric acid (GABA) is known as one of neurotransmitters that acts on the central nerve system. It could be used to synthesize bio-polymers such as polyamide, polyester and pyrrolidine. In this study, *Corynebacterium glutamicum* HH09 was used to develop GABA-overproducing strain. First, GABA-aminotransferase (*gaba-T*) gene was knocked out by homologous recombination using Cre/*loxP* system to construct the strain HH104. Second, one or multi-copies of glutamate decarboxylase (*gad*) gene was integrated to enhance the production of GABA in *C. glutamicum* HH104. In the flask scale culture, *C. glutamicum* HY208, containing multi-copies of *gad*, produced 8.12 g/L and *C. glutamicum* HH106, containing one copy of *gad*, produced 0.8 g/L of GABA, indicating that the production of GABA in HY208 was approximately 9.6 fold higher than HH106. To increase the copy number of *gad*, the plasmid pClik*gad* which expresses glutamate decarboxylase was introduced to HH106 and HY208 to construct the strain HH107 and HY209, respectively. The strain HY209 (25.4 g/L) produced 1.6 fold more GABA than the strain HH107 (15.6 g/L) with flask culture. The optimal condition for the enhanced production of GABA by the strain HY209 using 5L-jar fermentation is now under study.
Keywords : GABA, *Corynebacterium glutamicum*, Glutamate decarboxylase

D023

Identification of a Phenalamide Biosynthetic Gene Cluster in Myxococcus stipitatus DSM 14675

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Phenalamide is an antitumor and antiviral substance produced by *Myxococcus stipitatus*. We identified a 56-kb phenalamide biosynthetic gene cluster from *M. stipitatus* DSM 14675 by genomic sequence analysis and mutational analysis. The cluster consisted of 12 genes (MYSTI_04318 ~MYSTI_04329) that appeared to be transcribed as a single transcript. The first three genes were predicted to encode pyruvate dehydrogenase subunits. The fourth gene was predicted to encode polyketide synthetase modules carrying ketosynthase (KS), acyl transferase (AT), dehydrase (DH), enoyl reductase (ER), keto reductase (KR), and acyl carrier protein (ACP) domains. The fifth, sixth, seventh, and ninth genes were predicted to encode polyketide synthetase modules, each of which carried KS, AT, DH, KR, and ACP domains. The eighth gene was predicted to encode three polyketide synthetase modules. The tenth gene was predicted to encode a non-ribosomal peptide synthetase module, the eleventh gene a hypothetical protein, and the twelfth gene a flavin adenine dinucleotide-binding protein. Disruption of the second or third gene by plasmid insertion resulted in a defect in phenalamide production.
Keywords : Myxococcus stipitatus, myxobacteria, phenalamide

D024

Development of Novel Multiple Gene Integrative Cassette Sets Based on rDNA-NTS for construction of Vaccine-Grade Recombinant Yeasts

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The traditional yeast *Saccharomyces cerevisiae* has been widely used as a host system to produce recombinant proteins and metabolites of great commercial value. To engineer *S. cerevisiae* maintaining stably the expression cassettes, which were integrated into the host genome without antibiotic selection markers, we developed novel multiple gene integration cassettes exploiting the nontranscribed spacer (NTS) of ribosomal DNA (rDNA). Each terminal ends of rDNA-NTS (45 bp) were used as flanking sequences of the expression cassettes containing a set of *URA3* or *LEU2* selection markers with a truncated promoter in different lengths. The integration numbers of expression cassettes were shown to be proportional with the extent of decreased expression of the auxotrophic selection markers. Furthermore, simultaneous co-integration of expression cassettes with different target genes into the host chromosome could be obtained even using the same inactivated selection markers. The rDNA-NTS based expression cassettes were successfully applied to construct recombinant yeast strains overexpressing the capsid protein of red-spotted grouper necrosis virus in the form of virus-like particles, which showed a high potential to be developed as oral vaccines to prevent virus infection of fishes. The results demonstrated that our novel rDNA-NTS multiple gene integrative cassette sets combined with inactivated selection markers are useful tools for the construction of GRAS-grade recombinant yeasts.
Keywords : Oral vaccine, rDNA NTS, S. cerevisiae

D025

Transcript Levels of Two Subtilisin-Like Proteases of *Bacillus licheniformis* SCD B34 at Different Growth Phases

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Bacillus species is a key bacterium in fermenting the soybeans by excreting large amounts of proteases and glycosidases. In order to improve the quality of traditional Doenjang, we have isolated an industrially potential strain SCD B34 identified as *B. licheniformis*, by screening the strain optimized for the fermentation. The genome contained 89 genes coding for proteases in 4,789 genes. The largest type of the transcribed protease genes was a subtilisin-like protease group (AprE) with 11 different proteases, while the second was trypsin-like protease group. Transcript profiles of subtilisin-like proteases were analyzed at different growth phase by using RNA-Seq. The transcript level of a hypothetical protein with subtilisin-like protease domain was 4.9 times higher at the stationary phase than at the log phase. The transcript level of extracellular alkaline serine protease was increased up to 127-fold at the death phase compared with that at the stationary phase, which means that the enzyme can be strongly involved in the spore formation. From these results, we suggest that each extracellular subtilisin-like protease may play a special role in maintaining cell growth sequentially.

Keywords : *Bacillus licheniformis*, RNA-sequencing, subtilisin-like protease, transcript level, transcriptome

D026

Secondary Metabolites from Lichen-Forming Fungi of *Brigantiaea leucoxantha* Inhibited on the Stemness Expression in Human Stem Colon Cancer Cells CSC221

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Usnic acid was found the largest distribution compounds in lichen-forming fungi of *Brigantiaea leucoxantha*. The ethyl acetate extract (EAE) and secondary metabolites from *Brigantiaea leucoxantha* induced the human stem colon cancer CSC221 death by apoptosis. Based on MTT assay, usnic acid andsubcomponents of EAE have main role in cytotoxic effect on CSC221. In addition, usnic acid and EAE induced PARP and caspase-3 activation on CSC221 at lethal doses in a time dependent manner. In vitro assays, the lichen-forming fungal extract inhibited formation of colony and spheres of CSC221 at sub-lethal doses. Compared with its single compounds, usnic acid and unknown 3 of EAE inhibited the formation of colony and spheres at similar extend with EAE. In the other hands, other remained components showed weaker inhibition than EAE. EAE inhibited the expression of stem colon cancer markers (ALDH1, CD133, CD44, Lgr5, Msi-1, Hes1, Bmi-1, EphB-1) at transcription and translation levels. Moreover, EAE and usnic acid similarly showed inhibitory effects at mRNA and protein expression of the surface colon stem cancer marker, but other components revealed slight inhibition effects on some colon stem cancer marker (ALDH1, CD133, CD44, etc). Taken all things together, usnic acid and sub-components of lichen forming fungi of *Brigantiaea leucoxantha* extract played decisive roles in inhibition of self-renewal, proliferation and induced-apoptosis CSC221 cell line.

Keywords : anticancer, *Brigantiaea leucoxantha*, lichen-forming fungi, stem cancer marker, usnic acid

D027

Inhibitory Activity of Lichen Substance, Physciosporin Isolated from *Pseudocyphellaria coriacea* against Human Lung Cancer Cell

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Lichens produce various unique chemicals used for pharmaceutical purpose. With the aim of screening some new drug agents for cancer cell invasion and metastasis inhibition, we tested acetone extracts of 13 lichen samples collected in Chile. One of these samples, *Pseudocyphellaria coriacea* (CL09002 A1) was chosen as the candidate, which showed significant inhibitory activity of human lung cancer cell line (A549) by both wound healing assay and invasion assay. Physciosporin was identified to be the effective compound based on the TLC standard matching results. As the epithelial cell marker, E-cadherin protein level does not have significant change, but N-cadherin and β-catenin protein expression were dramatically decreased in a dos dependent manner after treatment of physciosporin. Except for E-cadherin, the quantitative real time PCR data also showed consistent results in EMT marker (N-cad, snail,twist, Foxc2 and vementin) but not so significantly. A slight decrease in MMP7 and increase level in matastasis inhibition gene KAI1 was detected. We also found that physciosporin significantly lowered the level of GTP-Rac1 and GTP-Cdc42, but not those of GTP-RhoA. These results implied that lichen secondary metabolites might be a potential source of cancer cell metastasis inhibitors, and physciosporin can be used for migration and invasion inhibitor for further research.

Keywords : anticancer, lichen substances, lung cancer cell, physciosporin, *Pseudocyphellaria coriacea*

D028

Optimization of Protein Extraction from Lichen Thalli

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Biologically active proteins of lichen-forming fungi have been poorly studied owing to a number of problems associated with their extraction. The presence of phenols, quinones, proteases and other components released during cell disruption are the largest problems related to protein extraction from lichens. To overcome these problems and maintain good electrophoretic resolution and high protein concentration, an extraction buffer containing polyvinil-polypyrrolidone (PVPP), ascorbic acid, triton X-100, polyethylene glycol (PEG), proteinase and oxidase inhibitors in sodium phosphate buffer was developed. This extraction buffer showed high efficiency for all lichen species tested in the study.

Keywords : electrophoresis, extraction, lichen, method optimization, proteins

D029

Development of Fermentation Technique for Production of Teicoplanin by Actinoplanes Teichomyceticus in Lab-Scale Fermenter

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Teicoplanin is a potent glycopeptide antibiotic used clinically for the treatment of methicillin-resistant strains. The production of teicoplanin by Actinoplanes teichomyceticus was studied in flask and laboratory-scale fermentation. The mutant strain TK-137 was obtained by consecutive selection of UV mutagenesis. The optimum fermentation conditions were determined to be 700 rpm, 0,5 VVM and 32°C in 7-l fermenter. A teicoplanin production of 3.8 g/L was obtained after 96 h of batch culture in a laboratory scale through optimization of medium composition and culture conditions. This report demonstrated a high efficiency fermentation technology for high-concentration teichoplanin production. The pilot-scale fermentation study is suggested for the industrial production of teicoplanin. This work was supported by from Next-Generation BioGreen21 Program of the Rural Development Administration, Repeblic of Korea (No. PJ009541).
Keywords : teicoplanin, streptomycetes, fermentation, mutagenesis, glycopeptide

D030

Engineered Biosynthesis of Glycosylated Derivatives: 12-Membered Macrolide Antibiotic YC-17

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Macrolides antibiotic are a class of antibiotics found in *streptomyces*, which are characterized by structures containing 12-, 14-, and 16-membered macrocyclic lactone decorated with one or more deoxysugar moieties. In a survey of microbial systems capable of generating unusual metabolite structural variability, we constructed *S. venezuelae* mutant strains, expressing sugar gene cassettes, and producing YC-17 and its glycosylated derivatives. Recently, diverse biologically active glycosylated narbomycin derivatives are produced by the heterologous expression and each mutant produced small amounts of YC-17 derivatives, but the YC-17 derivatives were not isolated in the previous study due to the small quantity. In this study, we report the engineered biosynthesis of four YC-17 analogs by the heterologous expression of various sugar biosynthetic genes in *S. venezuelae* YJ003 and their structural elucidation, and the evaluation of their antibacterial activities. The resulting recombinants produced macrolide antibiotic YC-17 analogs possessing unnatural sugars replacing native D-desosamine. Metabolites were isolated and further purified using chromatographic techniques and their structures were determined on the basis of 1D and 2D NMR and MS analyses
Keywords : Combinatorial biosynthesis, Macrolides antibiotics, heterologous expression, *Streptomyces venezuelae*, YC-17

D031

Structural characterization of Cyclosporin A, C and cylclosporin A analog AM6 by employing HPLC-ESI-ion Trap-Mass Spectrometry

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Cyclosporin A (CyA) is a secondary metabolite of the fungi *Tolypocladium inflatuman*, and has been one of immunosuppressive drugs. Cyclosporins are group of cyclic peptides containing 11 unusual amino acids. There are lots of CyA analogs reports as congeners and some analogs were produced by precursor directed biosynthesis, human CYP-mediated metabolites, or microbial bio transformed analogs. Reported here is an attempt to modify the CyA structure in order to discover new biological potentials and/or improve physicochemical properties of the existing cyclosporins. In this study, we describe an efficient HPLC-ESI-ion trap MS^n(up to MS^8) protocol for the structural characterization of CyA analogs using CyA and CyC, a (Thr^2) CyA congeners in which L-aminobutyric acid is replaced by L-threonine (Thr). We examined the fragmentation patterns of a CyA analog as obtained by supplementing CyA into recombinant of *Streptomyces venezuelae* strain used as biocatalyst. These datailed systematic fragmentation pathways confirm the structure of the analog as(γ-hydroxy-$MeLeu^6$)CyA (known as a human CYP metabolite AM6). This paper is the first report on both the MS^n Capability of the ion trap-aids identification of CyC and the structural characterization of a CyA analogs using HPLC-ESI-ion trap MS^n analysis
Keywords : Cyclosporin A, Cyclosporin analog, HPLC-ESI-ion trap MS^n, MS fragmentation patterns, (γ-hydroxy-$MeLeu^6$)CyA

D032

The Bioactive Properties of Marine Fungi Isolated from Korea Seaside

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Marine-derived fungi have commonly halotolerant activity inhabiting in oceanic environment. Halotolerance offers them an ability to survive under the hard conditions, and marine-derived fungi could be used in various industrial applications such as pharmaceutical, agrichemicals, and enzymes. Hence, antioxidant and antifungal activities were evaluated in the fungi with halotolerant ability. A total of 18 Marine-derived fungi were investigated. Samples were prepared with crude extracts. Antioxidant activities were then determined through both ABTS and DPPH radical scavenging activity assays. And antifungal test was measuremented through growth inhibition against *Fusarium oxysforum*, which was considered as a pathogenic fungal species. The halotolerance was determined by different concentration of NaCl. Among the tested fungi, the high antioxidant properties were demonstrated in three species of *Penicillium* through ABTS and DPPH assay. On the other hand, *Fusarium equiseti* FU46 was halotolerant in 3% NaCl which maximum concentration of NaCl, while it indicated low antioxidant activity. In case of *Arthrinium phaeospermum* FU03, it indicated high growth inhibition against *F.oxysforum*, while the fungus was not relatively antioxidant. Further studies considering with the antioxidant and halotolerance properties of the *Penicillium* species, it will be required to clarify the antioxidant substances through isolation and purification of bioactive compounds
Keywords : marine bioproducts, antioxidant activity, halotolerant

D033

Analysis of 6-Pentyl-Alpha-Pyrone (6PAP) Production from *Trichoderma gamsii* KUC1747 with Statistical Approaches

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The yield of 6-pentyl-alpha-pyrone (6PAP) from *Trichoderma gamsii* KUC1747 was evaluated in this study. Various carbon and nitrogen sources with different cultivation periods were examined. Detection of 6PAP extracted from the designed medium was performed by gas chromatography and mass selective detector (GC-MSD). Among the culture media compositions, xylose and casein were selected as key carbon and nitrogen sources, respectively. The maximum 6PAP production (410 μg ml^{-1}) was obtained after 15 days of incubation. The highest amount of biomass of *T. gamsii* (33 g l^{-1}) was collected after 23 days of cultivation using xylose and yeast extract as carbon and nitrogen sources. A weak relationship between the biomass and 6PAP contents was found. Using the optimal conditions with respect to these factors, 6PAP was obtained at a rate more than five times higher than that before the experiments. The interrelationships between selected nutrient sources and 6PAP production were determined using central composite design (CCD). The significant components were used variably, with five concentration levels in 11 trials. The potential for enhanced 6PAP production was determined using "contour fit plots". The optimal levels of each variable were run for confirmation. SAS software was used for the regression and graphical analyses.
Keywords : Central composite design, GC-MSD, Media composition, Optimization, 6PAP

D034

Isolation, Purification, and Characterization of Three Compounds from *Trichoderma gamsii* KUC1747

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Trichoderma gamsii KUC1747 was confirmed as an anti-sapstain compound producer in our previous study. The 6-Pentyl-α-pyrone (6PAP), derivated from *T. gamsii* was evaluated as a strong biological control agent to inhibit the growth of ophiostoma spp.. Except the 6PAP, various compounds were detected through the isolation and purification of compounds from *T. gamsii* after 7 days incubation at 25°C. According to the profiles obtained with thin-layer chromatography, fractions 1-30 were then combined respectively into five fractions (F1-F5). The fractions were separated by Sephadex LH-20, ODS-A column chromatography and prep-HPLC. From this, five sub-fractions were collected. The fraction F4 (63 mg) and fraction F5 (281 mg) were analyzed by ultraperformance liquid chromatography and quadrupole time of flight mass spectrometry (UPLC-QToF-MS) and NMR spectroscopy. At the end of the separation of crude extracts, seven compounds were collected. Among them, three of compounds, 167-2k, 168-1k and 168-3k in *T. gamsii* were reported in this study.
Keywords : Isolation and purification, UPLC-QToF-MS, NMR, Trichoderma gamsii

D035

Synthesis and Characterization of Microgravity Grown Penicillium Chrysogenum Culture Filtrate loaded Electrospun Polyurethane-Dextran Nanofiber Mats

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Electrospinning is a fabrication process that uses an electric field to control the deposition of polymer fibers onto a target substrate. Nanofiber possesses the characteristic features of high length-to-diameter ratio and specific surface areas, enabling it to be applied for protective clothing, filter, antibacterial membrane, reinforced composite, and tissue engineering, etc. In this study, we describe how electrospinning can be adapted to produce mats composed of culture filtrate of low shear simulated microgravity grown *Penicillium chrysogenum.* The antibacterial activity of the mat is attributed to the presence of secondary metabolites, especially penicillin in the culture filtrate. The presence of penicillin was confirmed by using Liquid chromatography-Mass spectroscopy. So in this study, we have adopted a technique of direct *in situ* electrospinning of culture filtrate of low shear simulated microgravity grown *Penicillium chrysogenum* with a suitable carrier polymer to aid in its electrospinning. We demonstrate the process, stability, and characterization of the biological properties of such nanofibrous scaffolds. The mat is found to be effective against gram positive bacteria. This represents useful information to improve the production of nanofibers with many other bioactive secondary metabolites not only in P. chrysogenum, but in other filamentous fungi as well.
Keywords : Electrospinning, secondary metabolites, microgravity, antibacterial, *Penicillium chrysogenum*

D036

Isolate of Pseudomonas sp. which have Antimicrobial Activity against E.coli

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To find a microorganism having antimicrobial activity on Gram-negative strains such as *Escherichia coli*, Samples were collected nearby Chonnam National University. strain selected by antibacterial activity screening, was identified as *Pseudomonas* sp. by 16s rRNA sequencing. Pseudomonas species have been known to produce various antimicrobial substances, such as rhamnolipids, pyrrolnitrins, pyoluteorins, phenazines and lipopeptides. We compared the antimicrobial materials of isolates and *P. aeruginosa*. The TLC patterns of isolates was different to *Pseudomonas aeruginosa*. It was confirmed that antibacterial substance is different from *P. aeruginosa* via HPLC.
Keywords : Pseudomonas, rhamnolipid, Escherichia coli, antimicrobial activity, Gram negative

D037

Characteristics of Lactic Acid Bacteria Isolated from Fermented Foods to *Pseudomonas aeruginosa* Antimicrobial Activity

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Pseudomonas aeruginosa is a Gram-negative, aerobic, *coccobacillus* bacterium with unipolar motility. An opportunisitic human pathogen, *P. aeruginosa* is also an opportunistic pathogen of plants. Biofilms *of P. aeruginosa* can cause chronic opportunistic infections, which are a serious problem for medical care in industrialized societies, especially for immunocompromised patients and the elderly. They often cannot be treated effectively with traditional antibiotic therapy. *Lactobacillus* is a genus of Gram-positive facultative anaerobic or microaerophilic rod-shaped bacteria. They are a major part of the lactic acid bacteria group, named as such because most of its members convert lactose and other sugars to lactic acid.The selected strains of lactic acid bacteria with antimicrobial activity against *P. aeruginosa* were isolated from fermented foods. The microorganism was cultured in MRS and extracted with an organic solvent. The paper disc diffusion method was used to determine the antimicrobial activity of the extract. Antibacterial activity was determined by using paper disc diffusion method. High antimicrobial activity was observed in the extract than ampicillin. Extracts were confirmed the single material possibility through TLC, MPLC, HPLC.
Keywords : Lactobacillus, Pseudomonas aeruginosa, antibacterial activity, Lactic acid bacteria, probiotics

D038

Medium Optimization of a Potential Herbicide from *Strepomyces scopuliridis* KR-001

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With environmental issues increasing from synthetic chemical herbicides, microbe-originated herbicides could be a fascinating alternative in current farming. In this study, we isolated a *Streptomyces* strain that could produce herbicidally active metabolites against a grass weed *Digitaria sanguinalis*. It was identified as a member of the *Strepomyces scopuliridis* cluster. In order to develop the medium composition, effects of ingredients including carbon sources, nitrogen sources, metal ions and phosphate were examined. The productivity was increased as the increasement of carbon sources and decreasement of nitrogen sources. The maximum productivity was reached approximately 1,100 ㎎/L in the optimized medium, which consist glucose 3%, potato starch 1%, soybean meal 1%, KH_2PO_4 1%, $ZnSO_4$ 0.1%, $FeCl_3$ 0.05%, $KHSO_4$ 0.05%. This research was supportd by a grant (B551179-13-02-07) from the R&D convergence program of NST (National Research Council of Science & Technology)
Keywords : herbicide, streptomyces, medium optimization

D039

Quality Properties of Soybean Products Fermented with Meju Fungi

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Traditional Korean Jang such as Doenajng and Ganjang has deep and rich taste and it is made with Meju which is fermented by naturally inoculated microorganisms. The unique taste and flavor of Jang was not standardized and industrialized. The study was aimed to develop fungal starters which make the taste and flavor of Korean traditional Jang. We isolated 1479 fungal strains from traditional Korean Meju in diverse regions in Korea and identified them as 26 genera and 101 species. We examined amylase and protease activities of 836 strains from them and selected 76 strains which showed high extracellular enzyme activities. We incubate the fungi on boiled soybeans for 14 days and we measured amylase and protease activities on soybean. Finally, we selected 13 strains which showed high enzyme activities on soybean and represented mycobiota of Meju. We made soybean products which had been fermented for 34 days with the 13 fungal strains selected and Aspergillus oryzae control strain, industrially used widely in Korea. The fermented soybean products were examined by physicochemical analyses such as pH, moisture content, reducing sugar and amino-type nitrogen. We also examined amino acids, organic acids, free sugars, and extra compounds of the fermented soybean products by 1H-NMR analysis. Some fungal strains showed high possibility for good starter, because they produced higher volume of useful amino acids and compounds than Aspergillus oryzae control strain.
Keywords : Meju fungi, Starter, Enzyme activity, quality properties

D040

An Indole Alkaloid from the Fruiting Body of Boletus Umbriniporus

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Mushrooms are valued as a nutritional food and also as an important source of useful medicinal components. They produce various secondary metabolites which have interesting biological activities and unique chemical structures. As part of our ongoing investigation on chemical constituents and bioactive components of Korean wild mushrooms, the fruiting body of Boletus umbriniporus was collected and investigated. B. umbriniporus is characterized by its yellow flesh, which changes to pallid blue when exposed to air, and its chemical constituents has not been reported. The methanolic extract of B.umbriniporus was concentrated to eliminate methanol and partitioned consecutively with hexane, chloroform, ethyl acetate, and butanol. The ethyl acetate-soluble portion was concentrated and separated by C18 Sep-pak cartridge chromatography, Sephadex LH-20 column chromatography, and preparative reversed-phase HPLC to provide an active constituent. Its chemical structure was determined to be flazine, an indole alkaloid, by the ESI-mass measurement and 1H NMR, 13C NMR, 1H-1H COSY, HMQC, and HMBC analysis. This compound was isolated from this mushroom for the first time.
Keywords : mushroom

D041

Antioxidant and Tyrosinase Activity of Fermented The Root of Chinese Liquorice (Glycyrrhiza uralensis)

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Korean traditional medicinal herbs have been reported to possess antioxidant and anti-hyperglycemic activities. The fermented medicinal herbs have some features that improve in the effect and higher absorptivity of useful ingredient. The root of chinese liquorice(*Glycyrrhiza uralensis*) was known that it is a therapeutic effect of Anemia, cancer, pain, wound healing, diabetes and hypertension. We tested the antioxidant and tyrosinase activity of medicinal herbs: Root of chinese Liquorice. The experiment of the extracts were examined by by α,α-diphenyl-β-picrylhydrazyl (DPPH) assays, Reducing power assays and tyrosinase activity. DPPH radical scavenging activities when fermented through, scored higher(more than 3~30%) than when not fermented. And tyrosinase activities when fermented through, scored higher (more than 16~47%) than when not fermented. Reducing power assays when fermented through, scored less than when not fermented. Based on these results, it was suggested that fermented medical herbs can be a useful and cost-effective for medicine.
Keywords : Chinese liquorice, antioxidant activity, tyrosinase activity, fermentation

D042

Antioxidant Activities of Extracts from Fermented burdock

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The ingredients of burdock are arctigenin, actiin, cynarin, phenolic compound, free radical, and lignan. It is be useful for anti-inflammatory activity, high blood pressure, arteriosclerosis and antimutagenic antioxidant activity, intended for diabetes and kidney disease. The antioxidant activity of fermented burdock (Lactobacillus helveticus, Lactobacillus plantarum, Lactobacillus brevis) extracts were analyzed using 1,1-diphenyl 2-picryl hydrazyl (DPPH), Reducing power, Total polyphenol compound, Total Flavonoid compound. Our results indicate that lactic acid bacteria fermentation-burdock have useful biochemical attributes, including antioxidant, total polyphenol, total flavonoid activities. Considering the high consumer demand due to the beneficial healte effects, fermented burdock and is fractions can be utilized to develop functional food, as well as health-promoting and pharmaceutical agents.
Keywords : Burdock, Fermentation, Lactic acid bacteria, Antioxident activity

D043

Antioxidant Activity and Reducing Power of Fermented Garlic

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The garlic has various physiological function, antifungal activity, anticancer activity, fall in blood pressure, lowering cholesterol, antioxidant activity. So it used regulate biological functions. In this study find out the functional foods potential for fermented garlic. It is fermented at a three Lactobacillus (L. helvericus, L. plantarum, L. brevis) and Bacillus sp. KSW. We compared the fermented garlic with garlic of antioxidant activity and reducing power. As a result of experiment the highest acticity of DPPH scavenging showed 40.07% (fermented by Bacillus sp. KSW) And reducing rower showed 1.45. So fermented garlic is practicable of functional food. Our results indicate that lactic acid bacteria fermentation galic have useful biochemical attributes, including antioxidant and total polyphenol, total flavonoid activities.
Keywords : Garlic, Antioxidant activity, Fermente, Lactic acid bacteria

D044

Antimelanogenic and Antioxidant Activities of Yeast Strains Isolated from Fruit

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Mushroom tyrosinase (EC 1.14.18.1) is a copper containing oxidase that catalyzes both the hydroxylation of tyrosine into *o*-diphenols and the oxidation of *o*-diphenols into *o*-quinones, and then forms brown or black pigments. In the present study, several yeasts were isolated from fruit such as banana, apple, and mulberry. Four isolates among all isolates were identified by 18S rRNA sequencing as belonging to *Bullera sp*, *Sporobolomyces sp*, and *Pichia sp*. Antioxidative activities of culture filtrates were examined by xanthine oxidant(XO) inhibition activity, reducing power, superoxide anion radical, and mushroom tyrosinase activity were also determined by following the ordinary methods. The mushroom tyrosinase inhibitor was also determined to be produced maximally when *Rhodotorula sp.* BCNU3008 have been cultured at 26¡É for 48 h in MG broth medium. The culture filtrate of *Rhodotorula sp.* BCNU3008 exhibited the highest mushroom tyrosinase inhibitory activities of 57.43%. The culture filtrate of *Rhodotorul sp.* BCNU3008 exhibited the highest superoxide anion radical of 90.62% . The culture filtrate of *Candida sp.* BCNU3005 exhibited the highest XO of 36.51%. Therefore, *Candida sp* BCNU3005*, Rhodotorula sp* BCNU3006*, Puccinia sp* BCNU3007*, Rhodotorula sp* BCNU3008*, Cryptococcus sp* BCNU3009. Isolates may be potential resources for the development of new cosmetics and for biomedical applications.
Keywords : Mushroom tyrosinase, Antioxidant activites, Fruit

E001

HBT-FEPD: Homology-Based Transfer based on Functionally Equivalent Proteins- and Domains-Detecting Algorithm

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In a functional annotation system, homology-based transfer (HBT) is a gold standard for transferring the functional description of homologs to the putative proteins. To search homologs for the putative proteins, sequence-based methods such as BLAST and HMMER have also been commonly used because of fast homolog searches against big databases. Although many putative proteins have been annotated by HBT using sequence-based methods with high confidence, meticulous attention is still required owing to the emergence of misannotated or unannotated proteins caused by missing functionally equivalent homologs. Thus, the performance of HBT-based annotation system using sequence-based methods is considerably dependent upon the ability of detecting functionally equivalent homologs. We developed an HBT program based on functionally equivalent proteins (FEPs) or domains (FEDs) using the FEP-BH algorithm, called HBT-FEPD. As FEP-BH algorithm is used to detect FEPs from the outputs of blastp and hmmsearch, HBT-FEPD can more precisely annotate the putative proteins.
Keywords : Genome annotation system, Bioinformatics, Functional annotation system, Homology-based transfer, Protein function

E002

Characteristics of a New Mid-High Temperature Adaptable Oyster Mushroom Variety『Heuktari』 For Bottle Culture

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'Heuktari' is a new variety of oyster mushroom for the bottle culture, It was bred by mating with monokaryons isolated from 'P11056' and 'MT07156' The optimum temperature for the mycelial growth was 23~26°C on PDA medium and that for the primordia formation and the growth of fruiting body of 'Heuktari' was 18~19°C on saw-dust media. It took 30 days to finish spawn running, 4 days to finish primordia formation, 5 days to finish fruitbody growth in the bottle culture. In the characteristics of fruit body, pilei were round type and dark grayish brown, stipe color was white color and stipe shape was short and thick. The yield per bottle was 180g/900ml and was 15% higher than that of control strain (Suhan-1ho). The physical properties of springness, cohesive, gumminess and brittleness of stipe tissue were 96%, 82%, 511g and 47kg, respectively.
Keywords : *Pleurotus ostreatus*(Heuktari), Bottle culture, New variety, mid-high temperature

E003

A *Salmonella* Leader Mrna Downregulates the Ferric Uptake Regulator Protein

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Virulence genes have to communicate each other to regulate a pathogen's capacity to cause disease. Here we report that the Salmonella Typhimurium virulence gene mgtC control expression of the fur gene, encoding the ferric uptake regulator. Specially, mgtC leader RNA corresponding to 1 to 113 is responsible for binding to the coding region of the fur gene. When we express leader RNA 1-113 from a heterologous promoter, it downregulates Fur protein levels. To identify regions required for this interaction. We searched for conserved regions from the RNA leader sequences of other enteric species and from a software prediction. Our finding shows that the leader RNA of the virulence mgtC gene has a potential to work as a trans-acting RNA to regulate other genes.
Keywords : *Salmonella* Typhimurium, Fur

E004

Rad52 is Required for Accurate Chromosome Segregation in *Saccharomyces cerevisiae*

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Chromosome bi-orientation is essential to maintain chromosome number and cell viability. For accurate chromosome segregation, eukaryotic cells monitor the state of spindle-kinetochore attachment by Aurora B kinase, which is a crucial factor of the spindle assembly checkpoint (SAC). In *Saccharomyces cerevisiae*, SAC is activated by the phosphorylation signal generated by yeast Aurora B kinase, Ipl1. Subsequently, activated SAC inhibits the metaphase to anaphase transition to correct mis-linked spindle-kinetochore connections. Recent studies have reported that various kinds of tumors in vertebrates have problems in SAC. Rad52 is a key subunit in homologous recombination machinery. During DNA replication in S phase, DNA double strand breakage caused by internal or external reasons is recovered by Rad52-dependent repair pathway. Although the function of Rad52 in DNA damage repair pathway is well studied, other functions are not uncovered yet. In this study, we found evidences that Rad52 plays a role in the regulation of SAC. Rad52 has genetic interaction with subunits of SAC and acts as a key factor to recover from spindle damage caused by nocodazole, a microtubule depolymerizing drug. We also found phospho-regulation pathway of Rad52 by Ipl1 and Mps1 kinase. Based on our results, we suggest that Rad52 functions as a major regulator of SAC and chromosome segregation in *S. cerevisiae*.
Keywords : Rad52, Spindle assembly checkpoint, chromosome segregation, Aurora kinase B, Mps1

E005

Functional Analysis of the VeA-Dependent Genes Identified by Differential Proteome in *Aspergillus nidulans*

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In *Aspergillus nidulans*, asexual development is inhibited by the conditions favorable for sexual differentiation, in which VeA is acting as a key regulator. Our previous proteomic analysis using VeA-deletion mutant revealed more than 200 proteins, of which expression level was affected by VeA. For functional analysis, 22 proteins were selected and designated Vdps (VeA-dependent proteins). Genes for 6 Vdps were successfully disrupted by homologous recombination and subsequent phenotypic analyses revealed that involvement of 3 Vdps in developmental process including growth. The deletion-effect of the *vdpA*, which encodes the homolog of the yeast survival factor, was the most dramatic and pleiotypic: reduced radial growth, production of dark-brown pigment, hyphae with hyper-branching and frequent anastomosis, abnormal conidiophores with reduced conidia, and block of cleistothecial development at primordial stage. When *vdpJ,* which encodes a protein of unknown function, was deleted, it did not reveal the involvement in development, but revealed arginine auxotrphy, which seems to be caused by deficiency in succinoarginate biosynthesis. The deletion of *vdpN*, which encodes the eukaryotic translation initiation factor 3 subunit, revealed defect in maturation of sexual organ by producing small cleistothecia. Further results on the function of above-mentioned 3 Vdps will be discussed.
Keywords : Aspergillus nidulans, VeA, proteome, development

E006

LAMMER Kinase Modulates the Co-operative Action of Cyclin-Dependent Kinase and MBF-Complex in Fission Yeast

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Previously we reported the involvement of fission yeast LAMMER kinase Lkh1 in control of the cell size and cell-cycle progression. Lkh1 phosphorylates Rum1 to activate this molecule as a cyclin-dependent kinase (Cdc2) inhibitor, which blocks cell cycle progression from the G1 to the S phase. In vitro mutagenesis and kinase assay with mutant proteins suggested multiple phosphorylation of Rum1 by Lkh1. Microarray analysis revealed that only four genes were cell-cycle related among the genes up-regulated by the *lkh1*-deletion. Interestingly, expression of those four genes is modulated by a transcription factor MBF (*Mlu*1 cell cycle box binding factor), that regulates cell-cycle genes in G1/S phase. In good agreement with the transcript analysis, pull-down assay confirmed the interaction between Lkh1 and an inhibitor for MBF-complex (MCI). *In vitro* kinase assay and peptide mass fingerprinting (PMF) also revealed that Lkh1 can phosphorylate the MCI. Consequently, our results indicated that LAMMER kinase affected the G1/S cell cycle progression at post-translational level by modulating Rum1 activity and at transcriptional level by modulating MBF activity in fission yeast. Further analysis to determine the cross-talk between Lkh1-dependent phosphorylation of Rum1 and of MCI is under investigation and the results will be discussed.
Keywords : Fission yeast, LAMMER kinase, Rum1, MBF

E007

An Easy, Rapid, and Cost-effective Method for DNA Extraction from Various Lichen Taxa and Specimens Suitable for Analysis of Fungal and Algal Strains

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Lichen studies, including biodiversity, phylogenetic relationships, and conservation concerns require definitive species identification, however many lichens can be challenging to identify at the species level. Molecular techniques have shown efficacy in discriminating among lichen taxa, however, obtaining genomic DNA from herbarium and fresh lichen thalli by conventional methods has been difficult, because lichens contain high proteins, polysaccharides, and other complex compounds in their cell walls. Here we report a rapid, easy, and inexpensive protocol for extracting PCR-quality DNA from various lichen species. This method involves the following two steps: first, cell breakage using a beadbeater; and second, extraction, isolation, and precipitation of genomic DNA. The procedure requires approximately 10 mg of lichen thalli, and can be completed within 20 minutes. The obtained DNAs were of sufficient quality and quantity to amplify the internal transcribed spacer (ITS) region from the fungal and algal lichen components, as well as to sequence the amplified products. In addition, 26 different lichen taxa were tested, resulting in successful PCR products. The results of this study validated the experimental protocols, and clearly demonstrated the efficacy and value of our KCl extraction method applied in the fungal and algal samples.
Keywords : genomic DNA, lichens, fungi, algae, rRNA

E008

The APSES Transcription Factor StuA Plays a Role in β-1,3-Glucan Biosynthesis during Development of *Aspergillus nidulans*

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The temporal and spatial regulation of β-1,3-glucan synthesis plays an important role in morphogenesis during fungal growth and development. Northern blot analysis showed that the transcription of *fksA*, the gene encoding β-1,3-glucan synthase in *Aspergillus nidulans*, was cell-cycle-dependent and increased steadily over the duration of the vegetative period, but its overall expression during the asexual and sexual stages was fairly constant up until the time of transcription cessation. In an *A. nidulans* strain mutated in the eukaryotic bHLH-like APSES transcription factor *stuA1*, the transcriptional level of *fksA*, and consequently the content of alkali-insoluble cell wall β-glucan, significantly increased at the conidial chain formation and maturation stage. Electrophoretic mobility shift assays revealed that StuA was bound to StREs (StuA Response Elements) on the *fksA* promoter region. Promoter analysis with sGFP-fusion constructs also indicated the negative regulation of *fksA* expression by StuA, especially during asexual development. Taken together, these data suggest that StuA plays an important role in cell wall biogenesis during the development of filamentous fungus *A. nidulans*, by controlling the transcription level of *fksA*.
Keywords : *Aspergillus nidulans*, β-1,3-Glucan, EMSA, GFP-fusion, StuA

E009

Genome Mining Reveals Distribution of 2-Pyridone Tenellin Biosynthetic Gene Cluster in Hypocrealean Fungi (Ascomycota)

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Our *in silico* predictions including phylogenetic analysis of A-domain sequences, domain structure of tenellin synthetase (PKS-NRPS), substrate prediction based on the extracted signature residues in A-domain core motifs, and syntenic comparisons of tenellin biosynthetic gene clusters revealed that the ability of synthesizing tenellin is inherent in the genomes of species in Cordycipitaceae including *Beauveria bassiana, B. pseudobassiana, Cordyceps militaris, C. pruinosa* and *Isaria farinosa.* In *Tolypocladium inflatum*, ACP was insignificantly observed while *Metarhizium anisopliae* lacked *C*-terminus R domain. Both *Metarhizium acridum* and *Fusarium oxysporum* lacked some core (KS and AT) and modifying (DH and cMT) domains in PKS region of *TenS* whereas *Fusarium verticillioides* possesses only a monomodular NRPS region. The predicted substrate specificity shows that *TenS* genes in the putative tenellin synthetase clade can synthesize tenellin except *T. inflatum*. We predicted twelve more genes in the flanking regions of the tenellin biosynthetic gene cluster (*tenA, tenB, tenC* and *tenS*) in *B. bassiana* covering a length of approximately 50 kb. Among the studied fungi, only *B. pseudobassiana* possesses a complete but scattered set of orthologs of tenellin biosynthetic gene cluster when compared with *B. bassiana*. Till date, only *B. bassiana* has been reported to produce tenellin. Further transcriptomic and metabolomic analyses will show the synthesis of tenellin by other hypocrealean fungi.
Keywords : gene cluster, hypocrealean fungi, PKS-NRPS, ortholog, tenellin

E010

Production of Cristazarin in the Lichen-Forming Fungus *Cladonia metacorallifera* Using Fructose as Carbon Source

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A rare lichen *Cladonia metacorallifera* var. *reagens* KoLRI002260 has been known to produce rhodocladonic, thamnolic, and didymic acid. However, these metabolites were not detected in the isolated mycobiont. In this study, we have investigated the effect of six different carbon sources on the biosynthesis of cristazarin in the *C. metacorallifera* mycobiont. Only fructose was observed to have an inducing effect on the production of these compounds. Transcriptional expression patterns were profiled by quantitative reverse transcription (qRT) PCR for thirty nine polyketid synthase (PKS) genes and two white collar (WC) genes as light sensor. The results suggest that different carbon sources may induce the polyketide biosynthetic pathway in culture of the *C. metacorallifera* mycobiont and various PKS genes are involve in production of cristazarin which promoted by light.
Keywords : lichen-forming fungus, fructose, cristazarin, polyketide synthase gene, light

E011

Phenotypic Characterization of Agrobacterium tumefaciens-Mediated Transformation of Lichen-Forming Fungus, *Umbricaria muehelenbergii*

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Umbricaria muehelenbergii is a dimorphic lichen-forming fungus that can be grown rapidly in a culture in the yeast-like form, while most other lichen-forming fungi grow extremely slowly in axenic culture. We generated transformants to understand gene function via the use of *Agrobacterium tumefaciens*. A number of phenotype-defective mutants were found in their color, growth and morphologies compared to wild-type. Among them, hyphal growth transformants are crucial to understand the mechanism of morphogenesis in this fungus. The scanning electron microscopy (SEM) represents hyphal form of transformant on PDA medium. A genomic southern blot analysis of phenotype-defective mutants showed a single T-DNA insert on their genome. We employed thermal asymmetric interlaced polymerase chain reaction (TAIL-PCR) to isolate genomic DNA segments adjacent to inserted T-DNA in the mutants. Sequence of the resulting amplicons showed an insertion of T-DNA in a gene with putative transcription factor. The metabolites of transformants were compared using High-pressure liquid chromatography (HPLC) to detect quorum sensing molecules. With efficient transformation methods, direct mutagenesis of candidate genes in lichen fungi via random insertional mutagenesis would be a more direct approach to discover and study such genes.
Keywords : ATMT, dimorphic fungi, insertional mutagenesis, lichen-forming fungus, *Umbricaria muehelenbergii*

E012

Molecular Sequence Analysis of Hydrolytic Gene Clusters from the Gut Microflora of *Hermetia illucens* using Metagenomic Library

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Among common environmental colonizers, the larvae of the black soldier fly, *Hermetia illucens*, are voracious feeders of various organic materials, and may thus be exploited as a simple system for processing daily food wastes. We constructed metagenomic fosmid libraries using the larval intestinal microbiome from the fly. The hydrolytic enzymes encoded by uncultured microorganisms were subjected to substrate hydrolysis analysis using carboxymethyl cellulose, starch, and tributyrin as a sole carbon source. Repetitive screenings identified 32 fosmid clones from metagenomic libraries with an average insert size of 34kb. Next-generation-sequencing was applied to retrieve total number of 1,082,186bp sequences from the fosmids. From them, 1,064 individual ORFs were identified, and subjected to Glimmer, Pfam, COG, BLOCK, UniPro, and InterProMotif analysis for putative functional annotations. Out of 18 cellulase-active fosmids, 9 microbial genome fragments were shown as novel. In addition, we isolated 7 novel amylase-active, and 3 novel esterase/lipase-active clusters, respectively. We found no significant pair-wise sequence similarities, suggesting non-overlapping gene clusters. Here, we present the structure of hydrolytic gene clusters obtained from yet unknown intestinal microorganisms.
Keywords : gene sequence, hydrolytic enzymes, metagenome, Hermetia illucens, gene cluster

E013

Interference of Bacterial Energy Generation and Quorum-sensing Regulator Folding by Indole

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Indole has received great attention because of its extensive effects on various biological functions. We demonstrated that many genes involved in energy generation and chaperones are highly expressed under indole treatment in both *Pseudomonas putida* and *Acinetobacter oleivorans*. Subsequent biochemical analyses have shown that indole increases the $NADH/NAD^+$ ratio and decreases the adenosine triphosphate (ATP) concentration inside cells, due to membrane perturbation and higher expression of TCA cycle genes. Interestingly, our in vitro protein-refolding assay demonstrated that indole interferes with protein folding. Indole enhanced *P. putida* biofilm formation and inhibited swimming motility, which were not observed when AHL was already bound to the quorum sensing regulator, thereby suggesting that the QS regulator PpoR–AHL complex masks the effects of indole. QS-controlled phenotypes in *A. oleivorans* appeared to be inhibited by indole and the *aqsR* mutant had the same phenotypes. Indole decreased the expression of two AqsR-targeted genes. The overexpression of AqsR in *Escherichia coli* was impossible with the indole treatment. Surprisingly, our ^{35}S-methionine pulse-labelling data showed that the stability and folding of AqsR protein decreased under indole without changing *aqsR* mRNA expression in *E. coli*. Here, we provided evidence showing that the indole effect on QS-controlled bacterial phenotypes is due to reduction of energy generation and inhibited QS regulator folding.
Keywords : Indole, Quorum sensing regulator, Acinetobacter, Pseudomonas, Chaperone

E014

***Pantoea agglomerans* R190, a Producer of Antibiotics against Phytopathogens and Foodborne Pathogens**

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Pantoea agglomerans (formerly *Enterobacter agglomerans*, *Erwinia herbicola*, and *Erwinia milletiae*) is a Gram-negative bacterium belonging to the Enterobacteriaceae. The saprophyte *P. agglomerans* has long been known to be an opportunistic pathogen of plants and humans, however, *P. agglomerans* has also been focused on as a beneficial bacterium due to its metabolic capabilities. *Pantoea agglomerans* R190, isolated from an apple orchard, showed antibacterial activity against various spoilage bacteria, including *Pectobacterium carotovorum* subsp. *carotovorum*, and foodborne pathogens such as *Escherichia coli* O157:H7. The draft genome sequence of *P. agglomerans* R190 comprised 5 contigs. The genome size was 5,002,566 bp at 309x coverage, with an N_{50} of 3,393,498 bp. The G+C content was 55.05%. There were 4,778 predicted open reading frames, 23 rRNA genes, and 77 tRNA genes. The total number of ORFs with predicted functions was 3,636. Among the five contigs, three (contigs 3, 4, and 5) were predicted plasmids. Contig 4 contained a gene cluster for a phenazine antibiotic. Genes related to other antibiotics known to be produced by *P. agglomerans*, such as pantocin or herbicolin, were not predicted, thus it is possible that all antibiotic activity of *P. agglomerans* strain R190 comes from phenazine antibiotics. This report will raise the value of *P. agglomerans* as an agent for biocontrol of disease.
Keywords : *Pantoea agglomerans*, biocontrol agent

E015

Genome-wide Analysis of Genes Induced by Metal Chelator TPEN and Zinc-Responsive Regulator Zur in *Streptomyces coelicolor'*

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Metals affect bacterial physiology in diverse ways, as catalytic cofactors, structural or regulatory components of proteins, etc. When in excess, they disrupt cell physiology as toxic agents, and hence its homeostasis needs be strictly achieved in all life forms. In *Streptomyces coelicolor*, several metalloregulators were identified, among which a zinc-responsive regulator of the Fur family has been reported to be a primary regulator for zinc homeostasis, which is important for differentiation and antibiotic production. In order to find genome-wide target genes of Zur, and more broadly an array of genes that respond to metal availability, we performed ChIP-chip and RNA deep sequencing analyses. Following treatment with TPEN that can chelate various divalent metals. The up-regulated genes contain all known Zur-target genes, and enriched in various transporter genes. Among them, the genes that exhibit strong Zur-binding (top 1% peak intensity; 172 peak sites) within 500 bp of their start codons were selected and further analyzed. About half of Zur binding sites were found within the internal region of ORF. A large number of genes that are induced by TPEN and have prominent Zur binding peaks were not affected by zur deletion mutation, suggesting involvement of additional metallo-regulators. Characterization of their regulation profile is under investigation.
Keywords : Zur, TPEN

E016

Transcriptome Analysis of *Lentinula edodes* depending on Cultures, Sawdust and Timber Log, by Next Generation Sequencing

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Lentinula edodes is one of most important edible mushroom in the Korea. Recently, breeding method has been researched to improve the quality of mushroom. For the purpose of molecular breeding, we investigated genetic polymorphism of L. edodes depending on cultures of sawdust and timber log by transcriptome analysis. To obtain an overview of the *L. edodes*, differently expressed genes of transcriptome during different development stages of timber log (Soohyangko) and sawdust (Sanlim 10), cDNA samples were sequenced using Illumina HiSeq TM 2000 sequencing platform. After filtering for adaptor sequences, de novo reconstruction of the high quality reads were assembled by 'Trinity'¯ software. Assembled 20,503 unigenes were annotated with Cazy (Carbohydrate-Active enzymes) pathway and Gene ontology. Hydrolases and oxidases in the degradation of carbohydrates and lignin were expressed on the mycelium of *L. edode*s. The level of genes related to lignin degradation was significantly increased in the hyphe than that of fruit body of Soohyangko. The results will be applied to the marker development for molecular breeding and genome acquisition of *L. edodes* mushroom.
Keywords : *Lentinula edodes*, Transcriptome analysis, Sawdust culture, Timber log culture

E017

Genome Features of *Streptomyces rubrolavendulae* Strain MJM4426 and *Streptomyces* sp. MJM8645 That Have Rice Bacterial Blight Suppressing Activities

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Streptomyces rubrolavendulae strain MJM4426 and *Streptomyces* sp. MJM8645 have been reported to suppress rice bacterial blight caused by *Xanthomonas oryzae* pathovar *oryzae*. The two genome sequences were determined by HiSeq2000 sequencer. The coverages were 194X and 93X and the sizes were estimated as 6.6 and 9.7 Mbp, respectively. The assemblies led to six and eighteen scaffolds. Predicted proteins were 5,673 and 8,091. Gene clusters responsible for the biosynthesis of antimicrobial compounds were identified.
Keywords : *Streptomyces*, genome

E018

Sulfur Assimilation Related Genes of *Puccinia horiana*, the Causal Agent of Chrysanthemum White Rust and the Comparison to those of *Puccinia graminis* f. sp. *tritici* the Wheat Leaf Rust Pathogen

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There are four *Puccina* genomes available on NCBI data base up to now. The genus *Puccinia* consists of obligate biotrophic and plant pathogenic basidiomycetes fungi. *Puccinia horiana* is the causal agent of Chrysanthemum white rust and *P. graminis* f. sp. *tritici* the wheat leaf rust pathogen. It is reported that the assimilation and the transportation of nutrients contribute to the obligate biotrophy nature. Comparison of the genes related to sulfur assimilation was carried out with draft geneme sequence of Korean isolate of *P. horiana*.
Keywords : *Puccinia*, sulfur assimilation, gene

E019

Global Regulator, Clp (Crp-like Protein) Gene Expression in *Xanthomonas oryzae* pathovar *oryzae* was not Changed by Rice Leaf Extract

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Xanthomonas oryzae pathovar *oryzae* (Xoo) is the causal agent of bacterial blight of rice, which is one of the most serious diseases affecting cultivated rice. It was reported that the addition of rice leaf extract (RLX) stimulates the secretion of some effector proteins from Xoo into the medium. Here, we report the clp gene's expression by RLX in Xoo. Most of the Clp-dependent genes are associated with virulence functions, and the deletion of clp abolishes virulence in Xanthomonas campestris pv. campestris. To study the clp, pathogenicity-related genes, expression the RLX was added into Xoo culture medium, and then total RNA was extracted by RLX treated Xoo cells. The extracted Xoo total RNA was used to quantitative Real-Time PCR. In this study, the clp gene expression was presented high level, but not different with control (untreated Xoo).
Keywords : *Xanthomonas oryzae* pv. *oryzae*, clp gene, expression

E020

Electrophoretic Karyotyping of *Hypsizygus marmoreus* and Evaluation of Variation Among Its Basidiospores

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Molecular karyotype of *Hypsizygus marmoreus* has been explored by contour-clamped homogeneous electric field (CHEF) gel electrophoresis. Eleven chromosomal bands were separated from the dikaryotic mycelia of *H. marmoreus* (strain Hm 3-10), and the sizes of the chromosomes were in the range of 1.9 to 5.8 Mb. The total genome size of the strain was estimated to be 36.3 Mb. The chromosome numbers were also confirmed by telomere fingerprinting, and 22 telomeric bands were identified. This result suggested that 11 chromosomes exist in Hm 3-10. The marker sequences for each chromosome were determined and were applied to identify each chromosome. Karyotyping and southern blot analysis revealed that the size of chromosomes in the basidiospores were largely different from those of parental dikaryon Hm 3-10 cells.
Keywords : Chromosome length polymorphism, CHEF gel electrophoresis, *Hypsizygus marmoreus*, Karyotype, Telomere fingerprinting

E021

Complete Genome Sequence Analysis of an H5N1 Avian Influenza Virus from a Domestic Quail Flock in Vietnam in 2014

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Genetic characterization of avian influenza viruses circulating in quail is important because quail is considered one of the intermediate hosts supporting the generation of reassortant influenza A viruses, which may result in human infection. However, there are few reports on the genetic characterization of H5N1 virus strains isolated from quail. Here, we reports the whole-genome analysis of an H5N1 virus isolated from an outbreak in a domestic quail flock in Central Vietnam during 2014. Genetic analysis revealed that the HA protein sequence possessed the sequence RERRRKR/G at the HA cleavage site. The receptor binding pocket of HA1 had amino acid residues 222G and 224Q (H5 numbering), which are known to preferentially bind to avian type (α-2,3 linked sialic acid) receptors. Alignment of the NA protein amino acid sequence indicated that it had the 20 amino acid deletion stalk. However, there were no mutations associated with drug resistance in the NA and M2 protein amino acid sequences. Phylogenetic analysis of the HA gene indicated that the virus derived from the A/goose/Guangdong/1/96-like lineage and was most closely related to other sequences of clade 2.3.2.1c, which was recently predominant in Northern Vietnam. This study provides updated genetic information on H5N1 viruses circulating in quail in Vietnam.
Keywords : H5N1, quail, Vietnam

E022

Analysis of Mating System in *Lentinula edodes* and Development of Mating Type-Specific Markers

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Mating of tetrapolar mushrooms is regulated by to chromosomal loci, A and B. A locus contains A gene that expresses a homeodomain protein whereas B locus contains multiple pheromones and receptor genes. In order to characterize the mating loci in Korean cultivated strains of *Lentinula edodes*, one hundred monokaryotic myclelia were isolated from the basidiospores of cultivated strains, including Cham-A-Ram, Sanjo701, and Sanjo707. Both mating loci were amplified using primer sets targeting conserved sequence regions for homeodomain (HD), pheromone, and receptor genes. Subsequent sequence analysis revealed that the Korean strains contained significant variations in the homeodomain of A locus, even within the same A1 or A2 mating type. Similarly, B locus was also highly diversified in the sequences of pheromones and receptors as well as gene organization. These results enabled us to design mating type-specific probes which can distinguish mating type of each strain. The specificity was confirmed by between intra- and inter-strain mating experiment.
Keywords : *Lentinula edodes*, Mating gene, Tetrapolar

E023

Development of Strain-specific SCAR Marker for *Pleurotus eryngii* Strains Adaptable to High-temperature by Bulked Segregant Analysis

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In this study, SCAR marker that differentiates *Pleurotus eryngii* strains adaptable to high-temperature from control strain was developed. Genomic DNAs of 7 control strains of *Pleurotus eryngii* and 7 *Pleurotus eryngii* strains adaptable to high-temperature were analyzed by bulked segregant analysis (BSA) using randomly amplified polymorphic DNA (RAPD). One-hundred twenty RAPD primers were screened on bulked DNA samples and a unique DNA fragment with the size of 385 bp was yielded by OP-A06 primer from the *Pleurotus eryngii* strains adaptable to high-temperature. A sequence characterized amplified region (SCAR) marker, designated as OP-A06-1-F and OP-A06-1-R, was designed on the basis of the determined sequence. The PCR analysis with the OP-A06-1 primer showed that this SCAR marker can clearly distinguish the *Pleurotus eryngii* strains adaptable to high-temperature from the control strains.
Keywords : Bulked segregant analysis, *Pleurotus eryngii*, High-temp. adaptable strains, SCAR marker, OP-A06-1 primer

E024

Development of Strain-specific SCAR Marker for High β-Glucan Producing Strains of *Pleurotus eryngii*

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In this study, SCAR marker that differentiates high β-glucan producing strains of *Pleurotus eryngii* from control strain was developed. Genomic DNAs of 9 control strains and 9 high β-glucan producing strains of *Pleurotus eryngii* were analyzed by bulked segregant analysis (BSA) using randomly amplified polymorphic DNA (RAPD). One-hundred twenty RAPD primers were screened on bulked DNA samples and a unique DNA fragment with the size of 300 bp was yielded by OP-R03 primer from the high β-glucan producing strains of *Pleurotus eryngii*. A sequence characterized amplified region (SCAR) marker, designated as OP-R03-1-F and OP-R03-1-R, was designed on the basis of the determined sequence. The PCR analysis with the OP-R03-1 primer showed that this SCAR marker can clearly distinguish the high β-glucan producing strains of *Pleurotus eryngii* from the control strains.
Keywords : *Pleurotus eryngii*, High β-glucan strains, SCAR marker, OP-R03-1 primer

E025

Recovery of Parental Monokaryons from 'Haemi'variety in Hypsizigus Marmoreus by Protoplast Regeneration

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Hypsizigus marmoreus is commercially the most important edible mushroom in Japan. Recently, some farmers successfully cultivate this mushroom called Backmansongi, Backilsongi in Korea. This study was carried out to obtain parental monokaryotic strains composed of 'Haemi' variety in Hypsizigus marmoreus. The mycelia was cultured depending on various conditions and protoplasts were released from mycelia cultured in MCM media. Homogenized mycelia was treated with commercial cell wall degrading enzymes to maximize protoplast yield from Hypsizigus marmoreus. The greatest number of protoplasts was produced at mycelia cultured in MCM for 3 days using novozyme enzyme. The isolated protoplasts were grown in regeneration agar media for two weeks. Regenerated colonies were picked and moved on separated dishes and observed on the microscope. Neohaplonts regenerated monokayotic strains were identified by the absence of clamp connections. We confirmed them each parental strains by reciprocal crossing. One of them will be used for genome sequence analysis.
Keywords : Hypsizigus marmoreus, protoplast regeneration, genome sequence analysis, parental monokaryons, 'Haemi'variety

E026

Molecular Analysis of Varicella-Zoster Viruses Isolated from Korean Patients: Subcluster Within Clade 2

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Varicella-zoster virus (VZV) is a causative agent for chickenpox in primary infection and shingles after reactivation from latency. Live attenuated vaccines have been developed based on Japanese Oka strain and Korean MAV/06 strain. At present full genome nucleotide sequences are available for 42 clinical isolates and 5 vaccine strains. Recently, 3 clinical strains were isolated from Korean patients and their genome sequences were completed by next generation sequencing technology. In this study it was attempted to analyze the single nucleotide polymorphism (SNP) of the VZV strains in order to understand the characteristics of Korean clinical isolates. Phylogenetic analyses with 42 non-vaccine and independent VZV strains including the 3 Korean strains YC01, YC02 and YC03 placed the 3 Korean strains to the clade 2 together with pOka and LAX1. Comprehensive SNP analyses identified 87 sites specific for each of the 5 VZV clades. Clade 2 could be further divided into 2 subclades: subclade 2a incuding pOka, LAX1 and YC01, and subclade 2b including YC02 and YC03. Subclade 2a and 2b differed at 7 SNP sites. The subclade 2b strains YC02 and YC03 also shared similar bootscanning pattern distinct from the bootscanning pattern of the subclade 2a strains.
Keywords : Varicella-zoster virus, Korean strain, Single Nucleotide Polymorphism, bootscan

E027

Characterization of the Repeat Sequences of Varicella-Zoster Virus

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Varicella-zoster virus (VZV) is a causative agent for shingles and herpes zoster. The genomes of VZV contain five reiteration (R) sequences and an origin of replication (ORI) sequences composed of tandem repeats whose numbers vary among different strains. Variation of the genome lengths among VZV strains could be attributed by the lengths of R sequences. There was a strong correlation between the lengths of VZV genome and R sequences, while variation of ORI did not contribute the variation of VZV genome length. The high G+C contents of The R sequences in ORF11, 14 and 22 influenced the codon usage of VZV in these ORFs. None of the most frequent 5 codons in R sequences was included in the top 5 most frequent codon in ORF11-14-22 or VZV genome, and vice versa.
Keywords: Varicella-zoster virus (VZV), Repeat Sequences, origin of replication sequences

E028

Construction of a Genetic Linkage Map Based on Simple Sequence Repeats of *Pleurotus eryngii*

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Pleurotus eryngii known as the king oyster mushroom is a commercially important edible mushroom due to its remarkable flavor, high nutritional value, and numerous medicinal features. Despite growing interest in the king oyster mushroom, little is known about its genetic characteristics. To improve the quality or productivity through breeding, a genetic linkage map is an important component. In this study, genetic linkage map of the *P .eryngii* was constructed using 98 monokaryotic progeny derived from dikaryon of parental KNR2312 strain derived from haploid meiotic spores. The whole genome sequence of P5 monokaryon from *P. eryngii* KNR2312 strain by Next Generation Sequencing (NGS) strategy was used to design the SSR markers. 484 primers pairs were identified by SSR Locator I and tested polymorphism via PCR. A total of 241 loci were mapped using Joinmap 4.0, comprising 222 SSR markers, 2 mating type factors, and the 13 INDEL markers. The map consisted of 14 linkage groups spanning 1003 cM at an average marker interval of 4.2 cM. The mating loci, *A* and *B* were mapped on linkage groups 4 and 11, respectively. The established linkage map and the genetic information based on NGS could be used for QTL mapping of agronomic traits, marker-assisted breeding that may ultimately lead to outstanding phenotypic characteristics. [Supported by a grant from the IPET (213003-04-WTI11), MIFAFF, Republic of Korea.]
Keywords : Pleurotus eryngii, Linkage mapping, QTL

E029

Multiple-locus Variable-number Tandem Repeat Analysis (MLVA) for Phylogenetic Analysis of *Campylobacter jejuni*

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Campylobacter jejuni is a causative pathogen of food-borne disease from sporadic cases or outbreak as well as systemic infections such as Guillain-Barré syndrome. For better controlling or source-tracing bacterial infections, efficient molecular genotyping methods are needed. In this study, we developed a multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA) for *C. jejuni* strains, which profile is determined by the combination of the repeat numbers at each VNTR locus. Out of 18 VNTR candidates predicted from a computational program using genome sequences of five reference strains, 10 VNTRs were selected for use in an MLVA assay and assessed with *C. jejuni* clinical isolates collected by the national surveillance program in Korea, 2012. By this approach, a total of 104 strains were classified into three groups including 11 MLVA patterns where MLVA pattern M.001 accounted for 49% (n=51) of the clinical isolates. Interestingly, this particular MLVA pattern was observed for twenty-one isolates received on the same date from Seoul and these isolates were resistant to tetracycline and quinolones, suggesting that an outbreak of *C. jejuni* infection occurred in Seoul. The *C. jejuni* MLVA scheme described in this study could be used as a rapid and reliable genotyping tool with high typeability and discriminatory powers in epidemiological studies and outbreak investigation.

Keywords : *Campylobacter jejuni*, MLVA, VNTR, genotyping tool, Pulsenet

E030

Complete Genome Sequencing of Gallid Herpesvirus 1 Using Next Generation Sequencing Technology

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Development of next generation sequencing (NGS) technology allowed to determine complete genome sequences from animals or microorganisms possessing large size of genomic DNA. In this study, complete genomic sequences of several strains of gallid herpesvirus 1 (GaHV1; Infectious laryngotracheitis virus) were determined using NGS. GaHV1 is a double stranded DNA virus that causes acute upper respiratory disease in chickens. One to five micrograms of purified GaHV1 genomic DNA was used to prepare fragment libraries that were sequenced in parallel using the Ion Torrent system (Life Technology). De novo assembly of the viral reads produced contigs that were aligned to the complete genome sequence of the global vaccine strain of GaHV1 as reference with the exception of the terminal repeat region. Genome sizes of each strain were found to vary, however all genomes had a G + C content of 47.5% and contained 79 predicted ORFs. In the complete genomic alignment between strains, virulent strains of GaHV1 showed high level of nucleotide sequence identity with that of vaccine strain. Variable numbers of single nucleotide polymorphism (SNP) were detected between the strains. Ongoing work in our laboratories is focused on using these sequence data to develop improved diagnostic tools and to better understand the epidemiology and pathogenesis of disease due to infection with GaHV1.

Keywords : Next generation sequencing, Gallid herpesvirus 1, Virus genome

E031

The *ndrA* Transcription Factor is Necessary for the Conidiation and Controlling Sclerotial Formation in *Aspergillus flavus*

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Aspergillus flavus is a saprophytic and pathogenic fungus that can infect animals and humans directly or indirectly by its secondary metabolites. It reproduces clonally by means of conidia (asexual spores), although recent evidence suggests the possibility of the sexual recombination. In eukaryotes, the helix-loop-helix (HLH) transcriptional factors play an important role in the developmental processes. One of these factors is *Aspergillus nidulans ndrA*, which is involved in the early stage of conidiophore development and sexual development. By protein BLAST of *ndrA*, we identified the *A. flavus ndrA* gene, which is its deletion associated with almost absence of the conidia with production of numerous sclerotia comparing to its parental wild type. The complementation of *ndrA* deleted strain by the intact *ndrA* ORF has restored the conidiation as in the wild type with disappearing of the sclerotia. Moreover, we found that, *ndrA* dose not affect the aflatoxin production as well as the antifungal drug sensitivity or resistance and it could not affect the growth in the fungus. The expression of *ndrA* is upregulated at 12 hours under asexual development favorable condition. Taken together, the *ndrA* gene could be considered as a one of the conidiation-critical and sclerotia controlling genes in *A. flavus.*

Keywords : *Aspergillus flavus*, conidiation, sclerotial formation

E032

***Aspergillus fumigatus ndrA* is Expressed at the Early Stage of the Asexual Development and Necessary for the Conidiation**

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Asexual reproduction in the Aspergillus spp. is a mean by which progeny arise from a single parent, and inherit the parental genes only and it does not involve meiosis, ploidy reduction, or fertilization (agamogenesis). The helix-loop-helix (HLH) transcriptional factor proteins that control cell growth and differentiation are playing key roles in a wide range of developmental processes. The intensive researches in the model Aspergillus speces disclosed some of genes that up regulate the asexual development. In a model fungus *Aspergillus nidulans*, one HLH gene, named *ndrA*, which is regulated by NsdD GATA factor has been isolated and characterized as a negative regulator of sexual development as well as positive regulator of asexual development. We performed BLAST search of this protein and identified the *A. fumigatus ndrA* gene, which is its knockout made this fungus unable to produce the conidia. The *ndrA* complemented strain was able to produce numerous amounts of conidia that same to the wild type strain. Northern analysis showed that the *ndrA* gene is highly expressed in the early stage in the conidiation. There was no difference between the wild type and *ndrA* deletion mutant when they subjected to antifungal sensitivity test. Moreover, there were no big differences in the growth rates between the wild type and *ndrA* deletion mutant. Taken together, in *A. fumigatus ndrA* gene could be considered as an important controller of conidiation.

Keywords : *Aspergillus fumigatus*, asexual development, conidiation, helix-loop-helix (HLH), transcriptional factor

E033

NGS Analysis Revealed that the *nsdA4* Mutation in NSD204 Strain is an Allele of *nsdC* in *Aspergillus nidulans*

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Sexual development and fruiting body production of fungi play pivotal roles in production of ascospores by meiosis as well as adaptation of various environmental changes. In a homothallic fungus *Aspergillus nidulans*, many environmental factors and genes affecting sexual development have been elucidated. To know sexual development further, NSD mutants, which are defective in the sexual process, have been isolated. The NSD mutants are divided into four different complementation groups, NSDA-D, and the two genes responsible for the *nsdC* and *nsdD* mutation have already been isolated and characterized. However, *nsdA4* and *nsdB5* mutations from NSD204 and NSD205 mutants, respectively, are remained to be unveiled. Whole genome sequence of NSD204 mutant obtained from Next Generation Sequencing (NGS) identified possible *nsdA4* mutation candidates. Recent intensive mutation analysis revealed that the NSD204 mutant strain carries missense mutations in *nsdC* ORF region, suggesting that phenotype of NSD204 mutant might be derived from the novel nsdC mutation, and eventually indicated that *nsdA4* is an allele of *nsdC* gene. To verify this, NSD204 was genetically crossed with test strain and check the correlation between *nsdA*$^-$ phenotype and *nsdC* mutation. As a result, all strains showing *nsdA*$^-$ phenotype carried *nsdC* mutation which is exactly same mutation found in NSD204 mutant strain, indicating that the nsdA is identical to nsdC.
Keywords : *Aspergillus nidulans*, Sexual development, Next Generation Sequencing

E034

Genome Sequence Analysis of *Chondromyces crocatus* KYC2823

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Chondromyces crocatus is a member of myxobacteria, which exhibits multicellular development and complex fruiting bodies with social behavior. The production of diverse bioactive compounds is a significant property of *C. crocatus*. *C. crocatus* KYC2823 strain was isolated from soil sampled in South Korea. GS FLX Titanium and Illumina Genome Analyzer were employed to determine the genome sequence of KYC2823. The genome sequence consists of a single circular chromosome of 11.4 Mb with 68.7% GC contents. The genome sequence was used to compare with genomes of other myxobacteria and to explore genes related to biosynthesis of secondary metabolites. The genome of KYC2823 contains an array of genes and gene clusters involved in secondary metabolism, which may be responsible for production of the bioactive compounds.
Keywords : myxobacteria, genome, secondary metabolism, bioactive compound

E035

Genome Sequence Analysis of the Flavobacteria Isolated from Tomato Rhizosphere

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The genus of *Flavobacterium* belonging to phylum *Bacteriodetes* is found in diverse environments. Five strains of flavobacteria which displayed specific phenotypes such as antifungal activity, plant growth promotion and biofilm formation, were isolated from soil around tomato roots. They were subjected to whole genome shotgun sequencing analysis using Illumina HiSeq 2000 system; they are *Flavobacterium daejeonense* RCH33, *Flavobacterium aqidurense* RCH62, *Flavobacterium beibuense* RSKmHC5, *Flavobacterium anhuiense* RCM74 and *Flavobacterium* sp. TCH3-2, respectively. Draft genome sequences of 2.8~5.3 Mb were produced and genes associated with the lifestyle in the rhizosphere were explored from the genomes. The genome sequences were used to compare with other flavobacterial genomes and to postulate genomic features specific to the rhizospheric *Flavobacterium*.
Keywords : *Flavobacterium*, rhizosphere, plant-associated bacteria, genome

F001

Synergistic Effect Between Baicalein and Antibiotics against Clinic Methicillin and Vancomycin-Resistant *Staphylococcus aureus*

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Baicalein is one of the major flavonoids in *Scutellaria baicalensis* Georgi, which has long been used in several biological effects, such as antiviral, anti-inflammatiom, anti-hepatotoxicity, and anti-tumor properties, have been reported. In this study, baicalein demonstrated strong antibacterial activity against clinic isolated MRSA and VRSA in this experiment. Baicalein was determined against clinic isolated MRSA 1-16 with MIC and MBC values ranging from 64 to 256 and 64 to 512 μg/mL; for MSSA 1-2 from 128 and 256 μg/mL and 128 and 512 μg/mL; for VRSA 1-2 from 64 and 128 μg/mL and 64 and 512 μg/mL, respectively. The combination effects of baicalein with antibiotics were synergistic (FIC index <0.5) against most of tested clinic isolated MRSA, MSSA, and VRSA except additive, MRSA 7 in oxacillin and MRSA 8 and 15 in vancomycin (FIC index <0.625-0.75). Furthermore, a time-kill study showed that the growth of the tested bacteria was completely attenuated after 2-6 h of treatment with the 1/2 MIC of baicalein, regardless of whether it was administered alone or with ampicillin, oxacillin, or vacomycin. The results suggest that baicalein could be employed as a natural antibacterial agent against multidrug-resistant pathogens infection. This research was supported by the Ministry of Knowledge Economy (MKE-R0002038).
Keywords : Baicalein, mrsa, vrsa, antibacterial activity, synergistic effect

F002

Draft Genome Sequence of *Listeria monocytogenes* NCCP15743 Isolated from Ovarian Cancer patient

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We report here the draft genome sequence of atypical *Listeria monocytogenes* isolated from an ovarian cancer patient in Korea. Genomic DNA was isolated from NCCP 15743strain and its whole genome was sequenced using an Ion PGM sequencer (IonTorrent, Inc.). A total of 3,268,134 single reads was generated with average read length of 142 bp. The raw sequences were pre-processed using CLC AssemblyCell version 4.0.6 (CLC bio, Denmark) with quality value over 20 (i.e. 99% base call accuracy) and minimum length cutoff 80bp. A total of pre-processed 1,531,462 reads, which comprises 46.7 fold coverage of the 2.94 Mbp genome size of the reference strain EGD-e (GenBank Acc. AL591824.1) serovar 1/2a. We performed the reference assembly of NCCP 15743 NGS reads against the EGD-e genome using CLC Assembly Cell. The draft genome of NCCP 15743 was 2,871,534 bp, i.e. 2,803,433 bp reference-assembled + 68,101 bp de novo assembled from unmapped reads, with GC content of 38.1%. Comparative analysis revealed 32,132 single nucleotide polymorphisms (SNPs) in NCCP 15743 genome, including 15,075 non-synonymous cSNPs to the EGD-e. Among 32,132 total SNPs, 14,903 (46.4%) were common with 07PF0776. In three strain comparisons, 16,973 SNPs were found to be NCCP15743 specific and 124,341 were 07PF0776 specific. Among 16,973 SNPs of NCCP 15743, 44 SNPs were found in *prfA* (peptide chain release factor1), 52 SNPs in *inlA* (internalinA) and 16 SNPs in *inlB* (internalinB) respectively.
Keywords : *Listeria monocytogenes*, Genome sequenceing, SNPs

F003

Enteric Bacteria Isolated from Diarrheal Disease in Korea 2013

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This study was performed to determine the characteristics of the diarrheal causing pathogens according to season, isolated regions, patient`s age and sex and to provide useful data for the prevention of diarrheal disease.Stool specimens from 20,984 patients with diarrhea were collected to identify the pathogenic bacteria from January to December 2013 in Korea. And 3,668 pathogenic bacteria were isolated and analyzed according to season, isolated regions, patients¡¯ age and sex. The proportions of isolated pathogenic bacteria were *Salmonella* spp. 523 (14.3%), pathogenic *E.coli* 954 (26.0%), *V.parahaemolyticus* 31 (0.8%), *Shigella* spp. 27 (0.7%), *Campylobacter* spp. 158 (4.3%), *C.perfringens* 731 (19.9%), *S.aureus* 972 (26.5%), *B.cereus* 243 (6.6%), *L.monocytogenes* 7 (0.2%), and *Y. enterocolitica* 22 (0.6%). Isolation rate showed highest ratio in summer season, from June to September for most of pathogenic bacteria, except for Gram positive bacteria. Isolation rate of pathogenic bacteria by patients¡¯ age showed highest ratio at 0 to 19 year for most of pathogenic bacteria. And Isolation rate by region, 26.4% isolated from cities and 11.3% isolated from rural provinces.Hygiene education should be addressed on diarrheal disease susceptible groups, such as age under 10, age of 10-19, and more than 70 years old, and ongoing monitoring for the pathogens is still required. In addition, efficient information system and surveillance project for infection prevention should be continued.
Keywords : Surveillance, Enter-Net Korea

F004

Bacteroides fragilis Enterotoxin Upregulates Heme Oxygenase-1 Expression in Dendritic Cells via Mitogen-Activated Protein Kinase and Nrf2-Dependent Pathway

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Enterotoxigenic *Bacteroides fragilis* (ETBF) produces an approximately 20 kDa heat-labile enterotoxin (BFT) that plays an essential role in mucosal inflammation. Although a variety of inflammatory cells are found at ETBF-infected sites and dendritic cells (DCs) play an important role in the regulation of inflammation, little is known about DC responses to BFT stimulation. This study was conducted to investigate the role of BFT on expression of heme oxygenase (HO)-1 in DCs. Stimulation of DCs with BFT resulted in the induction of HO-1 expression. BFT increased the signals of NF-κB, AP-1, and Nrf2. However, upregulation of HO-1 was independent on the activation of NF-κB and AP-1 signaling. In contrast, suppression of Nrf2 activity in DCs resulted in a significant reduction of HO-1 expression. In addition, HO-1 induction via Nrf2 was regulated by mitogen-activated protein kinases (MAPKs) including ERK and p38. These results suggest that a signaling pathway involving MAPKs and Nrf2 is required for HO-1 upregulation in DCs exposed to BFT.
Keywords : B. fragilis, dendritic cells, heme oxygenase-1, enterotoxin

F005

Active Immunization with Recombinant Japanese Encephalitis Virus Envelope Protein Fused with a Flagellin Elicits Mucosal Immune Responses against Japanese Encephalitis Virus Infection

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Japanese encephalitis virus (JEV), a mosquito-borne flavivirus, is a major human pathogen that cause viral neurologic disease. Given that there is no approved therapeutic agent for the viral disease, novel vaccine could provide an efficacious alternative to prevent the viral disease. In this study, we investigated whether intranasal immunization with recombinant fusion protein consisted of Domain III of JEV envelope protein and *Vibrio vulnificus* FlaB protein, E(DIII)-FlaB, elicit protective mucosal immune responses. Interestingly, nasal vaccination of mice with recombinant E(DIII)-FlaB induced a robust antibody response. Furthermore, antiserum raised from the E(DIII)-FlaB protein by nasal immunization showed neutralizing activity against JEV infection in plaque-reduction neutralization test. Taken together, these results suggested that the FlaB fused JEV E(DIII) may be relevant for developing a mucosal vaccine against lethal JEV infection.
Keywords : Japanese encephalitis virus, envelope protein, flagellin, vaccine

F006

Preventive Activity of intranasal Catalytic RNA-Hydrolyzing Antibody in Mice with an Influenza H1N1 Virus

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A novel anti-influenza H1N1 virus activity of the catalytic RNA-hydrolyzing antibody, "3D8 scFv", for intranasal administration was described. The recombinant 3D8 scFv can facilitate the degradation of viral RNA genome through its RNA-hydrolyzing activity. The preventive activity of intranasal 3D8 scFv was evaluated in BALB/c mice infected with an influenza H1N1 virus. Intranasal administration of 3D8 scFv (50 μg /day) demonstrated a 70% antiviral effect against H1N1 virus infection after 3D8 scFv protein administration for 5 days prior to infection. The antiviral activity of 3D8 scFv against H1N1 virus infection was not due to host immune cytokines or chemokines, but rather direct antiviral RNA-hydrolyzing activity against viral RNA genome. There was no difference in any cytokine expression level between 3D8 scFv-treated cells and control cells after H1N1 challenge. The ability of 3D8 scFv to penetrate into epithelial cells via the respiratory mucosal layer from bronchial cavity was confirmed by immunohistochemistry, qRT-PCR, and histopathological examination. Taken together, our results suggest that the RNase activity of 3D8 scFv, coupled with its cell-penetrating ability, is able to directly prevent H1N1 virus infection in a mouse model.
Keywords : 3D8 scFv, H1N1 influenza virus, intranasal administration

F007

In Vitro and *In Vivo* Preventive Effects against Murine Norovirus Infection of the Nucleic Acid-Hydrolyzing 3D8 scFv Protein Expressed by *Lactobacillus casei*

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The protein 3D8 single chain variable fragment (3D8 scFv) has potential antiviral activity due to its ability to penetrate into cells and hydrolyze nucleic acids. Probiotic *Lactobacillus casei* engineered to secrete 3D8 scFv for oral administration was used to test the antiviral effects of 3D8 scFv against gastrointestinal virus infections. We found that injection of 3D8 scFv into the intestinal lumen resulted in the penetration of 3D8 scFv into the intestinal villi and lamina propria. 3D8 scFv secreted from engineered *Lactobacillus casei* retained its cell-penetrating and nucleic acid-hydrolyzing activities, which were previously shown with 3D8 scFv expressed in *Escherichia coli*. Pretreatment of RAW264.7 cells with 3D8 scFv prevented apoptosis induction by murine norovirus infection and decreased mRNA expression of the viral capsid protein VP1. In a mouse model, oral administration of the engineered *Lactobacillus casei* prior to murine norovirus infection reduced the expression level of mRNA encoding viral polymerase. Taken together, these results suggest that *L. casei* secreting 3D8 scFv provides a basis for the development of ingestible antiviral probiotics active against gastrointestinal viral infection.
Keywords : 3D8 scFv, antiviral effect, Lactobacillus casei, oral administration, murine norovirus

F008

A nucleic-Acid Hydrolyzing Single Chain Antibody Confers Resistance to DNA Virus Infection in HeLa Cells and C57BL/6 Mice

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Viral protein neutralizing antibodies have been developed but they are limited only to the targeted virus and are often susceptible to antigenic drift. Here, we present an alternative strategy for creating virus-resistant cells and animals by ectopic expression of a nucleic acid hydrolyzing catalytic 3D8 single chain variable fragment (scFv), which has both DNase and RNase activities. HeLa cells (SCH7072) expressing 3D8 scFv acquired significant resistance to DNA viruses. Virus challenging with Herpes simplex virus (HSV) in 3D8 scFv transgenic cells and fluorescence resonance energy transfer (FRET) assay based on direct DNA cleavage analysis revealed that the induced resistance in HeLa cells was acquired by the nucleic acid hydrolyzing catalytic activity of 3D8 scFv. In addition, pseudorabies virus (PRV) infection in WT C57BL/6 mice was lethal, whereas transgenic mice (STG90) that expressed high levels of 3D8 scFv mRNA in liver, muscle, and brain showed a 56% survival rate 5 days after PRV intramuscular infection. The antiviral effects against DNA viruses conferred by 3D8 scFv expression in HeLa cells as well as an *in vivo* mouse system can be attributed to the nuclease activity that inhibits viral genome DNA replication in the nucleus and/or viral mRNA translation in the cytoplasm. Our results demonstrate that the nucleic-acid hydrolyzing activity of 3D8 scFv confers viral resistance to DNA viruses *in vitro* in HeLa cells and in an in vivo mouse system.
Keywords : 3D8 scFv, nuclease activity, pseudorabies virus, herpes simplex virus, nuclease acitivity

F009

Therapeutic Strategy for Preventing Pseudorabies Virus (PRV) Infection in C57BL/6 mice by 3D8 scFv with intrinsic Nuclease Activity

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3D8 single chain variable fragment (scFv) is a recombinant monoclonal antibody with nuclease activity that was originally isolated from autoimmune-prone MRL mice. In a previous study, we analyzed the nuclease activity of 3D8 scFv and determined that a HeLa cell line expressing 3D8 scFv conferred resistance to herpes simplex virus type 1 and pseudorabies virus (PRV). In this study, we demonstrate that 3D8 scFv could be delivered to target tissues and cells where it exerted a therapeutic effect against PRV. PRV was inoculated via intramuscular injection, and 3D8 scFv was injected intraperitoneally. The observed therapeutic effect of 3D8 scFv against PRV was also supported by results from quantitative reverse transcription polymerase chain reaction, Southern hybridization, and immunohistochemical assays. Intraperitoneal injection of 5 and 10 ¥ μg 3D8 scFv resulted in no detectable toxicity. The survival rate in C57BL/6 mice was 9% after intramuscular injection of 10 LD_{50} PRV. In contrast, the 3D8 scFv-injected C57BL/6 mice showed survival rates of 57% (5 μg) and 47% (10 μg). The results indicate that 3D8 scFv could be utilized as an effective antiviral agent in several animal models.

Keywords : 3D8 scFv, nuclease activity, pseudorabies virus, therapeutic effects, mouse

F010

Staphylococcus Aureus Produce Membrane Vesicles Which Inhibit Other Bacteria Biofilm Formation

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Membrane vesicles produced from the bacteria has been reported as playing a key role in a bacterial interaction. Although many bacteria use membrane vesicles for enhancing their biofilm, interestingly S. aureus hinder other bacteria biofilm formation by producing membrane vesicles. To elucidate the mechanisms of the S. aureus vesicle to block the biofilm formation, we investigated interference effect in variable ways. Membrane vesicles derived from S. aureus coated the substrate and change property of the surface to hydrophilic nature which would inhibit initial binding of the bacteria. We confirmed that hydrophilic surface by plasma treatment blocked the biofilm formation, as vesicles did. Furthermore, S. aureus membrane vesicles provided broad spectrum inhibition on other strain biofilm by changing the substrate property. These findings will provide interspecific interactions with membrane vesicles and their competition with other strains which can be a new strategy for controlling the pathogenic bacteria in medical aspect.

Keywords : Bacterial membrane vesicles, *Staphylococcus aureus*, Acinetobacter baumannii, Biofilm, Hydrophobicity

F011

Mitochondrial Uncoupling Protein-2 Expression is Decreased in Macrophages Infected with *Mycobacterium abscessus*

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Uncoupling proteins (UCPs) are mitochondrial anion carrier proteins that present in the inner membrane of mitochondria. UCP2 is expressed in many tissues including macrophages, and known to have dual functions in decrease of ATP synthesis and protection of cell function from oxidative stresses. *Mycobacterium abscessus* (Mabc) is an emerging nontuberculous mycobacterial strain to cause a variety of human diseases. Nevertheless, the expression of UCP2, mitochondrial reactive oxygen species (ROS), as well as inflammatory cytokine generation have not been fully characterized in macrophages infected with Mabc. In this study, we show that Mabc decreases the gene expression of mitochondrial uncoupling protein-2 (UCP-2), but increases inflammatory cytokine genes in macrophages, in bone marrow-derived macrophages (BMDMs). We also found that Mabc infection led to an increase of mitochondrial ROS in macrophages. Scavenging mitochondrial ROS led to an increase of Mabc-induced inflammatory cytokine expression in macrophages. Collectively, these findings indicate that Mabc inhibits UCP-2 expression, but increases inflammatory responses through mitochondrial ROS generation in macrophages.

F012

Modified siRNA Containing 5'-Triphosphate Targeting 3C Region of Enterovirus 71 Has A Potent Antiviral Effect in Vivo

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Enterovirus 71 (EV71) is a major pathogen associated with hand, foot, and mouth disease (HFMD). It also causes central nervous system disease and complications including encephalitis, aseptic meningitis, paralysis and brain stem encephalitis. Although EV71 infection has severe influence on many people, there are no established antiviral treatments to control EV71 infection. Previous our results have shown that modified siRNAs containing 5'-triphosphate targeting 3C region (H40-3p-siRNA) were able to significantly enhance antiviral effect in vitro. In this study, we observed antiviral effect of H40-3p-siRNA against EV71 infection in 1-day-old ICR mice. The antiviral effects were evaluated by mortality, degree of paralysis, weight, and viral RNA in each organ (brain, spinal cord, heart, liver, spleen, and muscle). The mortality, percentage of paralysis, and expression level of viral RNA of H40-3p-siRNA treated group is lower than those of H40-OH-siRNA treated group. These results demonstrate that H40-3p-siRNA has an antiviral effect in vivo, thus it could be a novel antiviral drug candidate against EV71 infections.

Keywords : Enterovirus 71, triphosphate(3p)-siRNA, antiviral effect, 3C protease, animal model

F013

Extended Longevity and Robust Early-stage Development of *Caenorhabditis elegans* by a Soil Microbe, *Lysinibacillus sphaericus*

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Caenorhabditis elegans, originally isolated from soil, is a nematode used in host-microbe interaction research. While human pathogenic bacteria have been actively studied in *C. elegans*, no bacterial species that provides beneficial effects on *C. elegans* has been reported. Here, we tested several bacterial soil isolates and further characterized the effects of *Lysinibacillus sphaericus* on *C. elegans* growth-related phenotypes. Worms fed with *L. sphaericus* lived significantly longer than those growing with *E. coli* OP50. Juvenile-stage growth was also highly stimulated by *L. sphaericus*. In addition, significantly elevated fertilization was observed in worms fed with *L. sphaericus*. Furthermore, growth with *L. sphaericus* resulted in the production of larger numbers of progeny than the growth with OP50. Worms grown with *L. sphaericus* were highly resistant to oxidative and osmotic stress. Together, our results reveal a novel mode of growth that involves healthy aging of nematodes.
Keywords : *Caenorhabditis elegans*, Lifespan, Longevity

F014

Genipin is a New Chemical Activator of Gammaherpesvirus Lytic Cycle

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Gardenia jasminoides is an evergreen flowering plant beloning to the family *Rubiaceae*. This plant is originated in Asia and most commonly found growing wild in Vietnam, Southern China, Taiwan, Japan, Myanmar and India. Genipin is an aglycone derived from an iridoid glycoside called geniposide, which is amply present in fruit of Gardenia jasminoides. Gammaherpesvirus includes Epstein-Barr virus (EBV, also HHV4) and Kaposi's sarcoma-associated herpesvirus (KSHV, also HHV8). EBV and KSHV causes several types of human cancers including Burkitt's lymphoma, nasophayngeal, gastric carcinoma, Kaposi's sarcoma (KS), and AIDS-related form of non-Hodgkin lymphoma, and etc. Antiviral agents can be categorized as virucidals, antiviral chemotherapeutic agents, and immunomodulators. In host cells gammaherpesvirus latently infected, genipin caused significant cytotoxicity (70-72.5 uM), arrested cell-cycle progress (S, G2/M phase), upregulated gammaherpesvirus genes (latent and lytic genes), stimulated viral progeny production, activated viral promoter for lytic gene expression (Fp promoter), and suppressed viral infection. These results suggested that genipin produces a strong antiviral activities in the life cycle of gammaherpesvirus by stimulating viral lytic replication cycle.
Keywords : Genipin, Gammaherpesvirus, Lytic activation

F015

Broadly Protective *Shigella* Outer Membrane Protein PSSP-1 against *Shigella* Infection

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In developing countries *Shigella* is a primary cause of diarrhea in infants and young children. Although antibiotic therapy is an effective treatment for shigellosis, therapeutic options are narrowing due to the emergence of antibiotic resistance. Thus, preventive vaccination could become the most efficacious approach for controlling shigellosis. We identified several conserved protein antigens that are shared by multiple *Shigella* serotypes and species. We evaluated cross-protective immunity of these proteins in mice. Among these, one antigen induced cross-protection and we have named it pan-*Shigella* surface protein-1 (PSSP-1). PSSP-1-induced protection requires a mucosal administration route and co-administration of an adjuvant. When PSSP-1 was administered with adjuvant intranasally, it induced cross-protection against *S. flexneri* 2a, 5a, 6, *S. boydii,* and *S. dysenteriae* 1. Intradermally administered adjuvanted-PSSP-1 induced strong serum antibody responses but failed to induce protection in the mouse lung pneumonia model. In contrast, intranasal administration elicited efficient local and systemic antibody responses and IL-17A and IFN-¥ã production. Interestingly, blood samples from patients with recent onset shigellosis showed variable but significant mucosal antibody responses to other conserved *Shigella* protein antigens but not to PSSP-1. We suggest that PSSP-1 is a promising antigen for broadly protective vaccine candidate against shigellosis.
Keywords : PSSP-1, IcsP, *Shigella*, Vaccine, Cross-protection

F016

Induction of Hummingbird Phenotype in Human Gastric Epithelial AGS Cells through Nuclear Targeting of *Helicobacter pylori* Urease Subunit A

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Urease is an essential enzyme for the survival of *Helicobacter pylori* under the acidic conditions in the stomach. We have previously reported that urease subunit A (UreA) of *H. pylori* targets the nuclei of COS-7 cells through nuclear localization signals. This study further investigated whether UreA of *H. pylori* targets the nuclei of gastric epithelial cells and then subsequently induces pathological changes in the host cells. UreA was detected in gastric epithelial cells in *H. pylori*-positive specimens obtained from patients with gastritis. *H. pylori* secreted outer membrane vesicles (OMVs), and UreA was translocated into gastric epithelial AGS cells through OMVs. UreA could target the nuclei of AGS cells. Nuclear targeting of rUreA did not induce cell death, but resulted in morphological changes such as cellular spreading and elongation in AGS cells, that is, the so-called hummingbird phenotype. AGS cells treated with rUreA¥ÄNLS proteins did not show the hummingbird phenotype. Nuclear targeting of UreA differentially regulated 102 morphogenesis-related genes, of which 67 and 35 were up-regulated and down-regulated, respectively. Nuclear targeting of *H. pylori* UreA induces a morphological change and regulates morphogenesis-related genes in gastric epithelial cells, which may be associated with the pathogenesis of *H. pylori*.
Keywords : Hummingbird phenotype, Morphogenesis, Nuclear targeting protein, Outer membrane vesicles, Urease

F017

Cytopathology of Human Epithelial HEp-2 Cells Induced by *Acinetobacter nosocomialis* Outer Membrane Vesicles

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Acinetobacter nosocomialis is an opportunistic pathogen that causes a variety of human infections, but pathogenesis of this microorganism has not yet been characterized. The aim of this study was to investigate the secretion of outer membrane vesicles (OMVs) from *A. nosocomialis* ATCC 17903^T and determine the induction of pro-inflammatory response and cytotoxicity of human epithelial HEp-2 cells treated with *A. nosocomialis*-derived OMVs. *A. nosocomialis* secreted spherical OMVs during *in vitro* culture. Using proteomic analysis of OMVs from *A. nosocomialis* ATCC 17903^T, 147 different proteins were identified. Among them, virulence-associated proteins, including CsuA, CsuC, CsuD, PilW, hemolysin, serine protease, and ferrous iron transporter B, were identified. OMVs prepared from *A. nosocomialis* delivered OmpA to HEp-2 cells, which could induce cytotoxicity in a dose-dependent manner. However, OMVs derived from isogenic Δ*ompA* mutant did not induce cytotoxicity. *A. nosocomialis* OMVs elicited gene expression of pro-inflammatory cytokines, including IL-1β, IL-6, IL-8, macrophage inflammatory protein-1α, and monocyte chemoattractant protein-1, in HEp-2 cells in a dose-dependent manner. In conclusion, OMVs secreted from *A. nosocomialis* are an important vehicle to deliver effector molecules to host cells and OMV-mediated delivery of effector molecules to host cells induces pro-inflammatory response and cytotoxicity, which may contribute to pathogenesis of *A. nosocomialis*.

Keywords : *Acinetobacter baumannii*, Outer membrane vesicles, Cytotoxicity

F018

Cordycepin is a New Chemical Suppressor of EBV Replication

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Cordyceps is known to produce many kinds of active components and used for a diversity of medicinal purpose due to various physiological activities. The physiological activities are anticancer activity, protection of liver damage, antidepressant effects, anti-inflammatory effects, hypoglycemic effect, antimicrobial activity, and etc. Cordycepin is a derivative of adenosine, differing from adenosine in that cordycepin lacks oxygen in the 3' position of its ribose part. This study would address to define how cordycepin makes anti-gammaherpesviral effects using epigenetic approaches. Our study demonstrated that cordycepin contains antitumor and antiviral activity against gastric carcinoma and EBV, respectively. First, comparison of CD_{50} between cordycepin and its analogues indicated that loss of 2'-hydroxyl group in cordycepin was critical of cordycepin to produce stronger cytotoxicity than its analogues. Treatment of cordycepin suppressed early apoptosis up to 64%, yet it increased late apoptosis/necrosis up to 31% in SNU719 cells. In same context, cordycepin significantly decreased frequencies of Qp and Fp promoter usages and H3K4me3 histone enrichment was significantly reduced by cordycepin from several important EBV genomic locuses. These results suggested that cordycepin is antiviral and antitumor against gammaherpesviruses and host cells virus latently infected.

Keywords : Cordycepin, Epstein-barr Virus, Gene expression, methylation

F019

Deaminase-independent Inhibition of PERV Reverse Transcription by APOBEC3G

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Porcine endogenous retroviruses (PERVs) present a unique concern associated with xenotransplantation because they have been shown to infect certain human cells in vitro. During mammalian evolution, a variety of mechanisms have arisen to limit retroviral replication. Retrovius replication can be restricted by cellular factors such as APOBEC3G, Fv1, TRIM5¥á, and tetherin. Human APOBEC3G (huA3G) is a single-strand DNA cytosine deaminase, which inactivates the coding capacity of the virus by deamination of cDNA cytosines to uracils. This reaction occurs within (-) DNA strand during reverse transcription, resulting in G-to-A mutation in the (+) strand. A recent report showed that huA3G also could inhibit viral replication by cytidine deaminase-independent mechanisms. To determine whether DNA deamination is required the huA3G and porcine APOBEC3G(poA3Z2-Z3)-dependent inhibition of PERV transmission, we established a new set of 293-PERV-PK-CIRCE (human 293 cells infected with PK15-derived PERVs) clones expressing huA3G or poA3Z2-Z3. 293T cells were infected with virus-containing supernatant from 293-PERV clones expressing huA3G or poA3Z2-Z3. To search for antiviral mechanism of huA3G and poA3Z2-Z3, Genomic DNA from 293T cells was prepared at 10h postinfection and the PERV gag gene from amplified PCR product was sequenced. We could not detect any G-to-A mutations. Based on these results, we conclude that the APOBEC3G restricts PERV replication by deaminase-independent mechanism.

Keywords : APOBEC3G, Cytosine deaminase, G-to-A mutations, PERV

F020

Combination of PFGE and MLST for Molecular Epidemiological Investigation in Salmonella Typhi

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Typhoid fever was not a problem in Korea for their low prevalence, but in recent years small outbreak related S. Typhi was occurred in Gyeongnam Province. In this study, we tried to reveal the epidemiological relationship among isolates by combination of PFGE and MLST. A total of 44 isolates obtained from the patients in Gyeongnam Province during 6 months (Jan.~June) in 2014. PFGE and MLST was performed according to the PulseNet protocol using Xba I as restriction enzyme and the method described in previous studies, respectively. Data were combined and applied to investigate epidemiological analysis.PFGE profiles differentiate into 5 types among 44 isolates. Especially SPPX01.051 (54.5%) and SPPX01.077 (38.6%) were the most prevalent in isolates. However, MLST profiles just differentiate into 3 sequence types, and most isolates belonged to ST2 (47.7%) and ST3 (50.0%). The combination of PFGE and MLST differentiate 7 types and showed 4 major types (51-2, 51-3, 77-2, 77-3). The 51-2 (n=12, 27.3%) was indigenous type that occurs in the Gyeongnam Province every month, while 51-3 (n=12, 27.3%), 77-2 (n=8, 18.2%), and 77-3 (n=9, 20.5%) were epidemic type which is spread around after exposure to specific carrier.We were obtained a meaningful data by the method according to the combination of PFGE and MLST. It might be a valuable tool to real the epidemiological relationship among undifferentiated strains.

Keywords : Typhoid fever, *Salmonella* Typhi, PFGE, MLST

F021

Bacteria Bacteria Interaction: Effects of Bdellovibrio Bacteriovorus on Gram Other Bacteria

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Pathogenic bacteria display various levels of host specificity or tropism. While many bacteria can infect a wide range of hosts, certain bacteria have strict host selectivity for humans as obligate human pathogens. In this study, we found that Host independent mutant of predatory bacteria Bdellovibrio bacteriovorus HD100 secretes proteins to surrounding media. Based on the shot gun proteomes analysis there are 17 protease including peptidase. Furthermore we evaluated the effect of extracellular proteases on Staphylococcus aureus membrane, result suggest that the extracellular secreted enzymes decrease the contents of outer membrane protein. Extracellular supernatant treated and untreated Staphylococcus aureus were co cultured with MCF-10a cells result suggest that supernatant treated Staphylococcus aureus invasion is 6 fold decrease compared to untreated. Furthermore we selected two protease which are detected in the supernatant of host independent mutant Bdellovibrio bacteriovorus HD100 and cloned into expression vector pET31b and purified using Ni NTA and tested their activity on milk agar plate result confirms the expressed protease is very active. Furthermore the ability of purified protease were tested on various pathogen including gram positive and gram negative pathogen.
Keywords : Pathogen, Protease, Iteraction

F022

A live Salmonella Enteritidis Vaccine Secreting LTB and its Protective Effects against Internal Egg Contamination

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This study was carried out to evaluate the effect of *Salmonella enterica* serovar Enteritidis (SE) secreting *Escherichia coli* heat labile enterotoxin B subunit (LTB) protein as an adjuvant for a live SE vaccine (JOL919) against virulent SE challenge in hens.The *eltB* gene encoding LTB was inserted into the Asd+ β-lactamase signal plasmid pJHL65. This plasmid was transformed into *△lon△cpxR△asd SE* to generate the LTB strain JOL1228. One-hundred female chickens were divided into five groups and chickens in immunised groups were primed and subsequently boosted with either JOL919 or a JOL919-JOL1228 mixture. Humoral and cellular immune responses were significantly higher in the immunised groups than the control group. On challenge with virulent SE, egg protection was 89.3% in immunised hens in group B (primed and boosted twice with JOL919 only), 89.3% in group C (primed with JOL919-JOL1228 mixture and boosted twice with JOL919), 100% in group D (primed and first booster with JOL919-JOL1228 mixture, then subsequently boosted with JOL919), 90.5% in group E (primed and boosted twice with JOL919-JOL1228 mixture) and 60.7% in group A (control group of non-immunised hens inoculated with phosphate buffered saline).The challenge strain was detected significantly less in all organs examined from hens in group D than those of the control group.
Keywords : Salmonella, LTB, protection

F023

Characteristics of *Salmonella* spp. isolated in Republic of Korea, 2013

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Salmonella spp. can cause a variety of infectious disease in animal and human. In recent, the occurrence was shown various trends contrary to existing pattern. In this study, we reported the characteristics of *Salmonella* serotypes isolated in Korea, 2013. A total of 777 *Salmonella* isolates were collected from acute diarrheal patients and environments in regional Institutes of Health and Environment and National Quarantine Stations in 2013. Serotypes were determined with Kauffman-White (K-W) methods and antimicrobial susceptibility test was performed with CLSI guideline. There were 777 isolates, including 748 domestic resident isolates and 29 from foreign travelers. *S.* Enteritidis (24.6%), *S.* Typhimurium (13.0%) and *Salmonella* I 4,[5],12:i:- (13.0%) were major serotypes. *S.* Montevideo (11.3%) was emerging strains, causing outbreaks of in December, and ranked 4th grade in 2013. Total of 223 serotypes was identified in Korea and 15 serotypes were firstly reported in 2013. Most isolates have resistance against ampicillin (42.2%), although there were different antimicrobial resistance patterns by each serovars. Because of the migration of population and distribution, the possibility of infection was increased and the pattern of infectious disease was changed. In this reason, we are necessary to perform thorough epidemiological survey according to the changed aspect of patients. Thus, the KNIH is continuous monitoring and research in progress at the national level.
Keywords : *Salmonella*, Serotypes, *S.* Montevideo, Outbreaks, Antimicrobial resistance

F024

Activation of Complement System in Acute Phase of KSHV-infected Endothelial Cells and its Role in Viral Persistence

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The complement system has critical roles in immune defense against pathogens that evade human body. The complement system can be activated by three main pathways, and it can induce inflammatory reactions, opsonization, and membrane attack complex(MAC)-mediated cell lysis. Besides immunological function, activated complement system on some eukaryotic cells have known to induce proliferation and survival of cells, especially in cancer cells. Kaposi's sarcoma–associated herpesvirus (KSHV) is associated with three different human malignancies, including Kaposi's sarcoma (KS), primary effusion lymphoma, and multicentric Castleman's disease. We investigated activation of complement system in acute phase of KSHV-infected primary human endothelial cells by using an immunofluorescence assay, flow cytometry and ELISA. In spite of highly up-regulated complement regulatory proteins, CD55, factor H, and KSHV ORF4, a definite C5b-9 deposition was observed on the surface of KSHV-infected human endothelial cells. Cell death by C5b-9 from complement activation was minimal and complement activation induced protective effects on the acute phase of KSHV-infected endothelial cells from the lytic replication over time. Therefore, complement activation on KSHV-infected endothelial cells might have a beneficial effect on the persistent infection of KSHV.
Keywords :

F025

Host pathogen Ineratactionn: Delivery of Virulence Factor by Invasive Pathogen *Yersinia pseudotuberculosis*

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Bacteria use a variety of secreted virulence factors to manipulate host cells, thereby causing significant morbidity and mortality. We studied a mechanism for the long-distance delivery of multiple bacterial virulence factors, simultaneously and directly into the host cell cytoplasm, thus obviating the need for direct interaction of the pathogen with the host cell to cause cytotoxicity. We identified extracellular proteins secreted by *Yersinia pseudotuberculosis* by LC/MS-MS, We identified multiple virulence factors, including rpoB, rpoS, and attachment and invasion locus protein which can be directly interact with the host cytoplasm. Finding suggest that invasive human pathogen *Yersinia pseudotuberculosis* secrets virulence factors. These virulence factors enter the cytoplasm of the host cell, where they rapidly distribute to specific subcellular locations to affect host cell. We studied effect secreted proteins on airways epithelial cell and found that it causes actin rearrangement followed by cytotoxicity on cells. We propose that secreted virulence factors are not released individually as naked proteins into the surrounding location where they may randomly contact the surface of the host cell

Keywords : *Yersinia*, Pathogenesis, Virulance

F026

Surveillance of Pathogens Causing Acute Viral Gastroenteritis in Korea, during 2013

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Acute gastroenteritis was more prevalent in children under 5 old, and the most important etiological agents are norovirus (NoV), group A rotavirus (RoV), enteric adenovirus (AdV), astrovirus (AsV) and sapovirus (SaV). In this study, we analyzed the laboratory surveillance of diarrhea induced by viral pathogens sporadically in Korea, during 2013. Fecal specimens were collected from acute gastroenteritis patients during 2013. NoV was detected by real-time RT-PCR and genotype was performed with RT-PCR and automatic sequencing system. RoV and AdV were detected their antigen by EIAs methods. AsV and SaV were specific gene detection by duplex RT-PCR methods. The overall occurrence of 18,908 sample investigated and viral infection were 3,379 samples (17.8%). NoV was found most frequently (8.5%), followed by RoV(7.6%), AdV(0.9%), AsV(0.6%), and SaV(0.3%). The highest viral positive rate (30.7%) was observed in children (≤ 5 years of age), and statistically significant relationship was observed when compared with other age groups (≥6 years of age) (8.0%). NoV was predominant in winter season but RoV was peaked in spring. The virus was infected in all age groups, but higher incidence of infection was in children under 5 old. In recent, AdV, AsV, and SaV associated outbreaks were reported, although these was shown low detection rate in sporadic case. These viruses also need to observe carefully.

Keywords : Acute viral gastroenteritis, Surveillance, 2013

F027

A New Licorice-derived Stimulator of Gammaherpesvirus Production

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Licorice is the root of Glycyrrhiza uralensis or G. glabra that have traditionally been cultivated in eastern part of Asia. Extracts of these plants are demonstrated anti-viral, anti-inflammatory, anti-atopic, hepatoprotective, anti-neurodegenrative, anti-tumor, anti-diabetic effects and so forth. Glycyrrhiza is known to produce a variety of bioactive compounds such as triterpene(glycyrrhizin, 18β(α)-glycyrrhetinic acid), isoflavan(glabridin, licoricidin), flavanone(liquiritin, liquiritigenin), chalconne(isoliquiritigenin, licochalcone A(B)), 3-arylcoumarin(glycyrol, glycyrin), and miscellaneous compounds. Quercetin is a flavonoid that found in fruits, vegetables, leaves and grains of most plants including licorice. It has greater inhibitory effect on HCV replication than other flavonoids. Isoliquiritigenin is a licorice-derived chalcone that plays a role of potent positive allosteric modulator for GABA-A benodiapine receptor. We tested antiviral and antitumor activities of quercetin and its structural analogue isoliquritigenin using in vitro systems such as SNU719 and iSLK-BAC16 cancer cells latently infected with EBV and KSHV, respectively. Both quercetin and isoliquiritigenin enhanced transcription of EBV latent/lytic genes, while isoliquiritigenin only was effective to induce KSHV latent/lytic genes induction. In conclusion, quercetin is one of antiviral agents that is good at stimulating EBV progeny production and isoliquiritigenin was good at KSHV progeny production.

Keywords : gammaherpesvirus, virus rogeny production, anti-viral agents, licorice-derived compound, quercetin

F028

Functional Analysis of *Pseudomonas aeruginosa* PA4834 Essential for Growth of *Pseudomonas aeruginosa* in Airway Mucus

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Pseudomonas aeruginosa causes chronic airway infections in patients with bronchiectasis including cystic fibrosis. Normal human nasal epithelial (NHNE) cells are known to secrete mucus. When various pathogens (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Vibrio cholera*, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Pseudomonas aeruginosa* (PA)) were cultivated in mucus, the number of PA was only increased. However, boiled mucus did not show increase in the number of PA. From microarray data showing change of PA gene in airway mucus, it was confirmed that the expression of iron-related genes is significantly increased. PA4834 showing increased expression was selected and its mutant was constructed. When deletion mutant of PA4834 was cultivated in mucus, its number was significantly decreased in comparison with the number of wild type. However, resistance of PA4834 mutant for various antibiotics was not varied. The addition of $FeCl_3$ in mucus recovered the growth deficiency of PA4834. PA can acquire this element by secreting iron-chelating compounds (siderophores) that scavenge iron and deliver it to the bacteria. Using siderophore assay, it was identified that PA4834 mutant have decreased siderophore production. Our data show that PA4834 is an important iron-scavenging gene for PA in airway mucus, indirectly or directly. To reveal the detail function of PA4834, the further study is performed at present.

Keywords : *Pseudomonas aeruginosa*, Airway mucus, Siderophore, PA4834, Iron uptake

F029

Enhanced Proliferation of Invasive Bladder Cancer Cell Lines by Infection of KSHV

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Kaposi sarcoma-associate herpesvirus(KSHV) is belonging to the gamma-herpesvirus subfamily. KSHV has been known to be associated with three disease: Kaposi sarcoma (KS), primary effusion lymphoma(PEL), and multicentric Castleman disease (MCD). A recent paper presented that KSHV was detected in about 50% of Croatian bladder cancer patients. To investigate the effect of KSHV to the development or progression of bladder cancer, we infected a recombinant KSHV, BAC16,to five different bladder cancer cell lines, RT4, 5637, T24, HT-1376, and TCC-SUP. RT4 is a papilloma-originated cancer cell line and the others are transitional carcinoma. Except RT4, all cell lines were infected with KSHV as much as primary human endothelial cells and KSHV-infected bladder cancer cell lines were isolated by selection with hygromycin B. The growth rate of the KSHV-infected bladder cancer cells were various depend on each cell line. Interestingly, KSHV-infected invasive bladder cancer cell lines, HT-1376 and TCCSUP, showed a significantly higher proliferation rate than that of uninfected cells. Our results suggests that KSHV might be related with the progression of invasive bladder cancer, though further study should be required.
Keywords : Bladder cancer, KSHV, Proliferation

F030

Seasonality and Relation with Environmental Factors of Norovirus Infection

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Norovirus is cause of acute gastroenteritis in all age. In recent, it was reported that climate change can affect several diseases. Norovirus infection also showed seasonality and affected the change of climate. In this study, we analyzed the accurate seasonality of Norovirus and relation with environmental factors to predict Norovirus prevalence. The data of Norovirus infection was provided from acute diarrhea laboratory surveillance (EnterNet-Korea) during 2011 to 2012. Detection of Norovirus was performed real time RT-PCR in 17 regional Institutes of Public Health and Environments. The collected environment factors were temperate and sunshine in climate factors, move attention and travel volume in population factors. The data offered from the KMA, KOFIC, MLT and KTO, recent two years. A total of 44,110 samples were screened, and 3,287 samples were determined as Norovirus positive. Seasonality of Norovirus infection was peaked in winter season but this was lasted in throughout year. On 2012, Norovirus infection rate was higher but average temperature was lower than 2011. On the more sunshine days, the more people movement such as traffic volume, movie attention, and amount of travel. Norovirus infection was primary affected by cold-weather. However, weather condition such as sunshine was influence people movement, respectively. To consider their secondary factors people movement, society and environment were affect with Norovirus infection.
Keywords : Norovirus, Climate change, Population factors, RT-PCR

F031

Analysis of *Vibrio vulnificus* Septicemia Occurrence with Temperature and Pathogen Isolation rate in Marine Environment

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Vibrio vulnificus (*V. vulnificus*) is a lethal human pathogen that can induce severe septicemia. In this reason, *V. vulnificus* septicemia was designated as a notifiable disease group III since 2000 in Korea. In this study, we analyzed the relation with occurrence of *V. vulnificus* septicemia, temperature of sea water, and isolation rate of *V. vulnificus* from marine environment. Temperature of sea water was provided from Korea Hydrographic Oceanographic Administration (KHOA). We calculated the average temperature of five days in Yeosu tidal station at 10 am. Isolation rate of *V. vulnificus* was provided from VibrioNet and patient data was obtained by website in KCDC. VibrioNet samples were collected by 11 Quarantine Station and 2 regional Institutes of Health & Environment. All data was collected and analyzed during 10 year period (2001-10). Recent data (3 year period 2012-14) was applied into our study. Our results show that the occurrence of *V. vulnificus* infection was closely related with the sea water temperature and isolation rate. Based on the 10 years data, we found that the patients not occurred at average temperature below 18¡ÆC and have already occurred at more than 19°C. The average temperature (18.5°C) was closely related with the begin and termination of *V. vulnificus* septicemia occurrence. However, the isolation rate (7%) was only related with the first occurrence of *V. vulnificus* septicemia. This result may be helpful to predict and prevent of *V. vulnificus* infection.
Keywords : *Vibrio vulnificus*, Septicemia, Marine environmental factors

F032

Determining Ferrichrome-Related Virulence Factors of *Pseudomonas aeruginosa* Biofilms

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Pseudomonas is a genus of bacteria that ubiquitous in the environment and can cause diseases. The most common species of *Pseudomonas* which causes human infections is *Pseudomonas aeruginosa*. *P. aeruginosa* is a gram-negative bacillus and an opportunistic pathogen. It usually causes mild infections in healthy individuals, such as Otitis media but the bacterium can cause very serious infections. For examples, bacteremic pneumonia, endocarditis, meningitis, burn wound infection, and sepsis, which caused by *P. aeruginosa*, are accompanied with very high mortality. Most of these infections are associated with biofilms. *P. aeruginosa* is known to be the powerful biofilm former. Biofilm infections have been getting more attention recently because it is the mode of infection for chronic bacterial infections and capable of causing many serious problems. The problems include evasion of immune defense of hosts and increased resistance against antimicrobial agents.In this experiment, we isolated few candidate genes that have effect on biofilm formation under sub-MIC antibiotics condition, and one of the candidates is the *fiuA*, ferrichrome receptor A, gene. We constructed *fiuA* deletion mutant, and conducted aerobic and anaerobic biofilm formation experiments, siderophore assay, elastase production test, autoinducer production test, and etc. According to the results, we postulated *fiuA* and its signal transduction system may have kin relationship with *P. aeruginosa* virulence
Keywords : Pseudomonas aeruginosa, biofilm, ferrichrome

F033

Incidence of Serovars and Virulence Factors of *Vibrio parahaemolyticus* Isolated from Clinical Specimens and Marine Environment in Korea between 2010 and 2012

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Vibrio parahaemolyticus is a major food-borne pathogen originated from marine environment. Since 1996, serotype O3:K6 and its serovariants are globally important pathogens that caused many outbreaks in the world. In this reason, we investigated sero-prevalence and virulence factors of *V. parahaemolyticus* which isolated from clinical and environmental samples in Korea during 2010 to 2012. Clinical isolates (n=631) were provided from EnterNet-Korea and marine environmental isolates (n=299) were from VibrioNet. The somatic (O) and capsular antigen (K) typing were done by slide agglutination test using commercial antisera and the virulence factors (*tlh*, *tdh*, *trh*, and *orf8*) were tested by PCR. In serotyping, 10 of O serogroups and 87 serovars were confirmed in this study. Environmental isolates divided into 9 of O serogroups and 50 of serovars, while clinical isolates were 10 of O serogroups and 66 of serovars. The pandemic O3:K6 and its serovariants were verified in environmental isolates (4, 1.3%) and clinical isolates (352, 55.8%), whereas pathogenic strains (*tdh* or *trh* genes) were detected in environmental isolates (74, 24.7%) and clinical isolates (572, 90.6%). In conclusion, our result indicates that pandemic and pathogenic *V. parahaemolyticus* were prevalence in Korea and especially clinical strains showed high prevalence of O3:K6 and its serovariants.
Keywords : *Vibrio parahaemolyticus*, O3:K6, Serovariants, *tdh*, *trh*

F034

$RpoB_{127-135}$ Peptide Derived from *Mycobacterium tuberculosis* is Processed and Presented to HLA-A*0201 Restricted CD8+ T Cells Via an Alternate HLA-I Processing Pathway

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Mycobacterium tuberculosis (M. tb) resides and replicates inside macrophages. We previously reported that CD8+ T cell-mediated immune responses specific for the peptide derived from M. tb RNA polymerase beta-subunit ($RpoB_{127-135}$) could be induced in TB patients expressing HLA-A*0201 subtype. In order to examine whether $RpoB_{127-135}$ specific CD8+ T cells can recognize M. tb infected macrophages *in vitro*, CD8+ T cell lines specific for $RpoB_{127-135}$ peptide were generated from peripheral blood mononuclear cells (PBMCs) of healthy HLA-A*0201 subjects by *in vitro* immunization. In this study, we observed $RpoB_{127-135}$ specific CD8+ T cells could recognize and destroy macrophages infected with M. tb for 2 to 4 days. Next, we investigated the HLA-I processing mechanism of $RpoB_{127-135}$ peptide in M.tb infected macrophages. As a result, the presentation of the $RpoB_{127-135}$ peptide, to CD8+ T cells was not inhibited by the treatment with brefeldin-A (Golgi-ER transport inhibitor) or lactacystin (proteasome inhibitor), which blocks the classical HLA-I processing pathway. However, $RpoB_{127-135}$ specific CD8+ T cell activity was blocked either by the blocking agent for the endocytosis (cytochalasin D) or by the blocking antibody (W6/32) for the HLA-I molecules. Therefore, the $RpoB_{127-135}$ peptide may be processed by the alternate HLA-I processing pathway. Understanding the processing and presentation mechanisms of the M. tb derived proteins will help to improve the efficacy of TB vaccines.
Keywords : *Mycobacterium tuberculosis*, $RpoB_{127-135}$ peptide, CD8+ T cells, macrophages, HLA-I processing pathway

F035

Clinical Features and Prevalence of Human Bocavirus Infections in the Patients with Respiratory Infections

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Human Bocavirus(hBoV) cause acute respiratory tract infections, particularly in infants and young children. And have been recently discovered virus. The aim of this study was to analyze the clinical features and molecular phylogeny of hBoV isolated in Busan, from January 2011 to December 2013. Total of 3,230 specimens(throat swabs) were collected from influenza-like illness patients and patients to detect eight respiratory virus¡²rhinovirus, adenovirus, human bocavirus, respiratory syncytial virus, human coronavirus, human metapneumovirus, parainfluenza virus and influenza virus¡³and detected 1,485(46.0%)cases. Among 1,485 positive specimens, 68(2.1%) cases were hBoV. HBoV was detected mainly in late spring and early summer. The 33.8 % of hBoV were found to co-infection with other respiratory virus. Genotypic distributions of hBoV positive specimens were analyzed by sequencing of VP1/VP2 region. HBoV type was identified as all hBoV 1.
Keywords : Acute respiratory infection, Human Bocavirus, Phylogenetic analysis

F036

Functional Characterization of *Cryptococcus neoformans KRE2/MNT1* and *OCiH1* Gene Family encoding Novel Mannosyltransferases involved in *O*-linked Glycans Biosynthesis

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Cryptococcus neoformans is an encapsulated basidiomycete that causes cryptococcosis in immunocompromised humans. Although the cell surface mannoproteins of *C. neoformans* were reported to be involved in fungal pathogenicity, their *O*-glycan biosynthetic pathway has not been elucidated. We had shown that the major *O*-glycans of *C. neoformans* were short manno-oligosaccharides that were connected mostly by α1,2-linkages but connected by α1,6-linkages at the third mannose residue. Here, we report three novel *C. neoformans* genes encoding mannosyltransferases involved in *O*-glycan extensions in the Golgi. *C. neoformans KTR3*, the only homolog of the *Saccharomyces cerevisiae KRE2/MNT1* family genes, was shown to encode an α1,2-mannosyltransferase that is responsible for the addition of the second α1,2-linked mannose residue to the major *O*-glycans without xylose. *C. neoformans HOC1* and *HOC3*, homologs of the *S. cerevisiae OCH1* family genes, were shown to encode α1,6-mannosyltransferases that can transfer the third mannose residue to minor *O*-glycans containing a xylose residue and major *O*-glycans, respectively. Moreover, the *ktr3*Δ mutant strain, which displayed increased sensitivity to cell wall stress, showed attenuated virulence in a mouse model of cryptococcosis. This suggests that the extended structure of *O*-glycans is required for full pathogenicity of *C. neoformans*, thus presenting the potential of Ktr3p as an ideal target for antifungal drug development.
Keywords : *Cryptococcus neoformans*, *O*-mannosylation, Glycan analysis, Cell wall protein, Mannosyltransferases

F037

Regulation of NF-κB Signaling by HCMV-encoded Tegument Proteins UL48 (DUB) and UL45 (inactive RR1)

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The human cytomegalovirus (HCMV) UL48 protein is a large tegument protein that is closely associated with the capsid. UL48 has a deubiquitinating protease (DUB) activity in its N-terminal region. UL48 interacts with another tegument protein UL45, an inactive ribonucleotide reductase R1 subunit (RR1) homolog. Since murine cytomegalovirus RR1 homolog M45 interacted with murine receptor-interacting protein kinase 1 (mRIP1) and inhibited mRIP1-mediated signalings, we investigated the effect of UL48 and UL45 on human RIP1-mediated signaling. UL45 interacted with RIP1 in a RHIM- and ubiquitin-independent manner. UL48 also interacted with RIP1 and deubiquitinated UL45 and RIP1. Overexpression of UL48 and UL45 synergistically decreased the RIP1-mediated NF-κB activation. During the early stage of HCMV infection, tegument UL48 inhibited the TNF-α-mediated NF-κB activation by modulating RIP1 ubiquitination. We produced a recombinant virus encoding HA-tagged UL45 and found that HA-UL45 forms a complex with UL48 and RIP1 in recombinant virus-infected cells. We also found that both UL48 and UL45 target to tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) and inhibit the TRAF6-mediated NF-κB activation. Collectively, our data demonstrate that HCMV UL48 and UL45 tegument proteins play a role in the regulation of NF-κB signaling.
Keywords : HCMV, UL48, UL45, RIP1, NF-κB

F038

Interaction of UL50 and UL53, the Components of the Nuclear Egress Complex of Human Cytomegalovirus, with ISG15

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Interferon-stimulated gene (ISG) 15 encodes a ubiquitin-like protein that is conjugated to a protein lysine residue via the sequential action of E1, E2, and E3 enzymes, which are also induced by interferons. Protein conjugation by ISG15 (ISGylation) inhibits the growth of many viruses. We recently found that the growth of human cytomegalovirus (HCMV) is inhibited by ISGylation. However, the mechanism by which ISGylation inhibits HCMV replication has not been fully understood. In this study, we identified UL50 and UL53 as the potential HCMV-encoded ISG15 targets. UL50 and UL53 are components of the nuclear egress complex (NEC) of HCMV and highly conserved in other herpesviruses. UL50 was isolated as an UBE1L (E1)-binding protein and UL53 was found as an ISG15-binding protein in yeast two-hybrid assays. Both UL50 and UL53 interacted with $ISG15_{AA}$, a conjugation-defective form of ISG15, demonstrating that ISG15 can non-covalently interact with UL50 and UL53. We investigated whether UL50 and UL53 are conjugated by ISG15. We observed that UL50 was covalently conjugated by ISG15 but UL53 was not. Furthermore, we found that UL50 inhibits ISGylation in cotransfection assays. The nuclear rim localization of UL50 was slightly inhibited when $ISG15_{AA}$ was overexpressed or ISG15 was induced by treatment of interferon-β. Our results demonstrate that UL50 and UL53 interact with ISG15 and that UL50 is conjugated by ISG15, suggesting that the NEC of HCMV may be regulated by ISG15.
Keywords : HCMV(human cytomegalovirus), ISG15, NEC(Nuclear Egress Complex), UL50

F039

Identification of Human Cytomegalovirus UL26 as a Target and a Regulator of ISG15 Modification

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Interferon-stimulated gene (ISG) 15 is an ubiqutin-like molecule that is strongly upregulated by type I interferons as a primary response to diverse microbial and cellular stimuli. Studies with several viruses have shown that ISG15 plays an important role in antiviral responses. But, the role of ISG15 and ISGylation during human cytomegalovirus (HCMV) infection has not been fully studied. We recently found that HCMV infection induces ISG15 expression and protein ISGylation and that ISGylation inhibits the viral replication cycle at multiple steps including viral gene expression and virion release. In this study, we performed yeast two-hybrid screening to identify HCMV-encoded ISG15 substrates. UL26 was isolated as an ISG15 and UBE1L-binding protein and an ISG15 substrate. UL26 also showed an inhibitory effect on ISGylation when it was transiently expressed or expressed by retroviral vectors. Consistently, cells infected with the UL26-deleted mutant virus showed a slightly increased ISGylation compared to wild type virus-infected cells. Our results demonstrate that UL26 is a target and a regulator of ISGylation.
Keywords : HCMV, ISG15, UL26

F040

Detection and Characterization of Human Enterovirus Isolated in Busan, 2013

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Human Enteroviruses (HEV) which belong to one group of the Picornaviridae family, can cause clinical symptoms such as aseptic meningitis, herpangina, hand-foot-mouth disease, and poliomyelitis. This study was performed to investigate epidemiological characteristics and diversities of HEV isolated from the pediatric patients with suspected EV infections in Busan from January 2013 to December 2013. Viral RNA was extracted from 1,032 samples of stool, throat swab, or cerebrospinal fluid. Among them 122 positive samples were collected by Real time RT-PCR and VP1 RT-PCR and classified into 17 of different genotypes. Most of HEV isolates was identified as EV71 (23 cases, 18.9%), Echovirus30 (21 cases, 17.2%), and Coxsackievirus B4 (18cases, 14.8%). They were from children <15 years old and positive rates (26.5%) had the highest in 5-9 years old. The positive rates of HEV were 15.8%, 10.4%, and 3.3% in stool, CSF, and throat swab, respectively. The results of sequence analysis of HEV isolates based on VP1 region showed that HEV-A contained 48 isolates including 7 genotypes, HEV-B contained 55 isolates including 10 genotypes.
Keywords : enterovirus, Picornaviridae, real time RT-PCR, genotype, sequence analysis

F041

Arbovirus Surveillance in Mosquitoes Collected from Airports, and Sea Ports during 2013 in the Republic of Korea

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This study investigates the distribution of mosquitoes and presence of arboviruses that cause encephalitis in humans and horses, which were collected as part of the mosquito monitoring program at airports and sea-ports in 2013. A total of 30,318 mosquitoes, representing 18 species and 7 genera were captured and the most frequently collected species was *Culex pipiens* (77.06%, n=23,364). A total of 6,598 mosquitoes were pooled to 584 samples and screened for West Nile virus (WNV), Japanese encephalitis virus (JEV), Eastern, Western and Venezuelan Equine Encephalitis Viruses (EEEV, WEEV and VEEV) by reverse transcription polymerase chain reaction. All of the 6,598 mosquitoes tested were negative except for 2 pooled samples (*Culex pipiens*), which were positive for JEV as confirmed by sequencing of the envelope protein coding genes and pre-membrane genes. WNV, EEE, WEE and VEE have never been reported in Korea and our study continues to support the view that the Republic of Korea is free from these diseases. However as climate change continues to raise the risk of introduction of these arboviruses into the country, continued vector monitoring will be needed to identify areas of risk and quickly detect new introductions.

Keywords : Mosquitoes, Arboviruse, Polymerase Chain Reaction

F042

Mouse Susceptibility to *Vibrio cholerae* Infection is Influenced by Altered Composition of Gut Microbiota

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Mammalian intestine is inhabited by trillions of commensal microbes, collectively termed gut microbiota. Its presence has been known to provide a protection against infections by intestinal pathogens. Molecular basis of such an antagonistic effect, however, remains largely unknown due to the lack of an appropriate model system. Here, we examined the effect of altered microbiota composition on mouse susceptibility to the infection by *Vibrio cholerae*, a well-known human enteric pathogen. When treated with mild concentration of streptomycin (Sm), microbes belonging to the Enterobacteriaceae family, later found to be a clonal expansion of an *Escherichia coli* variant (ECV), were abundantly recovered from the mouse intestine. Unlike the untreated control group, the Sm-treated mice became susceptible to *V. cholerae* colonization with clear manifestation of a cholera-like symptom. Likewise, *V. cholerae* infection occurred more readily in neonatal mice transplanted with the ECV strain, but not in those transplanted with a typical *E. coli* strain. The whole genome sequence of ECV revealed a wide range of atypical properties especially in carbohydrate metabolism and experiments are currently under way to understand its interaction with *V. cholerae* under host gut environment. Our results put a renewed emphasis on the role of gut commensals in regulating the extent of intestinal infections.

Keywords : gut microbiota, *vibrio cholerae* infection

F043

Characterization of Enterohemorrhagic *Escherichia coli* (EHEC) O157 and Non-O157 from Diarrheal Patients in Korea, 2003-2011

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Enterohemorrhagic *Escherichia coli* (EHEC) has been recognized as an important food-borne pathogen that causes several human gastro intestinal illnesses. To characterize the EHEC isolated from patients in Korea according to serotypes, we compared EHEC O157 with the EHEC non-O157 by several categories such as toxin gene, age, symptom and virulence genes. A total of 231 human clinical isolates were collected and separated 47 EHE O157 and 184 EHEC non0O157. In the EHEC O157 strains, *stx1/stx2* was detected most frequently than *stx1* and *stx2*, while *stx1* gene was most frequently than *stx2* and *stx1/stx2* in EHEC non-O157 strains. In children < 9 years of age, the isolation rate of two EHEC groups were higher than that among the other age groups (10~59 and > 60). The major symptoms of EHEC infections were diarrhea, bloody diarrhea and fever. Interestingly, in EHEC O157 group, bloody diarrhea was most common symptom (50%), while that was 16.8% in EHEC non-O157 group. Furthermore, in EHEC O157 group, eight virulence genes, *hlyA* (93.6%), *ehx* (85.1%), *eaeA* (91.5%), *iha* (93.6%), *efa1* (91.5%), *toxB* (91.5%), *tir* (95.7%) and *espA* (93.6%), had a higher frequency of occurrence than EHEC non-O157 group (72.3%, 56.2%, 73%, 69.3%, 66.4%, 57.7%, 63.5% and 63.5%). We confirmed there are some difference between two groups, such as distribution of toxin genes, virulence genes and major symptoms. This study highlights that the EHEC non-O157 is important pathogen, like EHEC O157.

Keywords : EHEC O157, EHEC non-O157, characterization, bloody diarrhea

F044

Serological Analysis of Motile- and Non-Motile Enteroinvasive *Escherichia coli* (EIEC) Strains Isolated in Korea, 2010-2013

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Enteroinvasive *Escherichia coli* (EIEC) cause dysentery by a mechanism that involves the epithelial invasion of the intestine. However, the relationship between the pathogenic mechanism and motility has been not well known. In order to characterization this relatedness, we investigated the profiles of serotypes, molecular characterization and antibiotic resistance of 24 EIEC strains based of motility character. We tested 24 EIEC isolates. The strains represented 7 serotypes, i.e. O124 (16.7%), O96 (16.7%), O28 (12.5%), O152 (8%), O42 (4%), O121 (4%), O164 (4%) and ONT (33%). The most frequent serotypes in the 15 motile strains were O96 (26.7%), O124 (20%), O152 (13.3%), O42 (6.7%), O121 (6.7%), ONT (26.7%), and in the 9 non-motile strains were O124 (11%), O164 (11%), O28 (33%), ONT (44.4%). All of the strains possesed *invE* and *ipaH* genes. The result of antibiotic resistance assay showed that relatively higher resistance to ampicillin and trimethoprim/ sulfamethoxazole (33.3%), while the resistance to gentamycin, ciprofloxacin, chloramphenicol were low (<4%). Interestingly, the resistance to nalidixic acid (22%) was found only in non-motile EIEC strains. In conclusion, EIEC strains are usually non-motile but in our study, motile EIEC strains were detected in 62.5% (15 of 24 strains) and O96, O124, O152, O42 and O121. To confirmation of the relationship between the motility and pathogenic mechanism, the study for comparison of motile- and non-motile EIEC was required.

Keywords : EIEC, motility, serotype, antibiotic resistance

F045

Loop-Mediated Isothermal Amplification for Rapid and naked-eye detection of Carbapenem-Resistant Pseudomonas Aeruginosa and Acinetobacter Baumannii

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Carbapenem-resistant pseudomona aeruginosa and acinetobacter baumannii are one of the leading causes of nosocomial infection at intensive care unit (ICU). Rapid and sensitive detection of CRPA/CRAB infection is in high demand for timely and suitable antibiotic treatment. Here, we optimized a distinct DNA-based diagnostic technique, loop-mediated isothermal amplification (LAMP) for rapid detection of the presence of *blaVIM-2* and *blaIMP-1* gene, a critical component of the gene cluster required for carbapenem resistance. Amplification efficiency of both two genes was optimal at 63 ˚C and with 2 mM $MgSO_4$. In case of *blaVIM-2* LAMP, the detection limit of the DNA template was 1 pg and LAMP amplicons were detected within 25 min. *blaIMP-1* LAMP of the detection limit was 10 pg and reaction took at least 40 min.; thereby suggesting a potential applicability of LAMP as a sensitive and urgent diagnostic method. Furthermore, positive LAMP reaction was directly detected with the naked-eye by monitoring the formation of a white precipitate. Finally, 120 clinical isolates were successfully tested with each *blaVIM-2* and *blaIMP-1* LAMP. The results were compared with PCR which detect same target genes. Together, our results clearly demonstrate the usefulness of LAMP for the diagnosis of CRPA/CRAB infection.
Keywords : LAMP, carbapenem resistance, rapid diagnostic technique, Pseudomonas aeruginosa, Acinetobacter baumannii

F046

Virus-like Particle Vaccine Confers Protection against a Lethal NDV Challenge in Chickens

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In this study, Newcastle disease virus (NDV) virus-like particle (VLP) was developed expressing NDV fusion (F) protein along with influenza virus matrix1 (M1) protein using the insect cell expression system. Specific pathogen-free chickens were immunized with oil-emulsion ND VLP vaccines containing increasing dosages of VLPs (0.4, 2, 10, or 50 μg of VLP/0.5m·dose). Three weeks after immunization, the immunogenicity of the ND VLP vaccines was determined using a commercial ELISA kit, and a lethal challenge using a highly virulent NDV strain was performed to evaluate the protective efficacy of the ND VLP vaccines. ND VLP vaccines elicited anti-NDV antibodies and provided protection against a lethal challenge in a dose-dependent manner. Although the VLP vaccines containing 0.4 or 2μg of VLPs failed to achieve high levels of protection, a single immunization with ND VLP vaccines containing 10 or 50 μg could fully protect chickens from a lethal challenge and greatly reduced challenge virus shedding. These results strongly suggest that utilization of VLP technology could a promising strategy for the better control of various pathogens in poultry species.
Keywords : Vaccine, Virus-like particle, Newcastle disease, Chicken

F047

A Study on Immunity of Hepatitis A in Gwangju Area

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Hepatitis A(HAV) is one of the most common acute viral hepatitis that has been remarkably increasing recently. Mostly, the clinical course becomes better soon, but some cases that show severe symptoms like liver transplantation and even death have been increasing. The change of seroprevalence rate of IgG anti-HAV has been well reported, however, vaccination and seroconversion rate of HAV are not well known. Therefore, we aimed to research the vaccination rate and seroconversion rate in the local community, Gwangju, based on the difference of seroprevalence rate of IgG anti-HAV according to age and sex. A total of 642 blood samples was collected from teenagers and adults between April and June, 2013. The average seroprevalence rate of IgG anti-HAV was 50.6%, and the result of women(51.7%) was higher than of men(48.7%). The results of 1st, 2nd, and 3rd decade were 31.2%, 21.4% and 44.2% respectively while the results of 4th decade and over fifty years old were 88.2% and 96.8%. The average 1st and 2nd of vaccination rate showed very low; 6.9% and 4.8%, and the average seroconversion rate after 1st, and 2nd vaccination were 76.9%, 87.1%. In this study, we can acknowledge the need of catch-up vaccination for teenagers and young adults. Furthermore, it is suggested that HAV vaccination should be considered as a mandatory schedule for immunizing children.
Keywords : Hepatitis A

F048

Greenhouse Evaluation of the Rhizobacteria for the Biological Control of Plant Pathogenic Fungi

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Five rhizobacterial isolates (*Paenibacillus polymyxa* PJ-1, *Alcaligenes faecalis* PJ-2, *Bacillus pumilus* PJ-3, *Bacillus megaterium* PJ-4 and *Bacillus amyloliquefaciens* PJ-5) isolated from different soil samples were evaluated against *Fusarium* wilt of tomato, anthracnose of pepper and white mold of lettuce under greenhouse condition. The results showed that isolate *Bacillus amyloliquefaciens* PJ-5 significantly inhibited *Fusarium* wilt caused by *Fusarium oxysporum* and 70% of the disease suppression was recorded. *Bacillus pumilus* PJ-3 and *Bacillus megaterium* PJ-4 reduced anthracnose disease on foliar and fruits of pepper as compared to pathogen-inoculated control and disease suppression rates of 74.8 and 67.5% were recorded in *Bacillus pumilus* PJ-3 and *Bacillus megaterium* PJ-4, respectively. In lettuce the percentage of diseased plants was significantly lower in *Paenibacillus polymyxa* PJ-1 and *Bacillus amyloliquefaciens* PJ-5 as compared to other isolates.
Keywords : Biological control, disease suppression, rhizobacteria

F049

Etiological and Epidemiological Surveillance of Enteric Virus of Sporadic Acute Gastroenteritis Patients in Gwangu

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Enteric viruses are recognized as the most significant etiological agent of acute gastroenteritis, accounting for approximately 70% of episode. Recently several studies revealed the epidemic changes and genetic variants of these enteric viruses including norovirus. The aim of this study was to provide useful epidemiological data on the gastroenteritis associated with enteric virus. 2,132 samples with acute gastroenteritis collected from 8 medical facilities were examined from January to December 2013. Rotavirus and Adenovirus were screened with an enzyme-linked immunosorbent assay. Astrovirus and Sapovirus were detected with duplex onestep reverse transcription polymerase chain reaction (RT-PCR). One step real-time RT-PCR was used for screening norovirus genogroups GI and GII. Overall, norovirus was detected in 20.6% of stool specimens (440 of 2,132); rotavirus was detected in 20.7% (314 of 2,132); sapovirus was detected in 1.5% (33 of 2,132); adenovirus was detected in 1.1% (23 of 2,132); and astrovirus was detected in 1.0% (22 of 2,132). Norovirus genotying was identified for 407 samples. Fifteen GII genotypes and seven GI genotypes were detected. Genotype GII.4 (47.4%) was the most prevalent, followed by GII.6 (12.3%), GII.2 (8.6%), GII.3 (7.6%), and GII.11 (6.1%). GII.4-2012 variant of GII.4 genotypes most predominantly circulate. Molecular epidemiological knowledge of enteric virus is critical for the development of effective preventive measures, including vaccines.

Keywords : Enteric virus, Norovirus, Genotype, Etiology, Epidemiology

F050

Oral Microbial Profiles in Healthy and Periodontitis Patients

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The oral microbiota presents a symbiotic relationship with the host and disturbances of microbial community may lead to the development of several oral diseases, such as gingivitis, different forms of periodontitis, cares and endodontic infections. Saliva and subgingivalspeimens were obtained from healthy and preriodontis patients. Bacterial culture were performed both in aerobic and anaeronbic condition using blood agar plates for isolate bacteria from saliva and subgingival specimens.The aerobic bacterial numbers in the saliva were 1.6×10^7 CFU/mL and $2.8\text{x}10^7$ CFU/mL in the healthy and periodontitis patients, respectively (P>0.05), and anaerobic bacterial numbers were 2.8×10^7 CFU/mL and 4.1×10^7 CFU/mL in the healthy and periodontitis patients, respectively (P>0.05). On the other hand, the aerobic bacterial numbers in the gum were 3.4×10^5 CFU/mL and 6.7×10^5 CFU/mL in the healthy and periodontitis patients, respectively (P<0.05), and anaerobic bacterial numbers were 3.5×10^5 CFU/mL and 7.9×10^5 CFU/mL in the healthy and periodontitis patients, respectively (P<0.05). There were more alpha-hemolysis producing bacteria both in the saliva and in gum of periodontitis patients compared with healthy subjects. This study might help to elucidate the causing factor of periodontitis.

Keywords : oral microbiota, gingivitis, periodontitis, Saliva, subgingival specimen

F051

Antibacterial Activity of Extracts from Spent Mushroom Substrate on Phytopathogenic Bacteria

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The production of different edible mushrooms in Korea was estimated to be 614,224 ton a year. Generally, about 5 kg of substrate are needed to produce 1 kg of mushroom, and consequently about 25 million tons of Spent mushroom substrate (SMS) are produced as agricultural waste each year in Korea. SMS from different edible mushroom species were screened for antimicrobial activity against the phytopathogenic bacteria, *Pectobacterium carotovorum* subsp. *carotovorum*, *Agrobacterium tumefaciens*, *Ralstonia solanacearum, Xanthomonas oryzae* pv. *oryzae, X. campestris* pv. *campestris, X. axonopodis* pv. *vesicatoria*. *X. axonopodis* pv. *citri, X. axonopodis pv. glycine*. Of different SMSs, water extract from SMS of *Hericium erinaceus* showed a clear inhibition zone against the tested pytopathogenic bacteria. The antibacterial compounds were semipurified from *H. erinaceus* SMC with ethylacetate and butanol. Futhermore, SMS extract of *H. erinaceus* was used to control tomato wilt disease caused by *R. solanacearum*. *Aknowlegement: this research is supported by research grant (Agenda project No. PJ009969) from Rural Department Administration, Suwon, Korea. Scholarship from BK plus project of National research foundation (NRF), Korea was supported for A Min Kwak, Kyong-Jin Min and Sang Su Kim

Keywords : antibacterial activity, disease control, spent mushroom substrate

F052

Suppression of Phytophthora Blight Disease on Pepper by Rye Green Manure Crop

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Green manure crop, rye have been tested to assess usefulness of soil improvement and disease control on pepper soil that was not rotated. This study was conducted to investigate the effects of rye green manure crop on Phytophthora blight and anthracnose. Rye green manure was treated to a Phytophthora-infested soil in fall of the first year of the trial. Pepper seedlings were planted with production crops in the spring (April) of the following year of the trial. Compared to control, pepper grown rye-treated soil had significantly greater stand counts and total biomass, respectively. In addition, pepper grown in rye-treated soils decreased over 20% of Phytophthora blight. The high microbial population (6.3×10^4CFU/g dry soil) of *Peudomonase* spp. was counted on rye-treated soil, while the low bacterial population (5.8×10^3CFU/g dry soil) was detected in control soil. The main *Peudomonase* species inhibited growth of *Phythopthora capsici* on PDA media. The bacteria were identified by16S rDNA sequence analysis. *Aknowlegement: this research is supported by research grant (Agenda project No. PJ009969) from Rural Department Administration, Suwon, Korea. Scholarship from BK plus project of National research foundation (NRF), Korea was supported for A Min Kwak, Kyong-Jin Min and Sang Su Kim

Keywords : phytophthora blight, rye green manure crop, suppression

F053

In Vitro and *In Vivo* Antifungal Activity of *Streptomyces* Species against Dark Mold

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Wood is widely used in construction and furniture due to its natural beauty. Unfortunately, wood quality is influenced by bacterial, algal, and fungal discoloration. Most molds discolor wood by forming masses of pigmented spores on the wood surface. Mold discoloration of coniferous wood is mostly removed by brushing or planning wood surface. However, molds tend to grow and establish mycelia below the surface and into the wood fibers. Therefore, effective and environmentally desirable control strategies have come under worldwide investigation. Using natural extracts from microorganisms is one of the most attractive alternatives to chemical treatment. The aim of this study was to screen *Streptomyces* strain extracts which have antifungal activity against dark mold such as *Alternaria*, *Cladosporium*, *Trichoderma*, and *Aspergillus*. *Streptomyces* sp. was completely able to inhibit all the tested molds *in vitro*. Furthermore, inhibition ability of the extract was determined on sapwood blocks of *Pinus densiflora*. After treatment of the crude extract, the treated-wood samples were inoculated with each mold and mold cocktail suspensions. During 8 weeks of incubation time, no growth of molds on the treated samples was observed, whereas untreated controls were entirely covered with the tested molds within 1 week. Accordingly, we report its potential for use as alternative to chemical fungicides. This is the first report of Streptomyces extracts against dark mold on wood.
Keywords : antifungal activity, crude extract, dark mold, *Streptomyces*, wood

F054

A Critical Role of Defective Viral Genomes Arising *In Vivo* for the Triggering of Innate Antiviral Immunity

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Respiratory virus infection is a critical causative for acute respiratory inflammation, cytokine storm, and acute respiratory distress syndrome. The innate immune response to viruses is initiated when specialized cellular sensors recognize viral danger signals. However, most viruses produce proteins that antagonize and effectively delay signaling by the primary viral oligonucleotide sensor molecules PRR (Pathogen Recognition Receptors), allowing the virus to replicate to high titers and produce large amounts of danger signals prior to host intervention. It is currently unclear how the host immune response overcomes viral evasion to initiate a protective antiviral response. Here we show that truncated forms of viral genomes that accumulate in infected cells potently trigger the sustained activation of the transcription factors IRF3 and NF-κB and the production of type I IFNs through a mechanism independent of IFN signaling. We demonstrate that these defective viral genomes (DVGs) are generated naturally during respiratory infections in vivoeven in mice lacking the type I IFN receptor, and their appearance coincides with the production of cytokines during infections with Sendai virus (SeV) or influenza A virus. Remarkably, the hallmark antiviral cytokine IFNβ is only expressed in lung epithelial cells containing DVGs, while cells within the lung that contain standard viral genomes alone do not express this cytokine. In conclusion, our data indicate that DVGs generated during viral replication are a primary source of danger signals for the initiation of the host immune response to infection.
Keywords : Respiratory virus, Defective viral genome (DVG), Danger signals, Innate antiviral response

F055

Clinical Evaluation of mixture antigens for Diagnosis of Scrub Typhus

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Scrub typhus is classified as one of 3rd class legal infectious diseases in Korea and is caused by the intracellular parasite *Orientia tsutsugamushi,* a Gram-negative bacteria. Symptoms are in common among other acute febrile illnesses causing that doctors have difficulties to differentiate this scrub typhus from others. If without early treatment, the disease is often fatal. Fatal cases have increased to 30%. Therefore, early and accurate diagnosis is very important for the treatment of patient. Currently, the gold standard method for diagnosis is indirect immunofluorescence assay (IFA), but it is difficult to interpret the result, false positive or negative is frequent. ImmuneMed Inc. developed a rapid and accurate diagnostics test for scrub typhus to overcome these difficulties. This kit is based on lateral flow immunochromatographic assay and it takes only 15 minutes to interpret the result.

F056

Development of Susceptible Mouse Model for *Helicobacter pylori* Infection

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Low dietary ascorbic acid has been proposed to negatively influence the clinical outcome of *Helicobacter pylori* infection in human studies. It was reported that ascorbic acid supplementation does not protect L-gulono-c-lactone oxidase-deficient (*gulo*$^{-/-}$) C57BL/6 mice from *H. pylori*-induced gastritis and gastric premalignancy. However, in our previous study, FVB mice were more susceptible to *H. pylori* infection than C57BL/6. In order to improve the experimental animal model to analyze whether dietary ascorbic acid would influence the outcome of *H. pylori* infection, we generated a FVB *gulo*$^{-/-}$ by backcross breeding of C57BL/6 *gulo*$^{-/-}$ and FVB wild type (*gulo*$^{+/+}$) in this study. We compared gastric colonization levels of *H. pylori* in *H. pylori*-infected *gulo*$^{-/-}$ mice supplemented with low (330mg/L) or high (3,300mg/L) ascorbic acid in drinking water for 16 or 32 weeks. This mouse model would be a useful tool for understanding of pathophysiological roles of *H. pylori* in the development of gastric disorders.
Keywords : *Helicobacter pylori*, ascorbic acid, L-gulono-c-lactone oxidase, gastric colonization level

F057

Outbreak of SHV-12-producing Enterotoxigenic *Escherichia coli* O64 Associated with Imported Kimchi in Republic of Korea, 2013

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During November and December 2013, Korea National Institute of Health investigated a foodborne outbreak of extended spectrum β-lactamase (ESBL)-producing enterotoxigenic *Escherichia coli* (ETEC) O64:H- at the four clusters (training center for teenager, packaged lunch store, meat buffet restaurant and Theological university) in Incheon city. Association between ESBL-producing ETEC O64:H- and specific food items was assessed among the four clusters. The ETEC isolates submitted from Incheon Research Institute of Public Health & Environment were characterized by pulsed-field gel electrophoresis (PFGE). All isolates with ESBL phenotypes were analyzed by PCR and nucleotide sequencing for the *bla* genes. Fifty-nine with illnesses were identified from the four clusters. All of patients consumed Kimchi and it was imported from China. Five ESBL-producing ETEC O64:H- were also isolated from imported Kimchi. All sixty-four ESBL-producing ETEC were identified as the SHV-12 ESBL and ETCX01.096 PFGE pattern.This study is the first report about ESBL-producing ETEC O64:H- outbreak resulted from consumption of imported Kimchi in Korea. Unheated food can cause food-poisoning highly. Although there is the recommendation about using fermented Kimchi, restaurant and school feeding facilities offer unfermented Kimchi. Therefore, food company should provide fermented or cooked condition food and customer is also needed to get some education for the risk of unfermented Kimchi.
Keywords : outbreak, enterotoxigenic *E. coli*, ESBL, SHV-12, imported food

F058

Occurrence of Extended-Spectrum β -Lactamases-producing *Salmonella* enterica serovar Typhimurium in the Republic of Korea, 2009 to 2012

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Salmonella spp. was routinely monitored for antimicrobial resistance by the laboratory-based surveillance. We carried out the prevalence for *S.* Typhimurium producing extended-spectrum ß-lactamases (ESBLs) in korea. A total of 358 clinical strains isolated from 2009 to 2012 were screened for the resistance to extended-spectrum cephalosporins and sequence analysis of the ESBL encoding *bla* genes was carried out. In the selected ESBL producing isolates were characterized by pulsed field gel electrophoresis (PFGE). Thirteen *S.* Typhimurium isolates were ESBL-positive, based on the synergistic effects between clavulanate and selected extended-spectrum cephalosporins. Sequence analysis of *S.* Typhimurium isolates revealed that they harbored bla_{TEM} (ten isolates) and $bla_{CTX\text{-}M\text{-}15}$ (seven isolates). *Xba*I-digested PFGE patterns of ESBL-producing *S.* Typhimurium isolates were classified into ten patterns and these patterns were different from each other.The emergence of ESBL production in *S.* Typhimurium could become a serious threat to public health. More active surveillance and effective controls for salmonellosis are clearly needed to minimize the spread of ESBL-producing *S.* Typhimurium isolates.
Keywords : *Salmonella* Typhimurium, antimicrobial resistance, ESBL, CTX-M-15, PFGE

F059

RNA Helicase A/DHX9 is Critical for DNA Virus-Induced Innate Immune Responses

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RNA helicase A (RHA) or known as aspartate-glutamate-alanine-histidine (DExD/H)-box helicase 9 (DHX9) has been characterized as a transcriptional regulator, consistent with its mostly nuclear localization in various cell types. Recently, RHA/DHX9 was reported to act as a cytosolic B-form DNA sensor in plasmacytoid dendritic cells. In this study, we investigated the role of RHA/DHX9 on a DNA virus, murine gammaherpesvirus 68 (MHV-68) infection in fibroblast and epithelial cells. Overexpression of RHA/DHX9 in HEK293T cells potentiated MHV-68 triggered activation of NF-κB and transcription of antiviral genes and inhibited MHV-68 replication. Knockdown of endogenous RHA/DHX9 in both NIH3T3 and HeLa cells significantly enhanced MHV-68 replication, as lower levels of type I interferon and proinflammatory cytokines were produced. The helicase domain, but not the DNA sensing domain of RHA/DHX9 was required for virus-induced NF-κB activation. Endogenous RHA was able to physically interact with both endogenous NF-κB/p65 subunit as well as RNA polymerase II (RNAP II) during virus infection. In RHA knockdown cells, interactions between NF-κB/p65 and RNAP II were abolished. Taken together, our results demonstrate a critical role of nuclear RHA/DHX9 in the innate immune response against MHV-68 infection, by functioning as a transcriptional co-activator that mediates the interaction of NF-κB/p65 and RNAP II to activate expression of antiviral genes.
Keywords : RHA, NF-κB, DNA virus, Innate immune response

F060

Construction of a FLAG-tagged Recombinant Virus to Identify Cellular Proteins Interacting with a Viral Immune Modulator

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Gammaherpesviruses, including Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV), are important pathogens as they are associated with various malignancies. Murine gammaherpesvirus 68 (MHV-68) is a natural pathogen of small animal model system for the study of human gammaherepsviruses due to its high homology in genome sequences and amenable experimental systems both in vitro and in vivo. Previously, we identified MHV-68 ORF11 as a viral immune modulator that inhibits production of type I interferon (IFN) via interacting with TANK binding kinase 1 (TBK1). In this study, we constructed a recombinant MHV-68 virus expressing 3×FLAG tagged ORF11 (FLAG-ORF11/MHV-68) using by lambda Red-mediated homologous recombination. The viral genome integrity of FLAG-ORF11/MHV-68 was confirmed by restriction enzyme mapping and its replication kinetics was similar to that of WT. When murine embryonic fibroblast (MEF) cells were infected with FLAG-ORF11/MHV-68, interaction and co-localization of ORF11 with endogenous TBK1 was confirmed during virus replication. To further identify cellular proteins interacting with MHV-68 ORF11, a pull-down proteomics approach was taken and candidate proteins were analyzed by mass spectrometry (MS) analysis. Our pull-down proteomics results will contribute to our understanding of how MHV-68 ORF11 may modulate the host immune responses.
Keywords : MHV-68, ORF11, Recombinant virus, Proteomics

F061

Influenza A Virus NS1 Proteins Suppress the NLRP3 Inflammasome

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Inflammasome is a molecular platform that stimulates the activation of caspase-1 and processing of pro-interleukin 1β (pro-IL-1β) and pro-IL-18 for secretion. NOD-like receptor family, pyrin domain containing 3 (NLRP3) is activated by diverse molecules and pathogens, leading to the formation of NLRP3 inflammasome. Recent studies showed that NLRP3 inflammasome mediated innate immune responses against influenza A virus infection. In this study, we investigated the function of the non-structural protein 1 (NS1) in modulating NLRP3 inflammasome. NS1 proteins derived from both a highly pathogenic strain and a non-pathogenic strain, efficiently decreased secretion of IL-1β and IL-18 from THP-1 macrophage cells treated with an NLRP3 agonist. NS1 significantly impaired the transcription of proinflammatory cytokines by inhibiting transactivation of NF-κB, a major transcription activator. Furthermore, NS1 physically interacted with NLRP3 and activation of the NLRP3 inflammasome was suppressed in NS1 expressing THP-1 macrophage cells. Our results suggest that NS1 down-regulates NLRP3 inflammasome activation by targeting NLRP3 as well as NF-κB, leading to down-regulation of inflammatory cytokines as a host immune evasion strategy.

Keywords : NS1, Influenza A virus, NLRP3, Inflammasome

F062

Virulence Characteristics of Clubroot Pathogen, Plasmodiophora Brassicae on Commercial Varieties of Kimchi Cabbage in Korea

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Clubroot is a disease characterized by gall formation on roots of cruciferous crops, caused by Plasmodiophora brassicae. We collected galls of clubroot in Pyeongchang(D1, D2 isolate) and Asan(B1, B2 isolate) in each of the two kimchi cabbage fields, which are the representative cultivating areas of kimchi cabbage in Korea. This study tested clubroot pathogen, P. brassicae from collected galls have different pathogenicity on 11 commercial varieties(CRmatt baechu, Bulam No. 3, Chungwang, Tamsrun baechu, Chunjeong, Alchandeul, Gaulmatt baechu, Bulam plus, Huiparamgold, Huangboc baechu, Norangbom baechu). The result was shown various patterns about clubroot symptom. B2 isolate generated a big size galls infected on all varieties of kimchi cabbage, while B1 isolate did not cause by clubroot disease on near varieties of kimchi cabbage tested. And D1 isolate formed galls much smaller than B2 isolate. In case of Pyeongchang isolate(D1, D2), CRmatt baechu, Bulam No. 3, Chungwang and Tamsrun baechu actually showed resistance, whereas the other varieties were susceptible on the same pathogens. Furthermore this could be provided that P. brassicae had various pathogenicity level on commercial varieties of kimchi cabbage in Korea, although collected pathogens from the same area were identified as same P. brassicae. Based on this result, it will be a useful information to select disease resistance varieties of kimchi cabbage.

Keywords : clubroot, Plasmodiophora brassicae, varieties, Virulence

F063

Structure-based Mutational Analysis of ORF49, a Virion Protein that Facilitates Gammaherpesvirus Lytic Replication

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Gammaherpesviruses such as Epstein-Barr virus, Kaposi's sarcoma-associated herpesvirus, and murine gammaherpesvirus 68 (MHV-68) are associated with various tumors. The ORF49 protein is conserved among gammaherpesviruses and shown to be a virion protein. ORF49 promotes the transcription activity of RTA, a master switch molecule for lytic replication. Our previous results showed that ORF49 directly bound to RTA as well as its negative cellular regulator, poly (ADP-ribose) polymerase-1 (PARP-1), disrupting the interaction between RTA and PARP-1. Here we generated multiple mutant constructs of ORF49, based on its X-ray crystal structure information and analyzed their effects on ORF49 function and interactions. Transcomplemetation of ORF49 deficient virus with mutants further confirmed the functional role of individual mutants. The results from the mutant studies showed the important domain of ORF49 for its function and interactions with PARP-1 and/or RTA. Taken together, our study highlights the importance of virus-host interactions in regulating virus lytic replication.

F064

Antimicrobial Immune Response in the Absense of HVEM

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Sepsis remains the leading cause of death in most intensive care units. Advances in understanding the immune response to sepsis provide the opportunity to develop more effective therapies. The herpes virus entry mediator (HVEM) is recently described as a molecular switch that plays different roles in immune response. However, the role of HVEM in the innate immune response to sepsis has not been addressed clearly. Here we show that $HVEM^{-/-}$ mice have significantly enhanced survival, bacterial clearance and cell infiltration than WT mice after polymicrobial sepsis induced by cecal ligation and puncture (CLP). The depletion of macrophages but not neutrophils induced HVEM KO mice susceptible to CLP as much as wild type mice, indicating macrophages are critical mediators in this model. Notably, we show here that the deficiency of HVEM prevents the downregulation of CCR2 induced by G-protein–coupled receptor kinase 2 in monocytes after CLP. Our data indicate that HVEM-mediated stimulatory signals aggravate polymicrobial sepsis and suggests a potential role for therapeutic blockade of HVEM in this devastating disorder.

Keywords : HVEM, sepsis, CLP, monocytes, inflammatory response

F065

Antiviral Mechanism of DBM2198 and AZPSONs against HIV-1 and its Variants

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DBM-2198, a six-membered azasugar nucleotide (6-AZN)-containing phosphorothioate (P=S) oligonucleotide (AZPSON), was featured in our previous report,(Lee et al., 2005) with regard to its antiviral activity against a broad spectrum of HIV-1 variants. This report describes the mechanisms underlying the anti-HIV-1 properties of DBM-2198. The LTR-mediated reporter assay revealed that the anti-HIV-1 activity of DBM-2198 is likely attributed to extracellular activity rather than intracellular sequence-specific antisense activity. Nevertheless, the antiviral properties of DBM-2198 and other AZPSONs were highly restricted to HIV-1. Unlike other P=S ONs, DBM-2198 caused no host cell activation during its administration into cultures. Once HIV-1 was pre-incubated with DBM-2198, virus did not show any infectivity to host cells, while host cells pre-incubated with DBM-2198 remained susceptible to HIV-1 infection, suggesting that DBM-2198 acts on the virus particle rather than cell surface molecules for the inhibition of HIV-1 infection. Affinity competition assays with anti-gp120 and anti-V3 antibodies revealed that DBM-2198 acts on the viral attachment site of the HIV-1 gp120, but not on the V3 region. This report provides a better understanding of the antiviral mechanism of DBM2198 and may contribute to further study of DBM-2198 for the development of a potential therapeutic drug against a broad spectrum of HIV-1 variants.
Keywords : DBM-2198, AZPSON, HIV-1, antiviral mechanism, gp120

F066

Development of *Leptospira* Rapid Kit

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Leptospirosis is one of worldwide zoonotic diseases. Leptospirosis is caused by infection with *Leptospira* serovars, which occurs as an acute febrile illness in human and animals. Symptom of leptospirosis is very similar to other acute febrile illness, such as scrub typhus, murine typhus, dengue fever and other viral hemorrhagic fevers. So it is difficult to differentiate from them. The currently used standard method of leptospirosis diagnosis is microscopic agglutination test (MAT), detecting antibody serologically against the *Leptospira* in a clinical specimen. However, the MAT is a complex test that requires a large panel of live-cell suspensions, specialized and expensive equipments and can be applied only by trained personnel. Therefore we developed a rapid and simple diagnosis kit with high sensitivity and specificity. The positive result was indicated by the formation of a red line within 15 min after sample application. The sensitivity of this diagnosis kit was 100% with 11 serum samples obtained from leptospirosis patients. The specificity were tested with healthy (n=16) and other acute febrile diseases (n=23). In 16 cases of healthy person`s serum, results were all negative. Among other febrile diseases sera, there were positive for 2 cases. Therefore, specificity of the diagnosis kit was about 95%. These results suggest this kit can be applied to potential rapid and precise diagnosis to leptospirosis.
Keywords : Rapid Kit, Diagnosis, Leptospirosis

G001

Protection against *Vibrio Vulnificus* Infection by Active and Passive Immunization with the C-terminal Region of the RtxA1/MARTX$_{Vv}$ Protein

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Vibrio vulnificus is a foodborne pathogen that is prevalent in coastal waters worldwide and causes septicemia with high fatality rates exceeding 50%, even with potent antibiotic therapy. Several vaccine studies have been attempted to prevent *V. vulnificus* infection with limited success. In this study, we identified a C-terminal region (amino acids 3491 to 4701) of the *V. vulnificus* multifunctional autoprocessing RTX (MARTX$_{Vv}$ or RtxA1) protein, RtxA1-C, as a promising antigen to induce a protective immune response against *V. vulnificus*. Vaccination of mice with recombinant RtxA1-C protein with adjuvant elicited a robust antibody response and a dramatic reduction of blood bacterial load in mice infected intraperitoneally, which resulted in significant protection against lethal challenge with *V. vulnificus.* Furthermore, intraperitoneal passive immunization with serum raised against the recombinant RtxA1-C protein demonstrated marked efficacy in both prophylaxis and therapy. These results suggest that active and passive immunization against the C-terminal region of the RtxA1 protein may be an effective approach in the prevention and therapy of *V. vulnificus* infections.
Keywords : *Vibrio vulnificus*, RtxA1/MARTX$_{Vv}$, Vaccine, Passive immunization, Therapy

G002

Prostate Apoptosis Response-4 Is Associated with Macrophage Apoptosis during Mycobacterial Infection

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Prostate apoptosis response-4 (par-4) is a tumor suppressor protein and ubiquitously expressed. It is known to cause apoptosis via caspase activation, and interaction between par-4 and glucose regulated protein 78 (GRP 78) is essential for apoptosis. Previously, we reported that ER stress-induced apoptosis plays a critical role as a host defense mechanism against Mycobacterium tuberculosis (Mtb).In this study, we have tried to understand the role of par-4 in ER stress-induced apoptosis. To determine the expression of par-4 in M. tuberculosis H37Ra-infected macrophages, we examined the induction of par-4 by western blot analysis. We found the expression of par-4 was induced by Mtb H37Ra infection, and induction of GRP 78 was measured at the same time point. Next, we investigated whether par-4 binds GRP 78 because binding par-4 to cell surface GRP 78 leads to apoptosis induction. As expected, par-4 was colocalized with GRP 78 in Mtb H37Ra infected macrophages. As macrophages induce apoptosis during Mtb infection to control intracellular survival of Mtb, we examined whether expression of par-4 decreased replication of Mtb H37Ra in macrophages. The Raw 264.7 cells were transfected with par-4 siRNA before Mtb H37Ra infection, and then evaluated the intracellular growth of Mtb H37Ra. Interestingly, the intracellular survival of Mtb H37Ra was increased in par-4 siRNA treated macrophages. These data suggested that par-4 plays a crucial role in the pathogenesis of tuberculosis.
Keywords : *Mycobacterium tuberculosis*, Par-4, GRP 78, ER stress, Apoptosis

G003

Intranasal Administration of *Lactobacillus* Species Isolated from Kimchi and bean-paste Facilitates Protection against Influenza Virus Infection in Mice

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Influenza virus infections continue to be a significant public health problem. Recently new subtypes of influenza virus represent a global pandemic threat. For improved therapies and preventive measures against influenza, the need for a broad-spectrum antiviral therapeutics such as probiotics has been increased. In this study, we assessed the protective efficacy of 15 *Lactobacillus* species isolated from kimchi and bean-paste, the Korean fermented foods, against lethal challenge with influenza virus in mice. The survival rate of mice receiving intranasal administration of live *Lactobacillus* species ranged from 10% (*Lactobacillus C*) to 90% (*Lactobacillus L*). And mean death time of *Lactobacillus* administered group was more prolonged than control group. However, there was no significant difference in body weight loss among all challenged mice. This result indicated that intranasal administration of live *Lactobacillus* species of Korean fermented foods could provide protection against influenza virus infection, and there were huge differences in protective effects of various *Lactobacillus* strains on influenza virus infection.
Keywords : *Lactobacillus*, Influenza virus, antiviral, kimchi, bean-paste

G004

Synergistic Effect of Oleanolic Acids on Aminoglycoside Antibiotics against *Acinetobacter baumannii* ATCC 17978

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Oleanolic acid (OA), a natural pentacyclic triterpenoid, has hepatoprotective, antitumor, anti-obesity and weak anti-HIV, HCV activities. OA appeared to decrease motility and generate free radicals in *A. baumannii* ATCC 17978. Fractional inhibitory concentration (FIC) measurement demonstrated that OA has synergistic effect only with aminoglycoside-antibiotics. Other antibiotics (ampicillin, rifampicin, norfloxacin, and tetracycline) have additive effect with OA. Our microarray and qRT-PCR confirmed that ATP synthesis, cell membrane permeability, glycosyltransferase, peptidoglycan-related and phage-related genes and DNA repair genes were up-regulated under OA. . Deletion of highly induced genes: *adk*, encoding an adenylate kinase and *des6*, encoding a linoleoyl-CoA desaturase, increased FIC showing that adk and des6 genes contributed to synergistic effect of OA with aminoglycosides. 8-anilino-1-naphthalenesulfonic acid (ANS), fluorescence-conjugated gentamicin (gentamicin–Texas Red, GTTR) and FAME analysis suggested that those genes (*adk* and *des6*) are involved in change of membrane permeability. Proton motive force (PMF) analysis and ATP synthesis test showed that those genes are involved in energy metabolism. Taken together, our data showed that the OA boosts up aminoglycoside uptake by changing membrane permeability and increasing energy for gentamicin uptake in *A. baumannii*.
Keywords : Acinetobacter baumannii, antibiotics, synergism with antibiotics, triterpenoid, plant extract

G005

Mycobacterium Abscessus MAB2560 Induces Maturation of Dendritic Cells Via Toll-like Receptor 4 and Drives Th1 Immune Response

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In this study, we showed that Mycobacterium abscessus MAB2560 induces the maturation of dendritic cells (DCs), which are representative antigen-presenting cells (APCs). M. abscessus MAB2560 stimulate the production of pro-inflammatory cytokines [interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-1β, and IL-12p70] and reduce the endocytic capacity and maturation of DCs. Using TLR4-/- DCs, we found that MAB2560 mediated DC maturation via Toll like receptor 4 (TLR4). MAB2560 also activated the MAPK signaling pathway, which was essential for DC maturation. Furthermore, MAB2560-treated DCs induced the transformation of naïve T cells to polarized CD4+ and CD8+ T cells, which would be crucial for Th1 polarization of the immune response. Taken together, our results indicate that MAB2560 could potentially regulate the host immune response to M. abscessus and may have critical implications for the manipulation of DC functions for developing DC-based immunotherapy
Keywords : Dendritic cells, Th1 polarization, MAPKs, Mycobacterium abscessus, MAB2560

G006

New *Tuberculosis* Vaccine Candidate Rv20xxc Strong *BCG* Boosting and Effective Protection against *Mycobacterium tuberculosis*

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With *Mycobacterium tuberculosis* continuing to be a major cause of global morbidity and mortality, a new vaccine is urgently needed. *Mycobacterium Tuberculosis* subunit vaccines have been shown to induce robust immune response. In this study, we investigated the protective efficacy of Rv20xxc novel *Mycobacterium tuberculosis* antigens using intratracheal mouse model of pulmonary tuberculosis. These antigens were tested as subunit vaccines formulated in dimethyl dioctadecyl ammonium bromide (DDA) – D(+) with monophosphoryl lipid A(MPLA) (DDA/MPLA) adjuvant administered alone as monovalent vaccines or in combination. were shown to consistently and significantly reduce bacterial burdens in the lungs of mice relative to non-vaccinated controls. This subunit protein boosting of *BCG*-primed immunity will be able to stimulate the known immune correlates of protective immunity against M. tuberculosis. TH1 cells mediated immune responses with cytokines such as elevated levels of IFN- α and TNF- α, IL-2. These findings suggest that Rv20xxc is a promising candidate subunit vaccine to enhance the protective efficiency of BCG.
Keywords : *Mycobacterium tuberculosis*, *BCG*, subunit vaccine, cytokine, monophosphoryl lipid A

G007

IgA Antibody Response Against Mycobacterial Antigens for Diagnosing and Screening *Mycobacterium* Bovis Infection in Cattle

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Antibody responses are useful indicators of *Mycobacterium bovis* infection in cattle. Many studies have evaluated the ability of IgG to serodiagnose bovine tuberculosis (TB). Here, we compared IgA and IgG responses against the MPB70 and MPB83 antigens of *M*. bovis, a 38 kDa antigen that is a well-known serodiagnostic *M. tuberculosis* antigen, and a newly identified Rv14xxc protein in *M. bovis*-infected and non-infected cattle as well as in field samples. The diagnostic utility of the IgA antibody to MBP70 and MPB83 in bovine TB was superior or comparable to that of the IgG antibody, and sensitivity for serodiagnosis increased when the results of antigen binding by IgA and IgG were combined. However, sensitivity of the IgA antibody to the 38 kDa protein was significantly lower than that of IgG. Sensitivities of both antibodies to Rv14xxc were significantly lower than those of other antigens, and no diagnostic utility for Rv14xxc was observed in field samples. Finally, the IgA antibody reacted strongly to the MPB70 and MPB83 antigens and discriminated cattle with TB from healthy cattle in a multiantigen printed immunoassay. Our results support the feasibility of using an IgA antibody against the MBP70 and MPB83 antigens to detect bovine TB. In addition, applying it with the IgG antibody may increase detection accuracy.
Keywords : Bovine tuberculosis, IgA antibody response, serodiagnosis

G008

***Mycobacterium Avium* Complex MAV20XX Induces the Apoptosis in Murine Macrophage RAW 264.7 Cells**

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Mycobacterium avium complex causes (MAC) disseminated infection in AIDS patients and several forms of infection in immunocompetent hosts. Recent studies have shown that *M. avium* infection of macrophages *in vitro* leads to apoptosis of significant numbers of infected cells. MAC and their sonic extracts induce a macrophage apoptosis. However, any components of MAC that are involved in inhibiting or triggering apoptosis are not identified. Recently we identified the MAV20XX protein fractionation of *M. avium* culture filtrate protein using multistep chromatography. In this study, we investigated the biological effects of MAV20XX on murine macrophage RAW264.7 cells. MAV20XX protein induced significant macrophage apoptosis via activation of caspase 3 and caspase 9, and poly (ADP-ribose) polymerase cleavage. Enhanced ROS production and Apoptosis signal-regulating kinase 1 (ASK1), JNK activation were essential of MAV20XX-medicated apoptosis and induced IL-6, TNF, and MCP-1 production. In addition, MAV20XX is targeted to mitochondria. Dissipation of the mitochondrial transmembrane potential ($\Delta\Psi_m$) and depletion of cytochrome *c*, in macrophages. Taken together, our data suggest that MAV20XX may act as a strong pathogenic factor to cause apoptosis of murine macrophage RAW264.7 cells.

G009

Dendritic Cell Vaccine to Foot-and-Mouth Disease is Likely Applied to National Contingency Plan Early In Infection

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It was thought that vaccination may be meaningful even in early infection if development of disease starts 1-2 days after vaccination. However, no routine vaccination have not solved the problem because the vaccines designed to elicit humoral immune response at least 7 days after inoculation of vaccine. But modern vaccinology of therapeutic vaccination to cancer may allow the protection even in early infection otherwise infected animals are diseased. Humoral immunity against foot-and-mouth disease plays important rolls in removing residual virus. If animals including pigs are generally vaccinated before infection, those animals are protected by neutralizing antibody but not before development of neutralizing antibody. Therefore it is important to keep pigs from protecting FMDV for 2-3 days because massive vaccination increases neutralizing antibody. If neutralizing antibody would appear 2-3 days earlier than their usual appearance, FMDV may be neutralized. To see whether DC vaccine induce neutralizing antibody within 2-3 days after infection, we infected DC line with Ad5FMDV and those DCs were analyzed with FACS for expressing FMDV-VP1 protein and antibody titer for humoral immune responses. We found that ELISA antibody to FMDV was appearing 3 days after infection having as much as PI of 20. It is suggested that DC vaccine against FMDV may be applied to contingency plan since the delayed response against FMDV early in infection may contribute to the acute infection.

Keywords : Foot-and-mouth disease, Adenoviral vector, Dendritic cell, humoral immunity, ELISA

G010

Anti-inflammatory Potential of Ursolic Acid in *Mycobacterium tuberculosis* Sensitized and Concanavalin A Stimulated Cells

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Ursolic acid (3-beta-3-hydroxy-urs-12-ene-28-oic-acid, UA) is a triterpenoid carboxylic acid with various pharmaceutical properties and it is commonly found in apples, basil, berries, rosemary, peppermint, lavender, oregano, thyme, hawthorn and prunes. Here, we studied the effect of UA on *Mycobacterium tuberculosis* H37Rv induced release of a panel of inflammatory cytokines (*TNF-α*, *IL-1β* and *IL-6*) from murine macrophages, raw 264.7; alveolar epithelial, A549 cells, and also in concanavalin A (con A) stimulated rat splenocytes. We also tested UA for its ability to reduce the expression of inflammatory mediators, cyclooxygenase 2 (*COX-2*) and inducible nitric oxide (*iNOS*) in stimulated cells. In addition, reduction of nitric oxide (NO) release by UA was also examined in stimulated cells. Interestingly, UA significantly inhibited cytokines *TNF-α*, *IL-1β* and *IL-6* mRNA expression in stimulated cells. The expression of *COX-2* and *iNOS* was also suppressed by UA, as well as NO release at significant level. This study represents the powerful effects of UA on different cell types, which may contribute to the development of an anti-inflammatory drug. In case of adjunct host-directed immune therapy for tuberculosis, UA may be used along with established antibiotic therapies to improve treatment efficacy and outcome due to their anti-inflammatory potential. However, further in detail studies are required to establish their potential in anti-inflammation.

Keywords : Ursolic acid, inflammatory cytokines, nitric oxide, *tuberculosis*, concanavalin A

G011

Anti-mycobacterial activity of tamoxifen against drug-resistant *M.tuberculosis*

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Tuberculosis (TB), one of the world's major health problems, has become more serious due to the emergence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) TB. In the hope of developing new classes of antibiotics that could overcome these resistant mechanisms, a variety of screening studies for natural products and commercial drugs have been conducted in attempt to find anti-mycobacterial substances. In this study, we tested 150 of single compounds derived from plants and commercial drugs for their anti-mycobacterial activity against TB H37Ra and found that tamoxifen has anti-mycobacterial activity against TB. In the following experiments conducted with 7 clinically isolated drug-resistant TB, tamoxifen showed 2- to 8 fold stronger activities against MDR and XDR TB compared to isoniazid or rifampin, which is first line drug for TB. In addition, the effects of combinations of tamoxifen with Isoniazid and rifampin against MDR and XDR TB were evaluated according to the calculated fractional inhibitory concentration (FIC) index. Synergism was detected in both of MDR and XDR between tamoxifen and isoniazid (FIC index: 0.265 and 0.32, respectively) but not rifampin. Altogether, our results suggest that tamoxifen might become an alternative anti-mycobacterial agent for the treatment of tuberculosis.

Keywords : Tuberculosis, MDR, XDR, Tamoxifen, FIC

G012

Mycobacterium Fortuitum-Induced Proinflammatory Cytokine Generation is dependent on Nuclear Factor-kappaB Activation and Intracellular Calcium Signaling In Macrophages

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Mycobacterium fortuitum (MF) is one of facultative-pathogenic nontuberculous mycobacterial strains, however, the molecular mechanisms by which MF induces proinflammatory cytokine production in macrophages have not been widely known. In mycobacterial infection, nuclear facter (NF)-κB signaling pathway plays a key role in the induction of inflammatory cytokine generation and antimicrobial protein synthesis in macrophages. In addition, previous studies have revealed that intracellular calcium signaling contributed to phagosome maturation in mycobacterial infection. In this study, we determined whether MF activate NF-κB and intracellular calcium signaling, both of which are required for inflammatory cytokine generation in macrophages. We found that Mf infection strongly up-regulated NF-κB reporter gene activities in bone marrow-derived macrophages (BMDMs). In addition, MF-induced tumor necrosis factor (TNF)- α and interleukin (IL)-6 production was dose-dependently abrogated in BMDMs by pretreatment with Bay 11-7085 or caffeic acid phenethyl ester, specific inhibitors of the NF-κB signaling pathway. Furthermore, MF-mediated TNF-α and IL-6 expression was dose-dependently inhibited by 1,2-bis-(2-)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid acetoxymethyl ester (BAPTA, AM), the intracellular calcium chelator. From the above results, we report that MF-induced NF-κB and calcium signaling activation is essentially required for proinflammatory cytokine generation in BMDMs.

Keywords : *Mycobacterium fortuitum*, N

G013

Generation of *Burkholderia pseudomallei* Expressing Luminescence and Fluorescence with Dual Reporter Plasmid DNA

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Burkholderia pseudomallei is a high-risk pathogen of gram-negative bacteria which can cause Melioidosis, severe infectious disease by infection with low CFU of the bacteria. With its low infection dose for pathogenicity, *B. pseudomallei* could be considered as a bio-weapon. Conventional analytic methods currently used for *B. psuedomallei* study cannot provide enough information about the time and location during infection to understand more detail of bacterial distribution in mouse. In this study, we established new analytic system which can provide the spatiotemporal information at organism level using fluorescent/luminescent dual-labeled bacteria. We constructed dual-labeled *B. pseudomallei* which express both fluorescent and luminescent signals for pathogen visualization, and analyzed each organ using non-invasive *in vivo* imaging method after infection with *B. pseudomallei*. We have utilized the backbone of the vector pBHR1, a derivative of the pBBR1 plasmid that was originally isolated from *Bordetella bronchiseptica*, to construct a set of vectors useful for gene expression in *B. pseudomallei*. Mice infected with 10^6 CFU of *B. pseudomallei* pBHRgfp-lux intraperitonealy were analyzed at 24hr after infection and signal of fluorescent/luminescent were observed in lung, liver and spleen. This newly developed imaging method using dual-signal strain of *B. pseudomallei* could provide the detail of bacterial distribution and sequential relocation for the pathogenesis in infected mouse.

Keywords : *Burkholderia pseudomallei*, fluorescent/luminescent, *in vivo* imaging

G014

Enhanced Antibody Neutralization against Botulism by Apoptotic Peptide Conjugates

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Botulinum neurotoxin (BoNT) is known to the most toxic substances to humans. It is a potential biowarfare threat and public health hazard. Here, we report practicable therapy consisted of antibody against BoNT. We established therapeutic fusion antibody conjugated with apoptotic cell targeting peptide-streptavidin (APS) and biotinylated anti-BoNT antibody (BABA). The conjugation of peptide and streptavidin was evaluated using SDS-PAGE assay and peptide mess fingerprinding (PMF) analysis and amount of biotin in anti-BoNT antibody was analyzed by biotin quantitation assay. In a mouse neutralization model of BoNT, fusion antibody gave significant neutralization against $5xLD_{50}$ BoNT chllenge in mice. These results demonstrated that fusion antibody targeting apoptosis could be a potent neutralization enhancer against BoNT in vivo. Therefore, we expect that these fusion antibody might be relevant for efficient therapy of Botulism bioterror patients.

Keywords : Botulinum neurotoxin, Apoptotic peptide, Biotinylation, Fusion antibody, Neutralization

G015

Three Dimensional Structural Design and Applications of Theragnostic antibody Alternatives against Botulism

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Botulism is a severe neuroparalytic disease, which is caused by botulinum neurotoxin (BoNT). Accurate detection/treatment of BoNTs (A to H) have been critical for food safety, pharmaceuticals, and biodefense. We have identified complementarity determining regions (CDR) of binding/ neutralizing monoclonal antibodies against Botulinum neurotoxins. Using three demensional structural modeling method such as SWISS-MODEL (swissmodel.expasy.org) and Pymol, antibody alternatives have been designed to replace human antibodies. In order to increase the stability and affinity of CDR peptides, we used abundant human template where CDR peptides can be incoporated. Peptide embedded antibody alternatives were confirmed by three dimensional molecular modeling. After surface plasmon resonance analysis, engineered antibody and alternatives have been proposed to be used for immunological detection and therapeutic application. Antibody alternatives might be applicable to theragnostic materials for biodefense against not only botulism but other infectious bioterror diseases.

Keywords : Botulism, Antibody alternative, Protein drug, Structure design, Bioterror

G017

The Poly-γ-D-glutamic Acid Capsule of Bacillus Anthracis Induces Nitric Oxide Production via the Platelet Activating Factor Receptor Signaling Pathway

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The poly-γ-D-glutamic acid (PGA) capsule, a major virulence factor of *Bacillus anthracis*, protects the bacillus from immune surveillance and allows its unimpeded growth in the host. PGA capsules released from *B. anthracis* are associated with lethal toxin in the blood of experimentally infected animals and enhance the cytotoxic effect of lethal toxin on macrophages. During the infection process, macrophage-generated nitric oxide (NO) plays an important role in host defense by eliminating microorganisms. Various *B. anthracis* virulence factors are involved in this NO production. Here we used macrophages to study the ability of *B. anthracis* PGA to induce production of NO. PGA induced production of NO production and it was markedly reduced by inducible NO synthase (iNOS) inhibitors, suggesting that iNOS is specifically involved in PGA-induced NO production. In addition, PGA-induced NO production was substantially inhibited in macrophages from TLR2-deficient mice and in RAW264.7 cells treated with PAFR antagonists or transfected with PAFR siRNA. PGA-induced NO production required ERK, JNK, and p38 MAPK pathways, as well as NF-κB activation, and was also mediated by PAFR/Jak2/STAT-1 signaling pathways in the absence of interferon-β production. Our results suggest a role for PGA, especially during the late stage of anthrax infection, in which high concentrations of PGA in the blood of infected animals may induce high production of NO, which enhances sepsis and can lead to death.

Keywords : poly-γ-D-glutamic acid, *Bacillus anthracis*, nitric oxide, PAFR, TLR2

G018

Peptide H Suppresses IL6 Expression

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Chronic inflammation is invovolved in cancers. Interleukin 6(IL6) plays main roles in inflammation. Chungkookjang, fermented soybean contains diverse peptides which can be bioactive compounds. Peptide H Glu-Val-Tyr-Tyr-Met-Tyr was derived from Chungkookjang. the activities of peptide H and Chungkookjang ethanol extract(CEE) to suppress IL6 expression in a human breast cancer cell, MDA-MB-231 was determined. IL6 expressions were reduced greatly by peptide H and CEE treatments. Proliferation of MDA-MB-231 cells was inhibited by peptide H and CEE. Application of this study will contribute to drug development for a metastatic breast cancer which is caused by excessive IL6.
Keywords : inflammation, Interleukin 6(IL6), breast cancer, MDA-MB-231, peptides

G019

Development of Bivalent Vaccine against Anthrax and Smallpox using Attenuated Vaccinia Virus

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Anthrax and smallpox, that are prime candidates for a bio-weapon, represent the most deadly bioterror entities. Anthrax, which affects both humans and animals, is lethal. Also, highly infectious smallpox virus is easily cultivable but many populations in the world are susceptible to it, and no known treatment. The attenuated vaccinia virus developed in KCDC (Korea Centers for Disease Control and Prevention) could provide the effective delivery systems of foreign antigens, and in this study, protective antigen (PA) of Bacillus Anthracis was inserted in attenuated vaccinia virus, so that bivalent vaccines against anthrax/smallpox was produced. These candidates were characterized the expression of the PA protein in vitro. When the bivalent vaccine candidates were injected to Balb/C mice though I.P., antibody-titer against PA of Bacillus Anthracis and smallpox were increased in the serum and the protection against anthrax spores was enhanced. The serum vaccinated by KVAC-thPA-K1L-C7L was validated higher anti-PA ELISA titer compared to the serum that was vaccinated KVAC-thPA-C7L absorbed Freund's adjuvant. The bivalent vaccine could make the possibility working simplifying the logistics management of storage, stockpiling, and field delivery.
Keywords : Vaccinia, Smallpox, Anthrax, Vaccine

H001

Molecular Characterization of a Novel Iron-Dependent GH16 β-agarase, AgaH92, from *Pseudoalteromonas* sp. H9

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Cells grew at 20–30°C, between pH 5.0 and 9.0, and in media containing 1–5% (w/v) NaCl. Based on 16S rRNA sequence and biochemical and chemotaxonomic characteristics, we designated it as *Pseudoalteromonas* sp. H9 (=KCTC23887). A putative agarase gene (*agaH92*) encoding a primary translation product (50.1 kDa) of 445 amino acids with a 19-amino-acid signal peptide and glycoside hydrolase 16 and RICIN superfamily domains was identified in H9. The heterologously expressed protein rAgaH92 in *Escherichia coli* had an apparent molecular weight of 51 kDa on SDS-PAGE, consistent with the calculated molecular weight. Agarase activity of rAgaH92 was confirmed by a zymogram assay. rAgaH92 hydrolyzed *p*-nitrophenyl-β-d-galactopyranoside, but not *p*-nitrophenyl-α-d-galactopyranoside. The optimum pH and temperature for rAgaH92 were 6.0 and 45°C, respectively. It was thermo-stable and retained more than 85% of its initial activity at 50°C after heat treatment for 1 h. The K_m and V_{max} for agarose were 59 mg/ml and 156 U/mg, respectively. rAgaH92 required Fe^{2+} for agarase activity and inhibition by EDTA was compensated by Fe^{2+}. A thin-layer chromatography analysis of the rAgaH92 hydrolysis products and the kinematic viscosity of the agarase revealed that rAgaH92 is an endo-type β-agarase belonging to GH16 and hydrolyzes agarose into neoagarotetraose and neoagarohexaose. [Supported by grants PJ009536 from the Next-Generation Bio Green 21 Program, Rural Development Administration, Republic of Korea]
Keywords : β-1,4-agarase, neoagarotetraose, neoagarohexaose, *Pseudoalteromonas* sp. H9

H002

Molecular Characterization of a GH16 β-Agarase AgaH71 from *Pseudoalteromonas hodoensis* H7

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AgaH71 was identified from *Pseudoalteromonas hodoensis*. The nucleotide sequence revealed that AgaH71 had significant homology to GH16 agarases. *agaH71* encodes a primary translation product (32.7 kDa) of 290 amino acids, including a 21-amino-acid signal peptide. The entire AgaH71 was expressed in a fused protein with GST at its N-terminal (GST-AgaH71) in *E.coli*. Purified GST-AgaH71 had an apparent molecular weight of 59 kDa on SDS-PAGE, which was consistent with the calculated molecular weight (58.7 kDa). Agarase activity of the purified protein was confirmed by zymogram assay. GST-AgaH71 could hydrolyze *p*-nitrophenyl-β-d-galactopyranoside, but not *p*-nitrophenyl-α-d-galactopyranoside. The optimum pH and temperature for GST-AgaH71 were 6.0 and 45°C, respectively. GST-AgaH71 retained more than 95% and 90% of its initial activity at 40°C and 45°C after heat treatment for 60 min, respectively. The K_m and V_{max} for agarose were 28.33 mg/mL and 88.25 U/mg, respectively. GST-AgaH71 did not require metal ions for its activity, but severe inhibition by divalent metal ions was observed. Thin-layer chromatography analysis and mass spectrometry of the GST-AgaH71 hydrolysis products revealed that GST-AgaH71 is an endo-type β-agarase that hydrolyzes agarose into predominantly neoagarotetraose and small proportions of neoagarobiose and neoagarotetraose. [Supported by Basic Science Research Program (NRF-2012R1A1B3002174) through the National Research Foundation of Korea]
Keywords : *Pseudoalteromonas hodoensis*, β-agarase, neoagarotetraose, neoagarobiose, neoagarotetraose

H003

Production of Short Chain Alkanes by Metabolically Engineered *Escherichia coli*

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Here we report a development of a platform *Escherichia coli* strain that is able to produce short chain alkanes (gasoline), free fatty acids (FFAs), fatty esters, and fatty alcohols. Short chain alkanes were produced in *E. coli* through fatty acyl-ACP to free fatty acid to fatty acyl-CoA pathway. First, the *fade* gene was deleted to block *beta*-oxidation to secure fatty acyl-CoAs. Promoted *fabH* gene, and deletion of *fadR* gene enhanced short-chain fatty acid biosynthesis. Starting from short-chain FFAs generated by a mutated thioesterase, the sequential reactions of *E. coli* fatty acyl-CoA synthetase, *C. acetobutylicum* fatty acyl-CoA reductase, and *A. thaliana* fatty aldehyde decarbonylase generaded 580.8 mg/L of short chain alkanes conclusively. [This work was supported by the Advanced Biomass Research and Development Center of Korea (NRF-2010-0029799) through the Global Frontier Research Program of the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF). Systems metabolic engineering work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012M1A2A2026556) by MSIP through NRF].
Keywords : Metabolic engineering, Biofuels, Short chain alkanes, free fatty acids

H004

Phenol Production by an Engineered *E.coli* Strain

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Despite the increasing demands and efforts for carbon-neutral and sustainable routes for production of industrial compounds, the biological production of phenol is considered non-viable due to its toxicity and complex biosynthetic network. To overcome this hurdle, we took advantage of sRNA technology to engineer 18 *Escherichia coli* strains simultaneously for the production of phenol. The knock-down of *csrA* and *tyrR* genes were found to be crucial for redirecting carbon flux toward tyrosine biosynthetic pathway. Together, the overexpression of *pps*A, *tkt*A, *aor*G, *aro*K, *tyr*A, *tyr*C and *Pasteurella multocida tpl* generated *E. coli* strains capable of producing phenol from glucose. The strain background had substantial effects on the precursor and phenol production, enzyme activity, and tolerance to phenol. The engineered BL21 strain showed the highest phenol titer: 419 mg/L by flask culture and 1.69 g/L by fed-batch culture. A biphasic fed-batch fermentation process using glycerol tributyrate was develop to minimize the toxicity of phenol and improved the titer and productivity to 3.79 g/L and 0.18 g/L/h, respectively. The metabolic engineering strategy presented in this study will provide a valuable framework for the microbial production of toxic chemicals. [This work was supported by the Intelligent Synthetic Biology Center through the Global Frontier Project (2011- 0031963) of the Ministry of Science, ICT & Future Planning through the National Research Foundation of Korea.]
Keywords : Metabolic engineering, Phenol production, sRNA technology

H005

The Microbial Production of 1-propanol *via* Threonine Metabolism

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The 1-propanol is one of the next generation biofuels used as a gasoline substitute as well as industrial products. In terms of energy density and combustion efficiency, it is advantageous than ethanol. Previously, Atsumi *et al*. reported that a metabolically engineered *Escherichia coli* harboring an engineered citramalate synthase (*CimA*), which converts pyruvate directly into 2-ketobutyrate, from *Methanococcus jannaschii* produced the highest reported concentration of 1-propanol, 3.5 g L^{-1}. We have previously reported an L-threonine overproducing *E. coli* TH20 strain, which was genetically modified to concentrate carbon fluxes towards L-threonine by systems metabolic engineering. The TH20 strain was further engineered for 1-propanol production. Toward this goal, novel synthetic pathway for 1-propanol production, deleting competing pathway and carbon source optimization based on the *in silico* flux response analysis was established.[This work was supported by the Advanced Biomass R&D Center of Korea (NRF-2010-0029799) through the Global Frontier Research Program of the Ministry of Education, Science and Technology (MEST) Further support by BioFuelChem is appreciated.]
Keywords : Metabolic engineering, 1-Propanol production, *in silico*

H006

Proteome Analysis of Recombinant *Escherichia coli* Central Metabolism for Enhanced Biosynthesis of Poly(3-hydroxybutyrate)

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In this study, we developed recombinant *E. coli* strains overexpressing the triosephosphate isomerase (TpiA) and fructose-bisphosphate aldolase (FbaA) genes and examined these strains for enhanced P(3HB) synthesis from different carbon sources such as glucose, sucrose, and xylose. Previous analysis of the proteome of recombinant *Escherichia coli* showed that expression levels of several enzymes are proportional to the amount of P(3HB) accumulated in the cells. As predicted, amplification of TpiA and FbaA significantly increased the P(3HB) contents and concentrations in W3110, XL1-Blue, and W lacI mutant strains. TpiA amplification in *E. coli* XL1-Blue lacI increased P(3HB) from 0.4 to 1.6 to g/l from glucose. Thus, information from the global proteome analysis of recombinant *E. coli* producing large amounts of P(3HB) can be applied for the manipulation of recombinant strains to obtain efficient production of P(3HB) by allowing increased glycolytic pathway flux. [This work was supported by the Technology Development Program to Solve Climate Changes (Systems metabolic engineering for biorefineries) from the Ministry of Science, ICT and Future Planning (MSIP) through the NRF (NRF-2012-C1AAA001-2012M1A2A2026556) is appreciated.]
Keywords : FbaA, TpiA, Poly(3-hydroxybutyrate), Proteome analysis, Recombinant *E. coli*

H007

Rapid One-step Inactivation Using Integration Helper Plasmid in *Escherichia coli*

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In this study, rapid one-step inactivation using integration helper plasmid in *E. coli* was developed. The integration helper plasmid, pCW611, has two recombinases which are expressed in reverse direction by two independent inducible systems, thus subsequent expression of each gene in a cell without plasmid transformation and curing is possible. By using this system, required time and effort can be significantly reduced. We could delete one target gene in 3 days by using pCW611. To verify the usefulness of this gene manipulation system, the deletion experiments were performed for knocking out four target genes individually (*adhE*, *sfcA*, *frdABCD*, and *ackA*) and two genes simultaneously for two cases (*adhE-aspA* and *sfcA-aspA*). Also, fumaric acid producing *E. coil* strain was developed by deleting four target genes (*fumB*, *iclR, fumA*, and *fumC*) in 10 days as a proof-of-concept study. (This work was supported by Technology Development Program (NRF-2012M1A2A2026556) to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation of Korea)
Keywords : Cre recombinase, *Escherichia coli*, Gene manipulation, Metabolic engineering, Red recombinase

H008

Implementation of Synthetic Small Regulatory RNAs in *Escherichia coli* for the Production of Tyrosine and Cadaverine Using Metabolic Engineering

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Regulatory noncoding RNAs, such as small regulatory RNAs (sRNAs), act fast and consume little energy, allowing its use in many organisms to identify and modulate the expression of target genes. Here we present an approach for constructing synthetic small regulatory RNAs for controlling gene expression. We developed synthetic small regulatory RNAs repressing the translation of DsRed2 mRNA at various levels and also constructed three different sRNAs for the mRNAs of LuxR, AraC, and KanR without cross-reactivity. The ability to fine-tune target genes with designed synthetic sRNAs provides substantial advantages with its easy implementation, ability to modulate chromosomal gene expression without modifying those genes and the lack of need for strain library construction. Using synthetic sRNAs for the combinatorial knockdown of four candidate genes in 14 different strains, we isolated an engineered *E. coli* strain capable of producing 2 g per liter of tyrosine. Using a library of 130 synthetic sRNAs, we also identified chromosomal gene targets that enabled substantial increases in cadaverine production. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012-C1AAA001-2012M1A2A2026556); the Intelligent Synthetic Biology Center through the Global Frontier Project (2011-0031963) of the Ministry of Education, Science and Technology (MEST) through the National Research Foundation of Korea]
Keywords : Small regulatory RNAs, Tyrosine, *Escherichia coli*, Metabolic engineering

H009

Development of Strategies to Sequential (Multiple) Gene Knockout in *Clostridium acetobutylicum*

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Clostridium acetobutylicum is considered as a promising microorganism for the efficient production of renewable chemicals and biofuels. However, due to the lack of efficient genetic manipulation tools, strain improvement has been rather slow. Fortunately, mobile group II intron was successfully applied to gene knockout of *Clostridium* species, such as *Clostridium perfringens* and *C. acetobutylicum* in 2005 and 2007, respectively. Nevertheless, construction of a multiple gene knockout mutant was not easy, due to the limit of resources for genetic engineering. Since the knockout system based on the mobile group II intron was constructed in a replicable plasmid, curing of the plasmid was required prior to the disruption of the next gene. However, the curing process is not easy, since the replication of the knockout vector is rather stable. We developed a multiple gene-knockout system that does not require marker pop-out process by using a mobile group II intron *Ll.ltrB* and two different antibiotics markers, erythromycin and thiamphenicol resistance genes. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012-C1AAA001-2012M1A2A2026556); and the Advanced Biomass R&D Center of Korea (NRF-2010-0029799) through the Global Frontier Research Program of the MSIP.]
Keywords : *Clostridium acetobutylicum*, multiple gene knockout, mobile group II intron

H010

Development of a Hyper-ABE Producing *Clostridium acetobutylicum* and Its Genome Analysis

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Acetone-butanol-ethanol (ABE) are naturally produced by a genus *Clostridium.* In order to develop a sustainable and economically viable ABE fermentation process, a hyper ABE producer is needed combined with the development of efficient bioprocess. To develop a hyper ABE producer, Mutagenesis of the *Clostridium acetobutylicum* PJC4BK strain was carried out by using N-methyl-N'-nitro-N-nitrosoguanidine (NTG) and screened on fluoroacetate plates to isolate a mutant strain. Among the mutatns, a hyper ABE producing BKM19 strain was isolated. The BKM19 strain produced 32.5 g/L of ABE (17.6 g/L of butanol, 10.5 g/L of ethanol, and 4.4 g/L of acetone) from 85.2 g/L of glucose in batch fermentation exhibiting the total solvent production capability 30.5% and 90.5% higher than the PJC4BK and ATCC824 strains, respectively. Furthermore, we resequenced the genome of the BKM19 strain to verify which genes relate with enhanced solvent production. We confirmed several potential mutant points for the BKM19 strain, which is the target for reverse engineering to verify its mutant effects. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556); and the Advanced Biomass R&D Center of Korea (NRF-2010-0029799) through the Global Frontier Research Program of the MSIP.]
Keywords : *Clostridium acetobutylicum*, ABE fermentation, hyper ABE producing mutant

H012

L-arginine Production using Metabolically Engineered *Corynebacterium glutamicum*

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L-Arginine is an important amino acid for diverse industrial and health product applications. *Corynebacterium glutamicum* ATCC 21831 was metabolically engineered for the production of L-arginine. First random mutagenesis is performed to increase the tolerance of *C. glutamicum* to L-arginine analogues, followed by systems metabolic engineering for further strain improvement, involving removal of regulatory repressors of arginine operon, optimization of NADPH level, disruption of L-glutamate exporter to increase L-arginine precursor and flux optimization of rate-limiting L-arginine biosynthetic reactions. Fed-batch fermentation of the final strain in 5 l and large-scale 1,500 l bioreactors allows production of 92.5 and 81.2 g l^{-1} of L-arginine with the yields of 0.40 and 0.35 g L-arginine per gram carbon source (glucose plus sucrose), respectively. The systems metabolic engineering strategy described here will be useful for engineering *Corynebacteria* strains for the industrial production of L-arginine and related products. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012-C1AAA001-2012M1A2A2026556). Further support from Daesang Corporation is appreciated.]
Keywords : L-Arginine, *Corynebacterium glutamicum*, systems metabolic engineering

H013

Computational Analysis of Functional Roles of Human Genes Having Different Numbers of Splice Isoforms

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Alternative splicing is a process during gene expression that allows the multi-exon gene to produce multiple mRNA products which may have different activities. At this point, our interest is to identify whether genes with different number of isoforms may have different functional roles or not. To answer the question, four annotated gene sets, including housekeeping, tissue-selective, essential, and non-essential genes, were used for controls. Two grouped genes, genes with a single and multiple isoform(s), were systematically compared with annotated four groups from genomic properties to network properties. In conclusion, we found that genes with multiple isoforms show similar characteristics with housekeeping and essential genes, fundamentally important. In contrast, genes with a single isoform are likely to be similar to tissue-selective genes. Furthermore, we updated gene-protein-reaction (GPR) association of human metabolic models at isoform level for future application such as isoform-specific constraint. This model will be useful to understand metabolism and future applications for genome-scale modeling and simulation. [This work was supported by the Bio-Synergy Research Project (2012M3A9C4048759) of the Ministry of Science, ICT and Future Planning through the National Research Foundation. This work was also supported by the Novo Nordisk Foundation.]
Keywords : Metabolic engineering, Splice isoforms, Alternative splicing

H014

Prediction of Novel Metabolic Pathways for the Production of Industrially Valuable Chemicals

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There are several systems developed in the past decades to predict the novel pathways for the production of industrially valuable chemicals. In this trend, we present the framework to predict pathways and enzyme candidates through screening process to evaluate feasibility of predicted pathways. The framework consists of two parts, route generation and prioritization process. Route generation process generates pathways based on the reaction rule sets which were constructed based on the logics acquired from analysis of reaction mechanism of existing biochemical reactions. After, five screening factors, binding site covalence, chemical similarity, thermodynamic favorability, pathway distance and organism specificity are evaluated to assess the feasibility of predicted pathways. For validation, novel metabolic pathways for isobutanol, 3-hydroxypropionate (3HP) and butyryl-CoA, were predicted using this system. This research will open a new gate to the production and engineering of the valuable chemicals which are not available in the realm of biochemical engineering before.[This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012-C1AAA001-2012M1A2A2026556).]
Keywords : Metabolic engineering, Novel metabolic pathways

H015

Use of Metabolic Model Reconstruction on Genome-scale and Gene Manipulation Target Prediction for Avermectin Biosynthesis in *Stretpmyces avermitilis* MA-4680

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Streptomyces species, the Actinobacteria family, are aerobic, gram-positive, mycelium-forming, and filamentous soil bacteria. *Streptomyces* species is characterized by the presence of gene clusters that biosynthesize a variety of secondary metabolites, including polyketides and terpenes. In addition, modeling of genome scale metabolic network and *in silico* simulation based on its model has been successful for improvement of production of target products in metabolic engineering. In this research, we constructed the genome-scale metabolic model of *S. avermitilis* which contain metabolic reactions related to biosynthesis of secondary metabolites such as avermectin. Furthermore, we predict manipulation gene targets for improvement of avermectinB1a production by using minimization of metabolic adjustment (MOMA) and flux response analysis algorithms. Under minimal medium condition, genes encoding succinyl-CoA synthetase and glucose-6-phosphate isomerase are predicted as the knockout targets, and genes encoding dihydrolipoamide dehydrogenase related to coenzyme A biosynthesis such as *dfp* and *coaA* genes are predicted as the overexpression targets. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556).]
Keywords : In silico prediction, Genome-scale metabolic network, Avermectin B1a

H016

Verification of screening methods using white rot fungi for biodegradation of PAHs

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White rot fungi are the most promising group due to their remarkable ability of degradation of recalcitrant xenobiotics including PAHs. And they are capable of producing extracellular enzymes to utilize carbon source from a variety of environmental pollutants due to their low substrate specificity. In accordance with their degrading ability, white rot fungi have been received attention for biodegradation of xenobiotics. Thus, to significantly degrade PAHs, a total of 120 white rot fungal species were characterized and selected via three methods, such as bavendamm's reaction, RBBR, and tolerance test to PAHs. In the results of the study, it was demonstrated that 31 fungal strains oxidized tannic and gallic acid with dark brown or brown color. Among them, 28 fungal strains were selected due to their complete decolorization of RBBR on the solid medium within 10 days. Tolerance test was carried out to the PAHs with these selected fungi in presence of 100 mg/L of PAHs mixtures including penanthrene, anthracene, fluoranthene, and pyrene. Ultimately, 14 fungal strains were selected from all tests and these were *Microporus vernicipes*, *Peniophora incarnata*, *Phanerochaete sordida*, *Phlebia acerina*, and *Phlebia radiata*. Among them, *P. incarnata* demonstrated the highest degradation rates of individual four PAHs in the liquid medium. This fungus was recently recognized as a remarkable species and verified in the current study as the most valuable species for biodegradation of pollutants.
Keywords : Biodegradation, *Peniophora incarnata*, PAHs, White rot fungi

H017

Processing and Biological Effects of Gelatin Hydrolysate from *Branchiostegus japonicus* Scales

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The potential utility of fish scales to the functional food industry has been investigated due to its antioxidant and antihypertensive characteristics. In this study, we report on the reactive oxygen species (ROS) scavenging and angiotensin I converting enzyme (ACE) inhibitory activities of gelatin hydrolysates processed from *Branchiostegus japonicus* scales, which are also high in protein content (about 46.1%). We prepared the enzymatic gelatin hydrolysates with four proteases from *B. japonicus* scale gelatin, which was prepared according to different reaction times, substrate/ enzyme ratios and substrate concentrations. Furthermore, gelatin hydrolysates of Neutrase and α -chymotrypsin showed the highest DPPH radical and H_2O_2 scavenging activities, respectively. However, the activities were not significant. We also observed that the four gelatin hydrolysates significantly increased ACE inhibitory activities from approximately 20% to 60%. Among them, the Alcalase gelatin hydrolysates showed the higher ACE inhibitory activity compared to the others. These results suggest that the enzymatic gelatin hydrolysates prepared from *B. japonicus* scales may possess a potentially useful function as an ACE inhibitory agent. This research was financially supported by the Ministry of Education (MOE) and National Research Foundation of Korea (NRF) through the Human Resource Training Project for Regional Innovation (NRF-2012H1B8A2025863)
Keywords : *Branchiostegus japonicus*, Fish scales, Reactive oxygen species, ACE inhibitory, enzymatic hydrolysates

H018

Identification of a Novel Endo-β-1,4-Glucanase From Gut Metagenome of Black Soldier fly, *Hermetia illucens*

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A metagenomic fosmid library was constructed using genomic DNA isolated from the gut microflora of *Hermetia illucens*, a black soldier fly. A cellulase positive clone, *CS10* gene was identified by the extensive Congo-red overlay screenings for cellulase activity from the fosmid library of 92,000 clones. The *CS10* gene was composed of the 996 bp DNA sequence encoding the mature protein of 331 amino acids. The deduced amino acids of *CS10* gene showed 72% sequence identity with glycosyl hydrolase family 5 gene of *Dysgonomonas mossii*, displaying no significant sequence homology to already known cellulases. The purified CS10 protein presented a single band of cellulase activity with a molecular weight of approximately 40 kDa on SDS-PAGE and zymogram. The purified CS10 protein exhibited the optimal activity at 50 °C and pH 7.0, and the thermostability and pH stability of CS10 were preserved at the ranges of 20 ~ 50 °C and pH 4.0 ~ 10.0. CS10 exhibited little loss of cellulase activity against various chemical reagents such as 10% polar organic solvents, 1% non-ionic detergents, and 0.5 M denaturing agents. Also, the substrate specificity and the product patterns by thin-layer chromatography suggested that CS10 is an endo-β-1,4-glucanase. From these biochemical properties of CS10, it is expected that the enzyme have the potential possibility for application in industrial processes.
Keywords : glucanase, metagenome, cellulase, black soldier fly, microbiology

H019

Fermentative Production of Deoxyviolacein and Construction of Its Producing Strain from Improved L-Tryptophan synthesis pathway *Escherichia coli*

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Vioalcein and deoxyviolacein are components of crude violacein from several strains such as *J.lividum*, *C.violaceum, Duganella sp., Pseudoduganella sp., Collimonas sp.* The usage of violacein in the bio-application field is various and sub-divided, needs of amount of violacein and its derivation will be increase. Nevertheless this outlook, the production of the violacein is not established well than biofuel field. In this reason, we make the crude violacein production strain, GPT vio, from modification of L-tryptophan producing *E.coli* strain called GPT1002. BBa J72214 BBa J72090, the violacein production plasmid containing the vioABCDE gene which known 5 major gene for production of violacein, is inserted in GPT 1002 strain with electroporation. Fermentation using GPT vio in the superbroth with 1g/L of L-tryptophan, is shown that production of crude violacein reach the around 196mg/L after 48h fermentation and its purity, measured by HPLC, is deoxyviolacein 95% over. The production rate per unit time is 4.1mg/L·h and 2.9 times enhanced rate recently reported 1.4mg/L·h. Moreover the temperature of fermentation is always 37°C that is improved cell growth and violacein production rate without low temperature or temperature switching.
Keywords : Violaciein, Deoxyviolacein, Fermentation, Tryptophan, Antibiotics

H020

Cloning and Overexpression of Carbonic Anhydrase in *Actinobaillus succinogenes* for Enhanced Succinic Acid Production

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Succinic acid is an important feedstock chemical used as the precursor of various industrial chemicals like pharmaceuticals, food additives, and green solvents. Currently, petrochemical processes produce most of the commercially available succinic acid. However, the petroleum-based succinic acid production has the problems of fossil resource depletion, high energy consumption, and carbon dioxide emission. Recently, several efforts were made for the synthesis of succinic acid from renewable carbohydrates by microbial fermentation. One of the most influential factors in succinic acid production by microbial fermentation is the supply of carbon dioxide. In this study, therefore, the carbonic anhydrase (CA) gene of a natural succinic acid producer, *Actinobacillus succinogenes*, was cloned and overexpressed during fermentation to enhance the ability of the strain to use carbon dioxide. The role of CA is to catalyze the reaction of $CO_2 + H_2O \rightarrow HCO_3^- + H^+$ and it can enhance the ability of the strain using carbon dioxide. Therefore, expression vectors harboring the CA gene were constructed and transformed into the producing strain. In addition, biological properties of the transformed strains were examined by RT-PCR and enzyme activity assay.
Keywords : biorefinery, succinic acid, microbial fermentation, carbon dioxide, carbonic anhydrase

H021

Heterologous Expression of Mushroom Peroxidases in *Escherichia coli*

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White-rot fungi decompose polyphenolic lignin components through the activities of lignin degrading enzymes, including laccase, lignin peroxidase, manganese peroxidase, etc. These enzymes can be applied to bioremediation of recalcitrant chemical compounds including polycyclic aromatic hydrocarbons, endocrine disrupting chemicals, and synthetic dyes due to their high catalytic powers and broad substrate specificities. Especially, peroxidases are known to catalyze non-aromatic compounds as well as aromatic rings. To make use of the power of peroxidase, we cloned 11 peroxidase genes from various wild mushroom species, including *Pleurotus ostreatus*, *Ganoderma lucidum*, *Coriolus versicolor*, *Dichomitus squalens*, and *Phanerochaete chrysosporium*, to an expression vector, pPICHOLI vector, for the heterologous expression in *Escherichia coli* (BL21DE3). Among the 11 peroxidases, 9 were expressed as soluble proteins as shown by SDS-PAGE and immunoblot analyses. The soluble enzyme fractions showed different degrees of peroxidase activity in ABTS assay, indicating the diverse nature of biochemical characteristics of peroxidases among mushroom species.
Keywords : Peroxidase, Protein expression

H022

Novel Method for Extraction of Violacein from Agar Plate and Characterization of Purified Violacein

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Nowadays human beings have many diseases like a cancer and infection by microorganism. In this situation human beings are trying to make new material can care above diseases. Hence, we consider about care material harmlessness. That is violacein have function such anticancer, antibiotics, antifungal, antiprotozoans. For Mass production of violacein, This study focus on the general property of natural violacein producing host such as *C.violaceum, J. lividum, Duganella sp.* The violacein production mechanism of these strains is quorum sensing molecule reported cviI/cviR or similar mechanism. In this study, we use *Duganell sp. NI28* strain for production of violacein, that shown dramatically increasing with colony state on the agar plate than liquid medium culture. Like this low production in the liquid culture decline the total production violacein. We dried the agar well spread producing strain plate directly in the 65°C oven 24hour. The agar was changed to film form peeled off from the plate. With Ethanol extraction, normal violacein extraction method, we can get the 1.1mg per plate of violacein which is around 2.5 time higher concentration of violacein than flask culture. The purity of extracted violacein was analyzed by HPLC. In the crude violacein, 95% of purified violacein is vioalcein other small amount is deoxyviolacein and violacein derivation compounds.
Keywords : Violacein, Deoxyviolacein, Antibiotics

H023

Development of Diagnostic Kit using the Antigenic Protein of the Helicobacter Pylori

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It is knowed infection of Helicobacter pylori that It cause gastritis, stomach ulcer and duodenal ulcer. furthermore, It seriously cause stomach cancer.Helicobacter pylori is detected more than 90% among patients with stomach ulcers, duodenal ulcer and gastritis. As, early diagnosis of H. pylori infection is required for fast treatment.As mentioned, The research Members are going to develop kit detecting H. pylori antibody how refine Cag7 that It is outer membrane of H. pylori type-1 with pathotoxin gene among H. pylori origin to korean. We take the E. coli expression system to obtain many Cag7 protein. Then attached conjugate with the antigen(Cag7) We attached it with pad. Next We injected artificial specimen and diagnosed infection of H. pylori type-1 several mins. As a result, we used a total of 25 antibodies, These released same positive result when compared commercialized product and our product. judging from the result of an experiment, We are going to commercialize After a test pass through in a clinical specimen in the future.
Keywords : Helicobacter pylori, antigenic protein, diagnostic kit

H024

Immobilisation Study on the Clostridial Biofuel: Optimisation of Polystyrene Sponge Packed-Bed Reactor for Increasing the Biobutanol Yield for *Clostridium beijerinckii* NCIMB 8052

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Oil is major resource for all the industries. It can be the raw material or maintaining tools, but using as energy source is the first priority due to its characteristic.But it continuously pointed out as the major cause of global warming and various environmental pollution. Many researchers especially from governments and the energy companies are in work for solve these issues, and one of the promising solutions is the biofuel. Biofuel is the alternative energy source which is derived from biomaterials, which can be easily reproduced and have the identical characteristic to ordinary fuel, which can make easy adoption to established industries. Butanol, Clostridium's major product, has highly concentrated energy compares to other possible alcoholic product, and easy to adopt into the diesel engine. But there is the problem that we should study for breakthrough is the yield. Here, I introduce the immobilisation study on polystyrene sponge that our group is performing, and may introduce how can we optimise this system to maximize the yield of the biofuel for *Clostridium Beijerinckii* NCIMB 8052.
Keywords : Biofuel, Reactor, Clostridium, Immobilisation

H025

Biotransformation of Geraniol by *Polyporus brumalis* and Transcriptome Analysis

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Biotransformation can be defined as the specific modification by the use of biological catalyst. In this study, biotransformation of monoterpene (geraniol) by white rot fungi (*Polyporus brumalis*) was performed to produce the valuable monoterpenoids. Geraniol known to the precursor of diverse monoterpenes was added into the cultures of *P. brumalis*. After 10 days, the cultures adding a geraniol were extracted by ethyl acetate with sodium chloride. The chemical analysis of extracts was performed by gas chromatograph due to strong volatility of monoterpenes. As the results, menthane-type monoterpenoids, isopulegol and ρ-menthane-3,8-diol, were transformed from geraniol by P. brumalis. Profiling of transcripts from *P. brumalis* was performed to understand catalytic function on biotransformation process. The cDNA library was constructed using the GS FLX titanium rapid library preparation Kit. Sequencing was carried out using the Illumina HiSeqTM 2500 platform.Unigenes were annotated across the GO (Gene Ontology) and KEGG (Kyoto Encylcopedia of Genes and Genomes). As the results, genes related to diverse secondary metabolism are expressed from mycelium of *P. brumalis*. Especially, Limonene synthase which is a cyclase of monoterpene precursor in the monoterpene synthesis were expressed. Also, gene encoding 'cytochrome P450 monooxgenase' play important role in terpene biosynthesis was increased after substrate addition.
Keywords : Biotransformation, Polyporus brumalis, Geraniol, Transcriptome analysis, Next Generation Sequencing

H026

In Vivo Reconstitution of Membrane Protein by Caveolin1 Co-expression : Towards Implantation of Eukaryotic Membrane Trafficking Pathways in a Prokaryote

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Caveolae is a membrane-budding structure which exists in many animal vertebrate cells. One of the important functions of caveolae is to form membrane curvature and endocytic vesicle. Recently, It was shown that caveolae could be formed in *Escherichia coli* by expressing caveolin-1 other membrane proteins overexpressed inside the cell. The heterologous caveolae may host other membrane proteins overexpressed inside the cell. We utilized this system for construction of proteo-liposome in *Escherichia coli*. SNARE proteins (Syntaxin1a, SNAP25, VAMP2) were introduced to prove our *in vivo* reconstitution system. Here, we show that the purified heterologous caveolae indeed contain the co-expressed membrane protein and the membrane protein were facing outward. The size of the purified caveolae with membrane protein reconstituted were measured by dynamic light scattering. The presence of VAMP2 & Syntaxin1a on this proteo-endosome was confirmed by Western blot analysis. Furthermore, membrane proteins (VAMP2 & Syntaxin1) embedded in caveolae retained its ability to form SNARE complex. Our study proposes in vivo membrane protein reconstitution system.
Keywords : Membrane protein, Caveolae, Reconstitution, Proteoliposome, SNARE protein

H027

Antioxidant Effects of Peptide Isolated from Aquacultural Flounder Fish in Zebrafish

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The flounder, *Paralichthys olivaceus*, is one of the most important commercial cultivated fish species, and accounts for 98% of the domestic aquaculture market in South Korea. In a previous report, a peptide isolated from the muscle of aquacultured flounder was found to possess antioxidant activity and to protect against 2,2-azobis-(2-amidinopropane) hydrochloride (AAPH)-induced oxidative stress in Vero cells. The sequence of this flounder-derived peptide (AFFP) is Cys-Ala-Ala-Pro and its molecular weight is 360.1 Da. In the present study, we examined the protective effect of AFFP against AAPH-induced oxidative damage in a zebrafish model. Zebrafish embryos pretreated with AFFP (25, 50 and 100 μg/ml) and 10 mM AAPH, were evaluated for the protective effect by heartbeat rate, survival rate, ROS generation, lipid peroxidation, and cell death. AFFP dose-dependently reduced AAPH-induced intracellular ROS and lipid peroxidation, and decreased cell death in AAPH-induced zebrafish. These results revealed that AFFP could be used as a natural antioxidant, and that the zebrafish provides an alternative *in vivo* model to efficiently evaluate the antioxidative effects of peptides on fishe.
Keywords Flounder fish, Antioxidant, Peptide, Zebrafish

H028

Comparison of Cellulase Activities of *Tyromyces palustris* from *Phanerochaete chrysosporium*

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Wood rot fungi have been researched as a biocatalyst for bioconversion and biodegradation of wood components such as cellulose and lignin. In this study, cellulase (Endo-β-1,4-glucanase, β-glucosidase and cellobihydrolase) activity were detected from the two wood rot fungi, *Tyromyces palustris* and *Phanerochaete chrysosporium*. The highest activity of Endo-β-1,4-glucanase was 0.609unit/mL from *T. palustris,* brown rot fungus, after 13 days cultivation. Depending on cultivation period, the activity of Endo-β-1,4-glucanase from *T. palustris* was increased in the sucrose supplement medium. However, the low level of activity from *P. chrysosporium* white rot fungus, was maintained except the medium containing galactose and sucrose,relatively. The highest activity of b-glucosidase, was 61.851unit/mL from *T.palustris* in the medium containing galactose. The β-glucosidase activity of *P.chrysosporium* was 26.079unit/mL at the one day of cultivateion and then decreased rapidly after third day that. Additionally, the cellobihydrolase activity will be measrued. Also, the correlation between gene expression and enzyme activity of cellulasefrom *T.palustris* and *P.chrysosporium* will be investigated.
Keywords : Cellulase activity, *Tyromyces palustris*, *Phanerochaete chrysosporium*

H029

Identification of Gene Encoding Precursor of Novel Antimicrobial Peptide BSAP-254 Isolated from *Bacillus subtilis* SC-8

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BSAP-254, an antimicrobial peptide produced by *Bacillus subtilis* SC-8 (BSSC8), was isolated from the Korean fermented soybean paste and exhibited narrow antagonistic activity against foodborne pathogens *B. cereus* group. It is a lipopeptide and in this study we have selected four putative gene encoding precursors such as BSSC8_08030, BSSC8_10350, BSSC8_10670 and BSSC8_36740. BSAP-254 was screened by anitibacterial activity testing and PCR with other *Bacillus* strains, *B. cereus, B. subtilis*, *B. thurigiensis*, *B. licheniformis* and *B. sonorensis*. Among them, only *B. sonorensis* have shown activity against *B. cereus*. And it also shown a PCR amplified band with BSSC8_08030 and the sequence of *B. sonorensis* had 80% identity with BSSC8. And also, these four putative precursors are heterologously expressed in *E.coli* with pOPINM vector and the expressed proteins are analyzed by western blot with rabbit antibody against BSAP-254 in progress
Keywords : Antimicrobial Peptide, *Basillus subtilis SC-8*, Antagonistic activity

H030

Study of Extracellular Enzyme Activity of *Phanerochaete chrysosporium* and *Tyromyces palustris* in Different Carbon Sources

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In this study, we investigated the extracellular enzyme activity from the *Phanerochaete chrysosporium* of white-rot basidiomycete and *Tyromyces palustris* of brown-rot basidiomycete. The activities of Lignin peroxidase (LiP), Manganese peroxidase (MnP) and Laccase (Lac) from *P. chrysosporium* and *T. palustris* were measured in mineral salt broth (MSB) medium with different carbon sources (glucose, galactose, sucrose, starch, and cellulose). As a result, the highest activities of LiP and MnP were shown in the *P. chrysosporium* with 35 u/mL and 3.8 u/mL in medium supplemented with starch, respectively. LiP and MnP activity of *T. palustris* was a 16 u/mL in the sucrose medium and 2.2 u/mL in the starch medium. The results showed stronger activity of *P. chrysosporium* than *T. palustris.* Lac activity of *P. chrysosporium* and *T. palustris* showed 2 u/mL with galactose and starch, respectively. There has been little disccusion about the ligninolytic enzyme from *T. palustris.* However, the activities of the enzymes were detected in this study.The results will be used for characterizing of gene encoding enzymes, as the basic data.
Keywords : *Phanerochaete chrysosporium*, *Tyromyces palustris*, Lignin peroxidase (LiP), Manganese peroxidase (MnP), Laccase (Lac)

H031

Identification of Gene Encoding Precursor of Novel Antimicrobial Peptide BSAP-254 Isolated from *Bacillus subtilis* SC-8

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BSAP-254, an antimicrobial peptide produced by *Bacillus subtilis* SC-8 (BSSC8), was isolated from the Korean fermented soybean paste and exhibited narrow antagonistic activity against foodborne pathogens *B. cereus* group. It is a lipopeptide and in this study we have selected four putative gene encoding precursors such as BSSC8_08030, BSSC8_ 10350, BSSC8_10670 and BSSC8_36740. BSAP-254 was screened by anitibacterial activity testing and PCR with other *Bacillus* strains, *B. cereus, B. subtilis*, *B. thurigiensis*, *B. licheniformis* and *B. sonorensis*. Among them, only *B. sonorensis* have shown activity against *B. cereus*. And it also shown a PCR amplified band with BSSC8_08030 and the sequence of *B. sonorensis* had 80% identity with BSSC8. And also, these four putative precursors are heterologously expressed in *E.coli* with pOPINM vector and the expressed proteins are analyzed by western blot with rabbit antibody against BSAP-254 in progress
Keywords : Antimicrobial Peptide, *Bacillus subtilis* SC-8, Antagonistic activity

H032

Production of 2,3-Butanediol from Cellobiose by Engineered *Escherichia coli*

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Biofuels derived from cellulosic biomass are an attractive alternative to fossil fuels, due to their potential for sustainability and reduction of greenhouse gas emissions. We produced 2,3-butanediol (2,3-BDO) using cellobiose, a disaccharide hydrolyzed from cellulosic biomass. 2,3-BDO is an important chemical feedstock and It can be used as a precursor molecule for many industrial applications including pharmaceuticals and food additives. Especially, 2,3-BDO can be converted into butadiene, one of the major building blocks of the chemical industry. In this study, we deleted several genes for preventing consumption of pyruvate and introduced plasmids containing β-glucosidase (*Bgl3C*), and 2,3-BDO biosynthesis operon (*budABC*) into *E.coli*. This work was supported by a grant (NRF-2012M1A2A2671831) from the National Research Foundation, and the Intelligent Synthetic Biology Center of Global Frontier Project funded by the MESTMSIP (2011-0031964), Korea.
Keywords : cellobiose, 2,3-butanediol, metabolic engineering

H033

The Inhibitory Effect of ROS Production of Fucoidan Isolated from Brown Algae in Zebrafish Model

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Fucoidan, extracted from brown algae include Ecklonia cava and Miyouekui, has been extensively studied because of their various biological activities. Therefore we evaluated in vitro and in vivo antioxidative activities of ECF in this study. ECF exhibited more prominent effects in peroxyl radical scavenging activity, compared to that of the other scavenging activities. Thus ECF was further evaluated for its protecting ability against 2,2'-Azobis dihydrochloride induced oxidative stress in Vero cells and strongly reduced the AAPH-induced oxidative damage through scavenging in intracellular reactive oxygen species. Furthermore, we evaluated protective effect of ECF against AAPH-induced oxidative stress in a zebrafish model. ECF significantly reduced ROS generation, lipid peroxidation and cell death in zebrafish in vivo model. These findings indicate that it was revealed to have antioxidant activities in vitro Vero cells and in vivo zebrafish model, even though ECF is not polyphenol or flavonoid compounds and does not contain benzene rings or conjugated structures..
Keywords : algae, fucoidan, antioxidant

H034

Production of Rotavirus Virus like Particles in *Pichia pastoris* System

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Rotavirus is the major cause of acute gastroenteritis in children younger than 5 years old, worldwide. Rotavirus virus-like particles (VLP) don't have viral genetic material or infectivity. Therefore, it is safer and gives stronger protective immunity, as a triple layer structured antigen, than attenuated viral vaccines. It is attractive to produce rotavirus VLP using the methylotrophic yeast *Pichia pastoris* expression system. *P. pastoris* can utilize strong promoters for expression of foreign genes, thus enabling production of large quantities of protein with a relatively easy technology and at lower cost than with other eukaryotic expression systems. In this study, rotavirus VLP was produced using the *P. pastoris* system. The sequences of CAU200 VP2, CAU200 VP6 and CAU200 VP7 genes, isolated from patients, codon optimized to fit the *P. pastoris* expression system, were synthesized. The genes of the rotavirus structural proteins CAU200 VP2, CAU200 VP6, and CAU200 VP7 were cloned in *P. pastoris* strain (GS115) using pPIC6, pPICZ vectors and recombination to express three proteins in the same cell. The insertion of all three genes in the (VP2/VP6/VP7) transformant was confirmed by PCR. The molecular weight of intracellularly expressed VP2/VP6/VP7was, respectively,105, 45 and 37 kDa and was confirmed by SDS-PAGE and western blot. In the future, production of VLP with *P. pastoris* may lead to development of a commercialized vaccine worldwide and is a usefulvaccine candidate.
Keywords : Pichia pastoris, Rotavirus, expression

H035

Optimization of Protoplast Preparation and Transformation Efficiency in *Lentinula edodes*

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Recent studies on *Lentinula edodes* have been focused on the development of new and efficient varieties. This could be accomplished through molecular breeding utilizing protoplast fusion and transformation methods. In this study, we made an attempt to incorporate antibiotic resistance trait in *L. edodes* through transformation. We prepared protoplasts from *L. edodes* using our method and succeeded in increased number of protoplasts of about 3.4 x 10^7/ml. These protoplasts were then incorporated with antibiotic resistance gene hygromycin B. Wild type *L. edodes* was observed not to grow on media containing 5 /ml hygromycin B, whereas our transformants containing hygromycin B were observed to grow on media containing 5 and 10 /ml hygromycin B. The presence of hygromycin B was further confirmed through PCR analysis of the gDNA of transformants.
Keywords : *Lentinula edodes*, protoplast, transformation

H036

***In Vivo* Reconstitution of Membrane Protein by Caveollin1 co-expression : Towards Implantation of Eukaryotic Membrane Trafficking Pathways in Prokaryote**

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Caveolae is a membrane-budding structure which exists in many animal vertebrate cells. One of the important functions of caveolae is to form membrane curvature and endocytic vesicle. Recently, It was shown that caveolae could be formed in *Escherichia coli* by expressing caveolin-1 other membrane proteins overexpressed inside the cell. The heterologous caveolae may host other membrane proteins overexpressed inside the cell. We utilized this system for construction of proteo-liposome in *Escherichia coli*. SNARE proteins (Syntaxin1a, SNAP25, VAMP2) were introduced to prove our *in vivo* reconstitution system. Here, we show that the purified heterologous caveolae indeed contain the co-expressed membrane protein and the membrane protein were facing outward. The size of the purified caveolae with membrane protein reconstituted were measured by dynamic light scattering. The presence of VAMP2 & Syntaxin1a on this proteo-endosome was confirmed by Western blot analysis. Furthermore, membrane proteins (VAMP2 & Syntaxin1) embedded in caveolae retained its ability to form SNARE complex. Our study proposes in vivo membrane protein reconstitution system.
Keywords : membrane protein, caveolae, reconstitution, proteoliposome, SNARE protein

H037

Reconstruction of Fructose Metabolic Pathway for D-psicose Production in Corynebacterium Glutamicum

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D-Psicose is a C-3 epimer of D-fructose, and suggested as an ideal sucrose substitute for reduced-calorie sweetener. However, D-psicose is rarely found in nature and difficult to be produced by chemical synthesis. Recently, biological process using enzymatic and microbial reactions become increasingly significant for D-psicose production. Corynebacterium glutamicum has a unique mechanism of fructose metabolism. Fructose is mainly trasported and phosphorylated by fructose-specific phosphotranferase system (PTS) in C. glutamicum. However, it has been reported that myo-inositol transporter (IolT) can transport fructose in an unphosphorylated form. When expressing heterologous DPEase gene in C. glutamicum, the recombinant strain could directly convert the intracellular fructose to psicose. Therefore, to further increase the intracellular unphosphorylated D-fructose concentration we deleted the fructose enzyme II gene (ptsF), and overexpressed myo-inositol transporter gene (iolT) as a non-PTS fructose uptake system. In addition, we also constructed mutants of a repressor gene(iolR) for myo-inositol transporter and putative genes for fructose export from the cell, and examined the effect of these genes on D-psicose production. These genetic modification of fructose metabolism via reconstruction of fructose metabolic pathways could be a good strategy for producing rare sugars like D-psicose in C. glutamicum.
Keywords : *ptsF*

H038

Trichoderma sp. Enhances Soil Mineral Solubilization and Plant Growth Promotion On Acidic Soils of Nigeria

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Solubility of phosphorus (P) and potassium (K) compounds is largely affected by soil pH. Moreover, soil mineral supplementation relies heavily on the use of chemical fertilizer, which has a considerable negative impact on the environment. Recently, the genus *Trichoderma* has received considerable attention as soil mineral solubilizers and plant growth promoters. A pot study was conducted to evaluate the effect of *Trichoderma* sp. on P and K solubilization and plant growth at different pH levels (4-6) of acidic soil. Soil was treated with calcium carbonate to change soil pH and sorghum-sudan grass was established along with *Trichoderma* sp. inoculation in the closed pots. After 3 months, contents of soil available ortho-P and K and plant total P and K, and plant dry biomass were studied. Results showed that although the contents of available P and K were significantly enhanced by the increase of soil pH in both control and fungus inoculated soils, the fungus inoculation further enhanced the available P (36-48%) and K (23-39%) in soil and the plant total P (32-53%) and K (27-41%) compared to control soil. Thus, the use of *Trichoderma* sp. may improve plant P and K absorption and so reduce the use of chemical fertilizer.
Keywords : Mineral solubilization, Posphorus, Pant growth, Ptassium, *Trichoderma* sp

H039

Efficient Production of a Large-sized Type I Antifreeze Proteins in Recombinant *Escherichia coli*

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Antifreeze protein is a potent additive in cryogenic preservation of food products. In this study, synthetic type I antifreeze proteins (AFPs) were designed as multimeric structures and produced by *Escherichia coli*, an ideal host for the large-production of heterologous proteins. In this investigation, the genes of AFPs were artificially synthesized by a codon usage optimized program and constructed as 4-mer or 8mer. The functional AFPs were expressed at a high level by a protease deficient host, *E. coli* M15. These results present opportunities for the design and synthesis of novel ice-growth-inhibiting and antifreeze compounds. This study also provides an efficient tool to produce recombinant food peptide additive at a high level and overcomes the problem of natural resources limitation as well. [This work was supported by the Basic Science Research Program (2010-0008826) through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology]
Keywords : antifreeze protein, ice-binding protein, thermal hysteresis, *Escherichia coli*

H040

A Bacterial Surface Display System using the *Escherichia coli* Protein YiaT

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In a bacterial surface display system, successful recombinant protein display is highly dependent on the choice of anchoring motif. In this study, we developed an efficient *Escherichia coli* display system using novel anchoring motifs derived from the protein YiaT. To determine the best surface-anchoring motif, full-length YiaT and two of its C-terminal truncated forms, cut at the R_{181} and R_{232} sites, were evaluated. Two industrial enzymes, a lipase from *Pseudomonas fluorescens* SIK W1 and an ¥á-amylase from *Bacillus subtilis*, were used as the target proteins to be displayed. SDS-PAGE, Western blot, immunofluorescence microscopy, and whole-cell enzyme activity measurements confirmed the expression of the fusion proteins on the *E. coli* surface. Using $YiaTR_{181}$ or $YiaTR_{232}$ as the anchoring motif, the fusion proteins showed very high enzyme activities and did not exert any adverse effects on cell growth or on the outer membrane integrity. Additionally, these fusion proteins were suitable for displaying proteins of large molecular size in an active form. These results suggest that YiaT can be used as an *E. coli* anchoring motif to efficiently display various enzymes; this system could be employed in a variety of biotechnological and industrial applications. [This work was supported by the Basic Science Research Program (2010-0008826) through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology]
Keywords : cell surface display, outer membrane protein, YiaT, *Escherichia coli*

H041

The Putative Endoglucanase PcEg5A from *Phanerochaete chrysosporium* is a Novel Manganese Dependent Endoglucanase

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The cDNA encoding a putative glycoside hydrolase family 5, which has been predicted to be an endoglucanase (*PcEg5A*), was cloned from *Phanerochaete chrysosporium* and expressed in *Pichia pastoris*. PcEg5A contains a carbohydrate binding domain and two important amino acids, E209 and E319, playing as proton donor and nucleophile in substrate catalytic domain. SDS-PAGE analysis indicated that the recombinant endoglucanase 5A (rPcEg5A) has a molecular size of 43 kDa which corresponds with the theoretical calculation. Optimum pH and temperature were found to be 4.5-6.0, and 50°C-60°C, respectively. Moreover, rPcEg5A exhibited maximal activity in the pH range of 3.0-8.0, whereas over 50% of activity still remained at 20°C and 80°C. rPcEg5A was stable at 60°C for 12 hours incubation, indicating that rPcEg5A is a thermostable enzyme. Manganese ion enhanced the enzyme activity by 177%, indicating that rPcEg5A is a metal dependent enzyme. The addition of rPcEg5A to cellobiohydrolase and β-glucosidase resulted in a 153% increasing saccharification of NaOH-pretreated barley straw, whereas the glucose release was 147% higher than that cellobiohydrolase and β-glucosidase treatment alone. Our study suggested that rPcEg5A is an enzyme with great potential for biomass saccharification.
Keywords : Endoglucanase, *Phanerochaete chrysosporium*, Manganases dependent enzyme, barley straw, saccharification

H042

Comparative Analyses of Two *Bacillus* Mannanases Produced from Recombinant *Escherichia coli*

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Since the 1990s, β-mannanases have emerged as key enzymes in the biotechnology industry including food, feed, paper, bioenergy and laundry industries. Many attentions have been paid on the acidic mannanases due to their potential for applications in feed and food industries. Two genes encoding the mannanase of *Bacillus* sp. YB-1401 and *B. amyloliquefaciens* YB-1402, which had been isolated in acidic pH as mannanase producers, were cloned and sequenced in *Escherichia coli*, respectively. Both mannanase genes consisted of 1,080 nucleotides encoding polypeptide of 360 amino acid residues. The deduced amino acid sequences of the two mannanase genes showed four amino acid residues different from each other, and were highly homologous to those of mannanases belonging to the glycosyl hydrolase family 26. Comparison of two mannanases produced from recombinant *E. coli* revealed that His-tagged mannanasse of YB-1402 was more stable than that of YB-1401 at acidic pH and high temperature with retaining greater than 50% of its maximal activity after pre-incubation at pH 3.0 for 4 h. It is also worthy to notice that thermostabilities of the two mannanases were enhanced in the presence of Ca^{2+} ion.
Keywords : *Bacillus*, Mannanase, Gene, Acidic stability

H043

Production and Properties of Mannanases from Two *Bacillus* Strains Isolated in Acidic Medium

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Mannanases, which catalyze the random hydrolysis of the β-D-1,4-mannopyranosyl linkages within the backbone of various mannan-based polysaccharides, are enzymes useful in food, feed, paper, and laundry industries. Two *Bacillus* strains capable for hydrolyzing locust bean gum were isolated as producer of the extracellular mannanase by direct screening or enrichment culture in acidic medium from homemade soybean pastes. The isolate YB-1401 showed biochemical identity of 61.1% with *Brevibacillus laterosporus,* while the nucleotide sequence of its 16S rDNA showed the highest similarity with that of *Bacillus amyloliquefaciens.* The other isolate YB-1402 has been identified as *Bacillus amyloliquefaciens* on the basis of its 16S rDNA sequence and biochemical properties. The molecular masses of mannanases produced by both isolates were identical to be approximately 38.0 kDa on SDS-PAGE. Their productivities were drastically increased by growing the strains in the medium supplemented with konjac. Thermostabilities, acidic stabilities and optimal temperature of the mannanases were somewhat different from each other, while no difference was detected between the hydrolyzates of locust bean gum and mannooligosaccharides with the mannanases.
Keywords : *Bacillus*, Mannanase, Production, Property

H044

Investigation of Physicochemical Fermentation Conditions for Enhanced Hyaluronic Acid (HA) Production by High-Yielding Mutant Strains of *Streptococcus zooepidemicus*

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Hyaluronic acid(HA) is a high molecular weight linear polysaccharide, used as a vitreous replacement, eye drop formula and a moisturizer in cosmetic formulations. In order to enhance HA productivity in bioreactor fermentations of *Streptococcuszooepidemis*, both strain improvement and bioprocess optimizations were carried out. High-yielding mutants screened through UV and NTG treatments showed significantly higher HA productivity compared to the corresponding mother strains. Medium optimization was also carried out in order to find an optimal composition of the production medium. For this purpose, efficient statistical methods were adopted, such as full factorial design (FFD), steepest ascent methods (SAM) followed by response surface methods (RSM). In addition, physiochemical fermentation conditions such as inoculum state, dissolved oxygen level, temperature and pH-control were intensively investigated using 5L stirred tank bioreactors. It was revealed that pH-control around 7.5 and sufficient supply of dissolved oxygen through optimization of agitation speed and aeration rate were crucial for remarkable enhancement in HA productivity. As a result, approximately 3-4 fold increase in HA production level was obtained in the 5L bioreactor cultures performed under the optimized fermentation conditions with the high-yielding mutant strains.
Keywords : Hyaluronic acid, Medium optimization, dissolved oxygen level, pH-control, mutant strains

H045

Addition of Cyanobacterial Carbonic Anhydrase as a Production-Medium Component for Overproduction Of Succinic acid by High-Yielding Mutant Strains of *Actinobacillus succinogens*

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Succinic acid, a four-carbon dicarboxylic acid, is produced as a major fermentation product during anaerobic metabolism by bacterial *Actinobacillus succinogenes,* and is used in a wide range of applications. In the previous paper, we reported that succinic acid productivity was significantly enhanced when the commercial enzyme of carbonic anhydrase (of human origin) was supplemented as a medium component into the production medium. In this study, because of too high price of the commercial carbonic anhydrase, it was undertaken to economically overproduce a cyanobacterial carbonic anhydrase by the use of a recombinant *Pichia pastoris*. An expression vector system was constructed with the carbonic anhydrase gene PCR-cloned from *Cyanobacterium synechocystis sp*., and introduced into *P. pastoris* for fermentation studies. About 96.5 g/L of succinic acid was produced in the production medium with 30 ppm of carbonic anhydrase, approximately 2 fold higher productivity compared to the parallel process with no supplementation of the enzyme. These impressive results were assumed to be due to the enhanced CO_2 gas mass transfer rate(k_La), and especially due to the increased reaction rate catalyzed by the supplemented enzyme (i.e. rapid conversion of aqueous CO_2 to HCO_3^-, which can be far more easily transported into the producing cells than the aqueous CO_2).
Keywords : Succinic acid, carbonic anhydrase, CO_2 gas mass transfer rate, *P. pastoris, Actinobacillus succinogenes*

H046

Rapid and Large Screening of Succinic Acid High-Yielding Mutants of *Actinobacillus succinogenes* through High Throughput System(HTS)

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Department of Molecular Bioscience, Kangwon National University

Succinic acid utilized as a precursor of many industrially important chemicals is a four-carbon dicarboxylic acid, biosynthesized as an intermediate of TCA cycle. Succinic acid is a major fermentation product by *Actinobacillus succinogenes*. In this study, in order to enhance the productivity of succinic acid, intensive and rational programs were performed for strain improvement with a large number of mutated strains. Rapid and large screening process was possible by adopting a high throughput system(HTS), which consists of CO_2- and humidity-controlled incubator and 24-well microplate-culture system for miniaturized fermentations inside the incubator. Above all, actively proliferating cells were selected based on the growth extent of each mutant strain in the miniaturized fermentations. A EMS-mutated strain (UK13) could be finally obtained through application of various rational screening strategies, producing almost industrial level of succinic acid (around 110 g/L) in batch bioreactor fermentations. Notably, a *PckA* gene (phosphoenol pyruvate carboxykinase) transformed mutant was revealed to produce almost same level of succinic acid as UK13 in a stable manner under the optimized bioreactor conditions.
Keywords : Succinic acid, HTS, EMS-mutated strain, *PckA* gene, *Actinobacillus succinogenes*

H047

Development of Succinic Acid Production Medium through Extensive Application of Statistical Optimization Process

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Studies on the production of succinic acid have been carried out in anaerobic fermentations of *Actinobacillus succinogenes*. Succinic acid utilized as a precursor for many industrially important chemicals is a four-carbon dicarboxylic acid, biosynthesized as an intermediate of TCA cycle and also as one of the fermentation products of anaerobic metabolism. In this study, for enhancing succinic acid production, extensive medium optimization process was carried out in order to find optimal medium compositions, using the statistical methods such as Plackett-Burman design, fractional factorial design(FFD), steepest ascent method(SAM), and finally response surface methods(RSM). Especially, in order to develop an economic production medium, corn steep solid and corn steep liquor known as very cheap carbon- and nitrogen-sources were intensively investigated. It was also found that the production level of succinic acid was significantly enhanced by optimal CO_2-supply into the fermentation broth, demonstrating that CO_2 gas mass-transfer rate(k_La) is a crucial factor in the succinic acid fermentation bioprocess.
Keywords : *Actinobacillus succinogenes*, Succinic acid, medium optimization, CO_2-supply, CO_2 gas mass-transfer rate

H048

Continuous Production Of Succinic Acid by *Actinobacillus succinogens* using Cell-Recycled Fermentors of Various Decantor types

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In order to enhance volumetric productivity of succinic acid produced by bacterial cells of *Actinobacillus succinogenes*, cell-recycled continuous fermentations were carried under the various operating conditions. Cell-recycled continuous operations were possible, since the efficient decantor system (i.e., cell separator) developed in our laboratory could separate high density of the suspended cells effectively from the outlet stream, thus overcoming the wash-out phenomenon encountered at relatively low dilution rate in the continuous fermentation process without cell-recycling. It was found that the decantor system could recover more that 65% of the suspended cells present in the culture broth of the outlet stream of the continuous fermentation system. The strategy of feeding increased amount of carbonate (i.e., $MgCO_3$) through the inlet stream of the cell-recycled continuous fermentation system (CRCFS) turned out to be very efficient for enhanced production of succinic acid, resulting in about 10 fold, and more than 6 fold higher volumetric productivity (DP, g succinic acid/l/hr) than the corresponding batch and continuous fermentation systems without cell-recycling, respectively. By employing the CRCFS for continuous production of succinic acid, a major reduction factor in the reactor size was found to be around 8.72, as compared to the corresponding batch fermentation system.
Keywords : *Actinobacillus succinogenes*, succinic acid, decantor system, $MgCO_3$, CRCFS

H049

Properties of β -Galactosidase from Three *Bacillus licheniformis* Isolates

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β-Galactosidase has been used in food industry for production of galactooligosaccharides as well as hydrolysis of lactose in milk and whey. Enzymes derived from food grade organisms are usually regarded to be beneficial for application in the food industry. In this work, three bacterial strains were isolated from homemade fermented soy products such as Cheongkookjang and Doenjang as producers of the β-galactosidase, capable of hydrolyzing lactose to liberate galactose and glucose residues. The three isolates have been identified as *Bacillus licheniformis* on the basis of their 16S rDNA sequences and biochemical properties. β-Galactosidase activities of the culture filtrates were the most active to *para*-nitrophenyl-β-D-galactopyranoside(pNP-βGal) under reaction conditions ranging pH 5.5-6.5 and temperature 50-60°C. The hydrolyzing activity of β-galactosidases for pNP-βGal was dramatically decreased by the addition of low concentration of galactose, but was marginally decreased by high concentrations of glucose. Interestingly, their activities were slightly increased by xylose within concentration of 100 mM.
Keywords : β-Galactosidase, *Bacillus licheniformis*, Isolation, Property

H050

Production and properties of *β*-Galactosidase from Two *Bacillus licheniformis* Isolates from Doenjang

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α-Galactosidase has been known to be an useful enzyme capable of hydrolyzing α-galactooligosaccharides such as mellibiose and raffinose causing occasionally flatulence to human body. It can also play an important role in complete degradation of galactomannan. Two strains of *Bacillus licheniformis*, isolated from homemade fermented soybean paste, produced extracellular α-galactosidase at different level according to carbon and nitrogen after late logarithmic phase. Wheat bran and yeast extract were the most effective for production of α-galactosidase from both strains. The enzyme production was increased more by insoluble fraction than soluble extract of wheat bran. α-Galactosidases produced by two isolates were the most active to *para*-nitrophenyl-α-D-galactopyranoside (pNP-αGal) under identical reaction condition of pH 5.5-6.0 and temperature 45°C. The hydrolyzing activity of α-galactosidases for pNP-αGal was significantly decreased by sugars including glucose, mannose, galactose, xylose and ribose. Particularly, the enzymes were drastically inhibited in the presence of low concentration of ribose and galactose. The enzyme was able to hydrolyze efficiently raffinose and stachyose contained in the soluble extract of soybean meal.
Keywords : β-Galactosidase, *Bacillus licheniformis*, Production, Property

H051

Molecular Cloning and Characterization of a β-Mannosidase from *Paenibacillus woosongensis*

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Mannan, a major component of hemicellulose, is a highly branched β-1,4-linked D-mannose polymer with substituents that include acetyl and galactosyl groups. Mannanase degrades mannan into manno-oligosaccharides by attacking internal mannosidic linkages on the mannan backbone, and β-mannosidase subsequently hydrolyzes mannobiose and short manno-oligosaccharides into D-mannose by endwise attack. Recently, numerous microbial mannanases were reported, but few β-mannosidases have been reported from microorganisms. A gene encoding the β-mannosdiase of *Paenibacillus woosongensis* was cloned and sequenced. The β-mannosdiase was fused to hexahistidine residues in its C-terminus. Expression level of the gene was higher in *E. coli* BL21(DE3) CodonPlus than *E. coli* BL21(DE3). His-tagged enzyme, purified from cell-free extract of recombinant *E. coli* carrying the β-mannosidase gene by Ni-NTA column chromatography, showed maximal activity at pH 5.5 and 45°C for hydrolysis of *para*-nitrophenyl-β-mannopyranoside. As results of hydrolysis for mannooligosacchrides including mannobiose, mannotriose, mannotetraose, mannopentaose and monnohexaose by β-mannosidase, the mannose was liberated from them, assuming the β-mannosidase is an exo-type enzyme. The enzyme activity was significantly inhibited by ribose, but slightly inhibited by hexoses including glucose, galactose and mannose.
Keywords : β-Mannosdiase, *Paenibacillus woosongensis*, Gene expression, Purification, Property

H052

Cellulolytic Enzyme Production by Marine-derived Fungi and Their Halo-stability

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Halo-stable cellulases can be utilized in high salt and high osmotic pressure environments thereby reducing water consumption. They have a great potential in treatment of agricultural wastes and bioremediation of cellulosic materials in conditions of low water activity. The aim of this study was to investigate the potential of marine-derived fungi for the production of halo-stable cellulases. Eighteen fungal strains including 11 different species were used, which included 17 ascomycetes, and a zygomycete. To screen valuable fungi producing halo-stable enzyme, cellulolytic enzyme activities were investigated. The majority showed endo-glucanase (EG) and β-glucosidase (BGL) activities, especially *Dendryphiella salina*, *Fusarium Equiseti*, *Penicillium chrysogenum*, *Stagonosporopsis heliopsidis*, and *Trichoderma hamatum* produced high level of EG. Among the fungi *T. hamatum* showed filter paper unit (FPU). Halo-stability was evaluated by EG derived from the above five fungi that produced high level of EG. Interestingly, EG activity derived from *P. chrysogenum* was increased up to 126% at 0.25 M NaCl assay. It was vastly superior to commercial enzyme under hypersaline condition. The present investigation assumes significance in the production of halo-stable cellulase from *P. chrysogenum*, which would contribute to overcome hypersalinity condition of various industrial applications.
Keywords : ascomycete, cellulase, marine, zygomycete

H053

Enhanced Thermostability and Cellulolytic Efficiency by Increasing Hydrophobicity via Introduction of Arginine and Proline on Catalytic Domain of Mesophilic Family 9 Endoglucanase

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Thermostability is important in cellulase during industrial saccharification process after pretreatment with steam. In this study, mesophilic endoglucanase EngZ from *Clostridium cellulovorans* was mutated with lysine (K) to arginine (R) at a 94 residues and serine (S) to proline (P) at a 365 residues by site direct mutagenesis. The thermostabilities of K94R and S365P at 70 °C were significantly improved residual activity compared with wild type. Thus, we created double mutant as a K94R/S365P. The K94R/S365P had 7.6-times cellulolytic efficiency at 42.5 °C as the optimal temperature of wild-type and 44.8-times higher residual activity at 70 °C than that of wild-type, respectively. Increasing structural hydrophobicity of the K94R/S365P, about 3.3-times higher than wild-type, was detected by differential scanning fluorometry analysis with hydrophobic interaction fluorescent SYPRO Orange at 25 to 95 °C. The increasing rate of aggregation temperature of K94R/S365P was 3.1-times higher in presence of calcium ion than wild type. Enhanced Ca-binding affinity could relate with increased thermostability at the high temperature of K94R/S365P. The K94R/S365P mutant induced synergic effect on improvement of thermostability with high cellulolytic efficiency and maintenance the structure related with Ca-binding at the high temperature.
Keywords : Thermostability, Cellulolytic efficiency, Family 9 endoglucanase, Hydrophobicity, calcium binding

H054

Domestic Silkworm Strain Screening using Bombyx mori Nuclear Polyhedrosis Virus (BmNPV)

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Bombyx mori nuclear polyhedrosis virus (BmNPV) expression systems is one of the most efficient methods for the easy, low-cost, and large-scale production of recombinant proteins such as enzymes, antibodies, hormones, vaccines and difficult expression proteins. For use as a silkworm factory, we have searched for high permissive silkworm strains with higher production levels of a heterologous protein. In this study, we constructed BmNPV vector with luciferase (Luc) as a marker and confirmed the translation and transcription expression by Luc activity and Western blotting and the RT-PCR, respectively. For a selection of superior silkworm strain as a host, we had selected ten high-permissive and five low-permissive strains from domestic seventy-five strains. Also, in order to find out which an F1 hybrid of these strains would have hybrid vigor or heterosis, F1 hybrids of ten strains have tested Luc activity. This time, an example of luciferase protein production using silkworm larvae was summarized in this report. * This work was supported by the Bio-industry Technology Development Program, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (No: 111062-03-1-HD110; 111116–01–1-SB010).
Keywords : Baculovirus, silkworm, luciferase, recombinant protein

H055

One-Step Synthesis of Cellulose/Silver Nano-Biocomposites using Solution Plasma Process and Characterization of Their Antimicrobial Activity

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Solution plasma process (SPP) is a one-step synthesis technique used for rapid generation of ultra-pure, stable, and uniform nanoparticles in solution with plasma application. In this present study, silver nanoparticle (AgNPs) biocomposites were synthesized in cellulose by discharging plasma for 180s using a pulsed unipolar power supply into the solutions of cellulose (1-3%) and $AgNO_3$ (1-5mM) at 800V with frequency of 30kHz. 3D scaffolds of cellulose/AgNP biocomposite were prepared by lyophilization and they were cross-linked by UV irradiation. Intensity and purity of the biocomposites were examined by UV-Vis and FTIR spectrum. . 3D scaffolds with microporous structures were observed using FE-SEM equipped with EDS and topographic evaluation evidenced that the spherical shaped AgNPs were encrusted on the cellulose matrix. TEM analysis showed that spherical AgNPs in the size range of 5-20 nm were well distributed throughout the matrix without agglomeration in both of the C3Ag3 and C3Ag5 biocomposites. The biocomposite of C3Ag5 had antimicrobial activity against various pathogens with minimal inhibition concentration of 20-40 ㎍/ml for bacteria and 81.5-340 ㎍/ml for fungi. Furthermore, qualitative and quantitative inhibitory patterns against the pathogens were studied by Kirby-Bauer assay and CFU reduction assays. From the results, the cellulose/silver nano biocomposites synthesized by eco-friendly SPP can be utilized as topical antimicrobial agents in the modern medicine.
Keywords : Solution plasam process, Siver nanoparticle, Cellulose, Biocomposite, Antimicrobial activity

H056

Characterization of a Highly Thermostable Endo-1, 4-β-Xylanase from *Thielavia terrestris*

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The filamentous fungus *Thielavia terrestris* which is classified as thermophiles, having a maximum growth temperature at or above 50°C, is well known to degrade plants cell wall. The Endo-1,4-β-xylanase TT (xynTT) gene belong to the fungal glycosyl hydrolase family 11 was cloned into pPCZαA and expressed in *Pichia pastoris* GS115. Sequence size of xynTT is composed of 672 bp encoded 222 amino acids with a molecular weight of 25 kDa. Enzyme assay of XynTT demonstrated that optimum pH and temperature were 5 and 70°C on beechwood xylan, respectively. XynTT showed wide range pH stability from 3 to 8. It showed a high level thermal stability since it retained about 90% activity at 50 °C for 1h. In the presence of metal ions such as Ca^{2+}, Li^{2+}, the activity of the enzyme enhanced. However, in the presence of metal ions such as Hg^{2+}, the enzyme activity was significantly inhibited. This result show that XynTT stability at high temperature and wide range pH make it potentially effective for industrial applications.
Keywords : Thermostable, Endo-1,4-β-xylanase, *Thielavia terrestris*, Characterization

H057

Increasement of Paromomycin Production by *Streptomyces* sp. AG-P1441 Mutants

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Streptomyces sp. AG-P1441 could produce an aminoglycoside antibiotics, paromomycin. As a biocontrol agent, *Streptomyces* sp. AG-P1441 shows antifungal, antioomycete activity against *fungi*, *phytophthora* and *pythium* species, due to the bioactivity of paromomycin. Ultraviolet radiation and N-methyl-N'-nitro-N-nitrosoguanidine (NTG) treatment were conducted for acquiring better mutants which could produce more paromomycin comparing with *Streptomyces* sp. AG-P1441. We obtained 28 mutants totally. Using test organism, each mutant's antibacterial ability was reflected from the inhibition zone sizes. Among them, mutant 4, 18, 19 and 23 show bigger inhibition zone than the original strain. And as final result, paromomycin production was increased much by mutant 19 ultimately. This research was supported by a grant (10045326) from the R&D program of MOTIE/KEIT of Republic of Korea.
Keywords : *Streptomyces* sp. AG-P1441, paromomycin, Ultraviolet radiation, NTG, mutants

H058

Novel Biosurfactant Produced by *Aureobasidium pullulans* L3 Isolated from Flower of Tiger Lily, *Lilium lanciforlium* Thunb.

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Yeast biosurfactants are important biotechnological products in the food industry, and they have medical and cosmeceutical applications owing to their specific modes of action, low toxicity, and applicability. Thus, we have isolated and examined biosurfactant-producing yeast for various industrial and medical applications. A rapid and simple method was developed to screen biosurfactants -producing yeasts for high production of eco-friendly biosurfactants. Using this method, several potential samples of biosurfactant-producing yeasts, such as wild flowers, were investigated. We successfully selected a yeast strain, *Aureobasidium pullulans* L3, with potent surfactant activity from a tiger lily, *Lilium lancifolium* Thunb. was cultured by addition of glycerol as a carbon source, and its culture filtrate exhibited high surface tension ability. By the bioassay-guided fractionation, two high-surface-tension compounds were isolated from the culture supernatant of *A. pullulans* L3. Their chemical structures were determined to be novel compounds with molecular formulae of $C_{21}H_{40}O_7$ and $C_{21}H_{40}O_6$, respectively, by mass and NMR spectroscopic analyses. These compounds exhibited potent biosurfactant ability with surface tension activity of 29.5 and 36.4 dyne/cm, respectively.

Keywords : Glycolipid, Biosurfactant, *Aureobasidium pullulans*, Wild flower

I001

GNB (galacto-*N*-biose) Reduces Adherence of Enteropathogenic *Escherichia coli* to the Intestinal Epithelial Cell

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Carbohydrates are fundamental components of a diversity of glycolipids, glycoproteins and human milk oligosaccharides. β-1,3 linked galactosides such as GNB (Galacto-N-biose, Gal β-1,3 GalNAc) are important carbohydrate structure in nature. It plays a crucial role in cell adhesion, involving signaling, fertilization, differentiation .
To efficiently produce GNB (galacto-N-biose, Gal β-1,3 GalNAc), inexpensive starting materials (UDP) are reacted by a sugar nucleotide cycling (SNC) with trehalose synthase, UDP-glucose-4-epimerase and β-1,3-galactosyltransferase and their recombinant E. coli whole cells. The transfer product secreted from three enzymes and from the recombinant E. coli BL21 whole cells of three enzymes is analyzed by TLC and HPAEC. The reaction product is isolated by paper chromatography and Bio-Gel P2 chromatography. The reaction product is identified GNB (galacto-N-biose, Gal β-1,3 GalNAc) by MALDI-TOF MS. The ability of GNB to inhibit attachment of microcolony-forming Enteropathogenic Escherichia coli (EPEC) is investigated. A 10~30mM GNB (galacto-N-biose) reduces the adherence of EPEC from 20.8% to 81.5%. GNB increases 1.4 times for the adherence of bifidobacteria. The present study suggests that GNB could be an effective sugar substitute in the industry as well as inhibit the adherence of EPEC and increase the adherence of bifidobacteria to the intestinal epithelial cell.

I002

Hepatoprotective Effect Of Probiotics in a Rat Model of Acute Liver Injury

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Probiotics are live microbial food supplements or components of bacteria which have been shown to have beneficial effects on human health. Our aim was to evaluate the hepatoprotective activity of *Bifidobacterium adolescentis* SPM0212 which has been reported to have potentially anti-hepatitis B virus activity in our previous study. The present study was carried out using male Wistar albino rats and the probiotics was treated orally for 9 days continuously and acute liver injury induced by a administration of carbon tetrachloride (CCl_4) on 7th and 8th days. As a result, this probiotics has significantly reduced the elevated levels of SGOT and SGPT by close to normal values. Also, the probiotics reduced the negative effect of the toxicant on body and organ weights. In the histopathological study, the livers of the CCl_4-intoxicated rats showed a nearly complete loss of normal hepatocytes architecture, but those of the rats treated with probiotics showed minimal damage and their hepatocytes architecture was normal. It should be concluded that *B. adolescentis* SPM0212 was able to protect from hepatic injury by CCl_4-intoxication and these effects of *B. adolescentis* SPM0212 was compared with silymarin, the known hepatoprotective drug. We suggest that *B. adolescentis* SPM0212 be considered useful probiotics for protecting the liver from xenobiotics and hepatitis B virus, and as well as useful as a functional food for maintaining human health
Keywords : Carbon tetrachloride, *Bifidobacterium adolescentis*, Hepatoprotective activity, Probiotics, Silymarin

I003

Broad Spectrum Antiviral Activities of Probiotics against Human Enteric Viruses

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Enteric viruses primarily infect the intestinal tract and they are the commonest causes of gastroenteritis worldwide. However, there are no effective antiviral chemotherapeutic agents or vaccines. Probiotics have been defined as viable microorganisms that have a beneficial effect in the prevention or treatment of specific pathologic conditions. The use of probiotics to enhance intestinal health has been proposed for many years. Therefore, we evaluated the potential antiviral activities of probiotics against enteric viruses such as rotavirus, adenovirus, coxsackievirus, and echovirus. The antiviral activities of probiotics isolated from Koreans on enteric viruses were investigated using plaque assay and quantitative real-time PCR (RT-qPCR) assay. As a result, plaque assay showed that *Lactobacillus ruminis* SPM0211 and *Bifidobacterium adolescentis* SPM1608 had strong inhibitory effects against rotavirus and echovirus, respectively. Also, RT-qPCR assay showed that *L. ruminis* SPM0211 and *B. anavdolescentis* SPM1605 had the greatest inhibitory effects against adenovirus and coxsackievirus, respectively. These findings suggest that *L. ruminis* SPM0211, *Bifidobacterium adolescentis* SPM1608, and *B. adolescentis* SPM1605 may be a useful for the treatment or prevention of viral disease by these enteric viruses.
Keywords : Rotavirus, Adenovirus, Coxsackievirus, Echovirus, Probiotics

I004

Monitoring of Hygiene Indicator and Food Poisoning Microorganisms in Salted Fermented Food

Ho-Won Chang, Young Ihl Kim, Hae Ji Kim, Minjung Lee, Jin-Hee Kim, Eunjung Kim, Tae-Hyeon Koo, Jinwook Jang, Ji Yong Kim, Hoyong Lee, Yeongmin Shin, Kisung Kwon, and Ju-Young Park*
Busan Reginonal Korea Food and Drug Administration

Jeotgal in Korea Food Code is defined as a traditional salted fermented seafood made of seafood, with variety of fish intestines. Various microorganisms are associated with the jeotgal fermentation process. The purpose of this study was to evaluate hygiene of jeotgal retailed in Korea market and to improve specifications through examination of the hygiene indicator and food poisoning microorganisms contamination. A total of 200 samples of jeotgal were collected from various producers and retailers. Based on the methods in Korea Food Code, we examined the aerobic viable bacteria, coliform group, Escherichia coli, Bacillus cereus, Staphylococcus aureus, Vibrio parahaemolyticus and Clostridium perfringens.Aerobic viable bacteria (100.0%), coliform group (12.0%) and Cl. perfringens (7.5%) in jeotgal were detected at the level of 1.0~7.4 log CFU/g, 0~2.3 log CFU/g and 0~1.2 log CFU/g, respectively. E. coli, S. aureus, and B. cereus V. parahaemolyticus were not detected in all samples. The results of this study can be utilized as basic data for improving standards and specifications in jeotgal.
Keywords : Jeotgal, Korea Food Code

I005

Increase of the Platycodin D Concentrations of the Balloonflower Extract, Caused by Fermentation and the Antibacterial Ativity Against *Klebsiella Pneumoniase*.

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Bioland

This study was to increase the concentrations of platycodin D, a major components of the balloonflower extract by fermentation. The only balloonflower extract with no medium used to the bacterial cultures for finding an appropriate bacteria. Examined the increasing of the platycodin D concentrations in the balloonflower extracts and the antibacterial activity against K. pneumoniase. cultrured the balloonflower extract seeded L. casei, L. plantarum, p. pentosaceus, B. subtilis and S. cerevisiae. After culture, there was not a significant growth of L. casei, L. plantarum, p. pentosaceus and B. subtilis. But *S. cerevisiae* cell increased from 107CFU/ml to 109CFU/ml of the balloonflower extract. While the unfermented balloonflower extract contained 2.56mg/g of platycodin D, the balloonflower extract fermented by S. cerevisiae contained 4.33mg/g of platycodin D. As we have seen, the platycodin D concentrations increased due to fermentation by *S. cerevisiae*. The result of antimicrobial activity against *K. pneumoniase* showed that bacterial growth rate fell by more than 80% at the over 3% balloonflower extract. Besides, 80% reduction rate at the 0.05% balloonflower extract. Highest rate of reduction (100%) was shown at the over 5% balloonflower extract.
Keywords : Platycodin D, Balloonflower, Ferment, *Klebsiella Pneumoniase*, Bacteria

I006

Detection of Antifungal Activities from Pomegranate

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To isolate a novel antifungal compound we obtained Pomegranate in Goheung, Junnam and examined their antifungal activities for 70% ethanol extracts. Seed, Peel and whole of Pomegranate were separated and extracted with 70% ethanol solution. Three kinds of extract showed similar antifungal activity against Candida albicans when they grew in liquid media. However extract of whole portion showed relatively high activity in solid media. We further fractionated the extracts with chloroform and then ethyl acetate. The ethyl acetate fraction exhibited the highest anti-fungal activities when those fractions were examined for the growth inhibition of Candida albicans with disc diffusion method. The ethyl acetate fractions of whole and peel portion each showed at least 36% and 25% growth inhibition of Candida albicans, Candida glabrata, Candida tropicalis, Candida lusitaniae in liquid media. These results indicate that Pomegranate contain antifungal compounds soluble with organic solvent.
Keywords : antifungal, Candida, ethyl acetate

I007

Anti-Inflammatory Activity of Probiotics Isolated from Fermented Foods

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In current, probiotics are considered as a useful microbe and they can balance microbial distribution in animal intestine. Actually, many of them are founded in Korea traditional fermented foods, such as Kimchi or Makgeolli. In this study, 23 kinds of microbes were isolated from Kimchi and Makgeolli and identified using 16S rDNA sequencing. The each identified bacteria, *Lactobacillus, Leuconostoc* and *Bacillus* species, was cultured for 48 h and then the supernatants were harvested for treatment. The cytotoxicity or nitric oxide (NO) production were investigated against mouse macrophage RAW 264.7 cells. NO production was significantly decreased in LPS-activated RAW 264.7 cells treated with supernatants of FG-1S, FG-6S or FG-13S microbes without affecting cell viabilities. Furthermore, the expression of pro-inflammatory genes such as *COX-2, iNOS* and *TNF-α* was dramatically reduced by the culture supernatants treatment. In conclusion, probiotics of Korea traditional fermented foods can be considered as an effective resources for development of functional foods or drugs which possess anti-inflammation activity.

I008

Anti-proliferative and Anti-inflammatory Activities of Solvent Fractions of Lees Extracts

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In the previous study, we prepared eighty-five different kinds of solvent fractions of lees extracts and investigated their effects on cell viability in human colorectal cancer HCT116 cells and NO (Nitric oxide) production in mouse macrophage RAW 264.7 cells. Among them, three solvent fractions (KSD-E1-3, KSD-E2-3 and KSD-E4-3) were selected for further experiments. And also, we prepared additional eleven fractions from KSD-E1-3 and KSD-E4-3 extracts using various organic solvents. And, we investigated the effects of those fractions on cell viability and NO production using HCT116 cells and RAW 264.7 cells, respectively. Among treated fractions, KSD-E1-3-3 dramatically decreased cell viability and increased expression of anti-proliferative genes such as *ATF3*, *NAG-1*, *DDIT3* and *p21* genes. And, KSD-E4-3-2 significantly decreased NO production in LPS-activated RAW 264.7 cells without affecting cell viabilities. Also, they reduced the expression of pro-inflammatory genes such as *COX-2*, *iNOS* and *TNF-alpha*. Overall, our results suggest that lees can be a novel resource for the development of functional foods and cosmetics which possess anti-proliferative and anti-inflammatory activities.
Keywords : Lees, Anti-inflammatory, Anti-proliferative, RAW264.7 cells, HCT116 cells

I009

Investigation of Microbial Contamination Levels in noodles, Job's Tears Teas and Salad Dressings

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This study was performed to investigate the microbiological contamination levels in noodles, Job's tears teas and salad dressings for evaluating the previous guideline and further re-establishing the microbial regulatory standards of foods. First, we collected 96 samples (30 noodles, 16 Job's tears teas and 50 salad dressings) according to the sampling plans recommended by The International Commission on Microbiological Specifications for Foods (ICMSF) in metropolitan region of South Korea. We next tried to check the levels of microbial contamination of indicator organisms (total aerobic bacteria, *E. coli* and *coliforms*) as well as the levels of food-borne illness organisms: *Staphylococcus aureus* (S.*aureus*), *Bacillus cereus* (B.*cereus*) and *Clostridium perfringenes* (C. *perfringenes*) with the specified protocols. The total aerobic counts of noodles, Job's tears teas and dressings were found at the level of 1.34, 2.10 and 0.19 logCFU/g, respectively. In case of food-borne illness organisms, B. cereus was detected in the Job's tears teas and dressings at the level of 0.22 and 0.01 logCFU/g, respectively. It is considered safe because the detected level of B. cereus is less than the criteria of standards provided by Korea Food Code. Other indicator organisms and food-borne illness organisms were not detected. These results of this study can be a basic data to reset the microbial standards in Korea Food Code.

I010

Microbial Contamination Level in Livestock Manure Compost and Liquid Pig Manure

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Organic foods may be more susceptible to microbiological contamination because of the use of livestock manure compost and organic fertilizers. This study was undertaken to assess the microbiological quality and prevalence of pathogens in livestock manure compost and liquid pig manure produced in Korea. Livestock manure compost and liquid pig manure were analyzed for the presence of total aerobic bacteria, *Escherichia coli,* coliforms, *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, and *Cronobacter sakazakii.* The total aerobic plate counts in the livestock manure compost and liquid pig manure were in the range of 5 to 10 log CFU g^{-1} and 2 to 9 log CFU g^{-1}, respectively. In the livestock manure compost, coliforms and *E. coli* were detected in samples obtained from 22 and 5 companies, respectively, in the range of 1 to 7 log CFU g^{-1} and 2 to 6 log CFU g^{-1}. In the liquid pig manure, coliforms and *E. coli* were detected in samples obtained from 14 and 8 companies, respectively, in the range of 1 to 5 log CFU g^{-1} and 1 to 5 log CFU g^{-1}. *B. cereus and C. sakazakii* were detected in 9 and 1 out 110 compost samples, respectively, while other pathogens were not detected. In 56 liquid pig manure, *B. cereus* was detected, while other pathogens were detected. Results from these studies provide useful information in identifying manure handling practices to reduce the risk of foodborne pathogens transmission to fresh produce.
Keywords : Livestock manure compost, Liquid pig manure, Foodborne pathogens, Microbial analysis

I011

Role of *flgA* for Biofilm Formation and Motility in *Campylobacter jejuni*

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Campylobacter jejuni remains a major foodborne pathogenic bacterium worldwide. It can form biofilm which can pose a significant threat to public health. Random transposon mutants were generated from *C. jejuni* NCTC11168 strain using transposon containing Tn903 kanamycin resistance gene. Screening of 60 mutants identified a *flgA* insertional mutant with significantly decreased biofilm formation on polystyrene surface of 96-well microtiter plate in crystal violet assay. The *flgA* mutant had also about 30-fold decreased biofilm formation on the surface of stainless steel compared to 11168 wild-type. Transmission electron microscopy analysis revealed that the *flgA* mutant lack flagella. In motility assay using soft agar, the *flgA* mutant had significantly reduced motility (0.6 ± 0.1 cm) compared to 11168 wild-type (4.2 ± 0.4 cm) when motility halo was measured in diameter. This study demonstrates that *flgA* of *C. jejuni* is involved in biofilm formation and motility. It also strongly suggests that the motility may play a significant role in biofilm formation of *C. jejuni* on food contact surfaces.
Keywords : *Campylobacter jejuni*, *flgA*, biofilm, motility, transposon

I012

Trehalose Synthase from *Pyrococcus* and Expression in *Bacillus subtilis*

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Trehalose-synthesizing glycosyltransferase from *Pyrococcus* (TreT) has been found to synthesize trehalose or trehalose analogues with nucleoside diphosphate-glucose including UDP-Glc as a donor and glucose as an acceptor. TreT was proved to have a reversible activity with trehalose and NDP-Glc synthesis. There are many reports about cloning and expression of trehalose synthase in *Escherichia coli*. However, *E. coli* is pathogenic and is not safe for using directly on the food industry. However, compared with *E. coli*, *Bacillus subtilis* is one kind of safe expression host. In this study, TreT gene from *Pyrococcus* was amplified by PCR and subcloned into the *Bacillus-E.coli* suttle vector pLip with Hpa¥±promoter. A TreT gene was expressed in *B. subtilis*. We investigated that recombinant enzyme directly secreted into the culture. Compared with extracellular enzyme of host cell, recombinant enzyme indicated markedly a protein band with molecular weight of 37~50KDa by SDS-PAGE analysis. The recombinant enzyme was partially purified by a combination of procedures such as precipitation with ammonium sulfate and anion chromatography on Q-sepharose. Like expression of TreT in *E. coli*, the recombinant enzyme expressed in *B. subtilis* was able to partly hydrolyze the trehalose to glucose and the enzyme also catalyzed the reverse reaction with such nucleotide acceptors as UDP, GDP and ADP and trehalose donor, producing nucleotide-sugar.
Keywords : *Pyrococcus sp*., trehalose synthase, *Bacillus subtilis*, trehalose, nucleoside-diphosphate sugar

I013

Survey on Norovirus and Indicator Microorganisms contamination in Agricultural Produce, and Their Environmental Factors

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This report describes the results of a nationwide survey on norovirus and indicator microorganisms. Contamination levels of norovirus, E.coli, coli-form bacteria, and male-specific bacteriophage in agricultural produce along with their environmental factors, including soils, agricultural water, human feces, livestock feces, and streamwater, all need to be investigated in order to elucidate their correlations with norovirus. Samples from 90 sites in Korea were analyzed. Samples were collected and shipped refrigerated overnight to the laboratory. All samples were analyzed for the presence of norovirus by RT/nestedPCR. Levels of indicator organisms were also determined. Environmental conditions such as temperature, rainfall etc. were also considered in the correlation analysis. Their copy numbers were counted with real-timeRT-PCR, and the sequences were compared with BLAST and Clustal for analysis. It seems reasonable to infer that the low rate of positive detection was due to high temperature, as all positives were from samples collected during April or beforemid-May. While detection of norovirus was generally greater from April and May than summer (June,July,August), the low detection frequency obscured the seasonal effect. Low numbers of positive samples limited statistical analysis for correlating the respective data. These data provide guidelines that can be used to further validate risk assessment predictions and determine the effectiveness of new control measures.
Keywords : norovirus, detection, agricultural produce

I014

Hypocholesterolemic Effects of Weissella Confusa JB PML-111

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Jeonju Biomaterials Institute

The aim of the present study was to select lactic acid bacteria(LAB) with the anti-hypercholesterolemic effect. LAB were well known to enhance the intestinal health of human. For the development of hypocholesterolemic LAB, it were isolated from fermented soy bean sauce. JB PML-111 was identified as Weissella confusa and had the deconjugation of bile salts and cholesterol-removal activity in vitro. Also, the anti-hypercholesterolemic effect was evaluated on HepG2 cells using heat-killed(HK) LAB. The JB PML-111 demonstrated a decrease in cellular total cholesterol content and cellular triglyceride content at cell level. The present results suggest that treatment of W. confusa JB PML-111 can have hypocholesterolemic effects on cell lipids and be very useful ingredient for functional food.(This research was support by the Regional Specialized Technology Convergence R&D Program funded by the Ministry of Trade, Industry and Energy.)
Keywords : C

I015

A study on Breeding of New *Lentinula edodes* Strains by Mono-Mono Hybridization using Sawdust Cultivation Techniques

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Korea Forest Research Institute

In this study, we attempted to find a new *Lentinula edodes* strains for sawdust cultivation. Tweenty-four new strains were made by the method of mono-mono mating between monokaryotic strains derived from five strains to develop strains suitable for different three sawdust cultivation techniques. Among these new strains, six strains were selected by productivity and shape of fruiting bodies. In 100 days incubation, the productivity rate of A,J,L and M strains have higher values above 20 percent of sawdust medium weight. In 120 days incubation, B,K,L,M,N,W and X strains have higher values above 20 percent of sawdust medium weight.
Keywords : hybridization, *Lentinula edodes*, strains, mono-mono mating, shiitake

I016

Mycelial Growth Characteristics of Shiitake(*Lentinula edodes*) Strains on Solid and Liquid Media with Different Carbon Sources

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Korea Forest Research Institute

The objective was to investigate the effects of medium components with different carbon sources on mycelial growth of Shiitake(*Lentinula edodes*). To assess the effect of different carbon sources on mycelial growth, the cultures were grown in mushroom complete medium (MCM media). However, carbon source in MCM media was replaced by five different carbon sources. Different carbon sources including; Dextrose, sucrose, galactose, arabinose and xylose at the concentration of 20g/L were used. The results showed that in solid media, the highest mycelial growth was obtained when dextrose was used as carbon source. Similarly, highest mycelial growth was observed in liquid media with dextrose carbon source among 5 different carbon sources tested.
Keywords : *Lentinula edodes*, Mycelial growth, carbon source, shiitake, strains

I017

Developing Single Nucleotide Polymorphism Typing Method for Epidemiological Investigation of Multistate Peanut Better Outbreak of *Salmonella* Tennessee in United States

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Salmonellosis usually occurs via consumption of contaminated animal product such as eggs, undercooked meat, or unpasteurized milk. However, *Salmonella* outbreak associated with processed food such as milk powder, cereals, pistachios also reported in recent outbreak. In 2007, a nationwide *Salmonella* Tennessee outbreak occurred due to contaminated peanut butter. In this study, we developed a new molecular subtyping method to differentiate *S.* Tennessee isolates based on Single Nucleotide Polymorphism (SNP) markers. Whole genome sequencing of three representative S. Tennessee strains were performed and 94 SNP sites were selected. The SNP markers were applied to a total of 179 *S.* Tennessee strains associated with or without 2007 Peanut butter outbreak. S. Tennessee strains from various human, animal, and environment were collected. Among 179 S. Tennessee isolates, 52 human clinical samples and 7 food samples (59 isolates; 33.0%) were associated with Peanut butter outbreak. Based on the SNP typing data, phylogenetic analyses identified 7 clades and 16 subtypes. All outbreak- associated strains were belonged to clade 1. Most of the isolates (42 isolates; 71.2%) were belonged to subtype 1 in both human and food samples. These findings revealed that the SNPs successfully typed S. Tennessee strains linked to the large and multistate outbreak. The SNP typing method may be useful for establishing genetic relationships of S. Tennessee strains especially for epidemiological investigation.

I018

Functionality an Safety of Lactobacillus Strains Isolated from Korean Fermented Food

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The objective of this study was to evaluate the safety and functional properties of eight potential probiotic strains isolated from traditional Korean fermented food. Based on being higher tolerance to bile salts and showing higher acid tolerance or hydrophobic properties, five *Lactobacillus brevis* (BCNU 9037, BCNU 9098, BCNU 9101, BCNU 9134 and BCNU 9135), one *L. arizonensis* (BCNU 9032) and two *L. sakei* (BCNU 9131, BCNU 9132) were selected in the screening experiment. All strains can survived up to 99% after 3h culture in pH 2.5 and resistant to 1% bile salts. These strains also showed good antimicrobial activities against a number of food borne pathogens, especially against *Echerichia coli* and *Shigella sonnei*. In addition, L. brevis BCNU 9098 and BCNU 9101 showed higher adherence to Caco-2 cells (12.76 and 11.86%, respectively) than *Lactobacillus rhamnosus* GG, a commercial probiotic strain used worldwide. Their ability to lower cholesterol levels was demonstrated by bile salts hydrolytic activity, and cholesterol assimilation tests in vitro. The results suggest the probiotic potential of these strains.
Keywords : *Lactobacillus* sp. strains, Potential probiotics, Adhesion abilities, Cholesterol assimilation

I019

Characterization of a Newly Bred Shiitake(*Lentinula edodes*) Strain "Baekhwahyang"

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Korea Forest Research Institute

A new *Lentinula edodes* strain "Baekhwahyang" was bred by the method of Di-mon crossing. Mycelial growth was the highest in 27°C. Mycelial growth rate was 9.4mm, which was faster than "Chunbaegko" as control. "Baekhwahyang" was the first middle-low temperature type cultivar in korea, and total productivity was 18 kg/m^3 log in a dry weight. It is 1.4-fold of productivity of low temperature cultivar, which is 13 kg/m^3 log in general. The pileus of fruiting body was brown and hemispherical shape, diameter was 59.7mm. The optimum temperature of fruiting body development was 12~23°Cand the fruiting was sporadic.
Keywords : Breed, Fruit body, *Lentinula edodes*, New strains, Shiitake

I020

Preliminary Screening Method for Biogenic Amines-Degrading Bacteria

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Biogenic amines-degrading lactic acid bacteria can be potentially useful in fermentation to reduce the biogenic amines contents. But rapid screening method for biogenic amines (BAs)-degrading microorganisms is not currently available. New developed screening method for BAs-degrading microorganisms is based on differential media containing a pH indicator. BAs degradation is detected as an decrease in pH. Here we describe an easy, rapid and reliable method for detecting BAs-reducing strains using plate. Positive selection using the plate method was achieved within 24 h. False- positives might be screened by a further study. Of the 180 strains examined using new method, sixty-three strains had BAs-degradative capabilities. The suitability and accuracy of the new developed screening method for BAs-degrading microorganisms was confirmed by high-performance liquid chromatography detection of BA reduction
Keywords : Biogenic amine, Lactic acid Bacteria, fermented food

I021

Use of Lactic Acid Bacteria in Degradation of Biogenic Amines

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Biogenic amines (BAs) are frequently found in raw and fermented foods, such as kimchi, jeotgal and doenjang, in which they appear as a result of the microbial decarboxylation of amino acids. Because of their toxicities, their presence in high concentrations in foods should be avoided. In this work, BA-degrading lactic acid bacteria (LAB) were screened from samples of various kinds of Korean fermented foods. Their potential as probiotics were also investigated. Sixty-three isolates were found that were able to degrade tyramine and histamine in broth culture. Six isolates among all isolates were identified by 16S rRNA sequencing as belonging to *Leuconostoc mesenteroides, Lactobacillus arizonensis, brevis, Bacillus subtilis*, respectively. These strains were selected to quantify BAs and monitor the strains' growth over the cultivation period by HPLC and in the usual method, respectively. These strains were found to reduce accumulation of BAs. These strains might have potential for use as functional starter cultures for reducing the presence of BAs in various kinds of fermented food.
Keywords : Biogenic amine, Lactic acid Bacteria, fermented food

I022

Degradation of Histamine by Lactic Acid Bacteria from Kimchi

Bo Ram Lim, Bo Ra Lim, Yeo Jin Park, Hye Jung Choi, Woo Hong Joo*
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Histamine, one of biogenic amine (BAs), is commonly found in various kinds of Korean fermented food, such as kimchi, jeotgal and doenjang, in which they appear as a result of the microbial decarboxylation of amino acids, and may have adverse effects on the health of consumers. The purpose of this study was to investigate the probiotic properties and histamine-reducing activities of Lactic acid bacterial strains isolated from Kimchi, Three isolates among all isolates were identified by 16S rRNA sequencing as belonging to *Leuconostoc mesenteroides*, *Lactobacillus arizonensis, Weisiella hellenica,* respectively. They were confirmed as safe bioresources because of their non-hemolytic activities and non-production of harmful β-glucosidase, β-glucuronidase, tryptophanase and urease. These isolates were also highly resistant to acid (at pH 2.5) and bile acids. The strains also inhibited the cell growth of food-borne pathogens including *Listeria monocytogenes* and *Shigella sonnei*. These strains also exhibited remarkable histamine-degrading activities. These strains might have potential for use as functional starter cultures for reducing the presence of biodenic amines.
Keywords : Biogenic amine, Lactic acid bacteria, starter culture

I023

Use of Probiotic Bacteria for Biogenic Amine Reduction in Soy Sauce

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The biogenic amines (BAs) were commonly found in various kinds of Korean fermented food, such as kimchi, jeotgal and doenjang, in which they appear as a result of the microbial decarboxylation of amino acids. Because of their toxicities, their presence in high concentrations in foods should be avoided. In this study, BA-degrading lactic acid bacteria (LAB) were screened from samples of Daenjang, Korean traditional fermented soy bean paste. These bacteria exhibits remarkable histamine-reducing activities. Three isolates were identified by 16S rRNA sequencing as belonging to Bacillus amyloliqufaciens, Bacillus sultilis, Bacillus licheniformis, respectively. Their potential as probiotics were also investigated. The strains also inhibited the cell growth of food-borne pathogens including Listeria monocytogenes and Shigella sonnei. These strains might have potential for use as functional starter cultures for reducing the presence of BAs, especially histamine, in soy sauce making.
Keywords : Lactic acid bacteria, Biogenic amine, starter culture

I024

***Bacillus subtilis* Strains from Doenjang and their Use in Reduction of Histamine**

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Histamine, one of biogenic amines, is found in many fermented food products and may have detrimental effects on the human health. In this study, the probiotic strains were screened from Doenjang, Korean traditional soy bean paste. Foremost, their potential as probiotics were investigated. In a second experiment, cell-free supernatants of *Bacillus subtilis* strains were tested for their ability to degrade biogenic amines, especially histamine. in different doenjangs. The highest levels of histamine reduction were obtained with cell-free supernatant of one *Bacillus subtilis* strain. The *Bacillus subtilis* strain extracts described in this study may be useful in Doenjang-making to reduce the biogenic amines content of Doenjang, thereby enhance food safety levels in Doenjang.
Keywords : Lactic acid bacteria, Biogenic amine, Histamine reduction, Doenjang

IO25

Novel *Bacillus* sp. Mk22 for Use as a Probiotic against *Vibrio* and White Spot Syndrome Virus Infecting Black Tiger Shrimp (*Penaeus monodon*)

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The present study was made to prevent *Vibrio parahaemolyticus* and white spot syndrome virus (WSSV) diseases in black tiger shrimp (*Penaeus monodon*) by dietary administration of *Bacillus* sp. Mk22. This halophilic strain was isolated from saltpan environment and identified by 16S rDNA gene sequencing method. Shrimps were tested for 45 days on three experimental diets; experiment-I was a control diet alone, experiment-II diet with for commercial probiotics and experiment-III diet with *Bacillus* sp. Mk22. The shrimp treated with the bacterial isolate showed a significant improvement of growth (7.1 ± 0.21 g), survival (94.3 ± 0.58 %), weight gain (178 ± 4.93 g), less fed conversion ratio (0.8 ± 0.03), lower Total Vibrio Count (0.02 ± 0.01×10^2 CFU ml^{-1}) and reduced mortality compared to the other experimental diets I and II. Infection study revealed that no mortality was observed in shrimps with *V. parahaemolyticus* infection and WSSV infection while there was 68% survival on water infection and 20% survival on oral feeding infection at 45th day. Antioxidant enzymes such as catalase and superoxide dismutase were tested with the infected *P. monodon*; in experiment-III, the enzyme activities decreased compared to experiment-II and I.
Keywords : *Bacillus* sp. Mk22, probiotic, *Vibrio*, white spot syndrome virus, antioxidant enzymes

IO26

Enzyme IIA^{Ntr} is Associated with Regulation of *prpBCDE* Involved in Propionate Metabolism in *Salmonella enterica* Serovar Typhimurium

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In addition to the sugar phosphotransferase system for carbohydrate uptake, many Gram-negative bacteria have a parallel system named as nitrogen-metabolic phosphotransferase system (PTS^{Ntr}). PTS^{Ntr} is composed of enzyme I^{Ntr}, NPr, and enzyme IIA^{Ntr} (EIIA^{Ntr}, encoded by *ptsN*). It has been reported that EIIA^{Ntr} shows varieties of regulations associated with metabolism of carbon and nitrogen, potassium homeostasis, and virulence of some pathogens. In order to understand roles of EIIA^{Ntr} further, we analyzed transcriptome of wild-type and a mutant *Salmonella* strain lacking *ptsN* by RNA sequencing. Many genes showing noticeable differences of expression levels were selected and verified by quantitative Real-time PCR and β-galactosidase assay. Interestingly, one of the highly down-regulated genes in the *ptsN* mutant was the propionate catabolism operon (*prpBCDE*). *Salmonella* can degrade propionate into pyruvate via 2-methylcitric acid cycle when the environment lacks preferred carbon sources. Components of 2-methylcitric acid cycle are encoded by the *prpBCDE* operon whose expression is regulated by PrpR. Even though *rpoN* encoding sigma factor 54 (σ^{54}) is required for the expression of the propionate operon, the transcriptional levels of *prpR* and *rpoN* were not affected by the *ptsN* mutation. These results may suggest direct regulation of PrpR activity by EIIA^{Ntr}
Keywords : *Salmonella* Typhimurium, nitrogen-PTS, enzyme IIA^{Ntr}, propionate metabolism, propionate catabolism operon

IO27

Functional Activity of Fermentation *Hukma* by Lactic Acid Bacteria

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Yams (*Dioscorea* spp.) have been used as medicinal and edible resources worldwide. It has been used in traditional medicine to treat numerous diseases and reported to have anti-diabetes and anti-tumor activities. *Hukma*, as named balck yam have made prepared by repetitive steaming and drying process. In the present study, methanol extracts were prepared from fermented-hukma the antioxidant and α-glucosidase activities of these extracts were evaluated. The concentrations of total polyphenols, total flavonoids, DPPH, reducing power and α-glucosidase activity lactic acid bactria fermentation-*hukma* were 73.6 ppm, 30ppm, 78.3%. 3.1, and 98% respectively. Our results indicate that lactic acid bacteria fermentation-*hukma* have useful biochemical attributes, including antioxidant and α-glucosidase activities.
Keywords : Fermetation, Hukma, Lactic Acid Bacteria, Yam

IO28

Study on the Various Beer Yeasts for Microbrewery

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Recently, Micro-brewery pubs are rapidly increasing in Korea especially in Itawon, Seoul. Micro-breweries has their own brewing systems which they produce 60~300kl a year. Micro-breweries are very common in many countries including United States, Germany, Japan and others that are known as 'Beer belt' countries in Europe. In 2010, the new Korean liquor tax law has been approved that every micro brewery which has about 75,000L of brewery size can have Beer brewing licenses, which was a trigger that Micro-brewery in Korea could increase rapidly. Lately, even people make the beer by themselves at home. So beer yeast consumption will be exponentially increased. Every beer yeasts has their own characteristics such as flavors, smells which are very crucial deciding what the beer made will taste. That is why it is important to study beer yeasts scientifically. Therefore in our laboratory, we selected Beer yeasts such as California Ale Yeast, Belgian Saison Ale Yeast, San Francisco Lager Yeast, Bavarian Weizen Yeast, London Ale, Conan Ale which are common to micro-brewery pubs, and with them we especially focused on the yeast cells themselves growth tendency in 48 hours, and secondly we focused on how beer yeast could effect in human body, so we checked if the yeast cells produces protease, and if they have antibiotic effects as well.
Keywords : Microbrewery, Yeast, Beer, Enzymes, Protease

I029

Genetic Diversity of the Edible Mushroom *Pleurotus ostreatus* Isolated from Korea Using ITS Sequence Analysis and RAPD Fingerprinting

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Genetic diversity of *Pleurotus ostreatus* strains isolated from Korea were investigated using ITS sequence analysis and RAPD fingerprinting. Sequence analysis of the ITS1–5.8S rDNA–ITS4 region of 5 strains of *P. ostreatus* were revealed that the DNA region share mostly 99 % sequence identity, indicated that sequence-based analysis was verified of closely related *P. ostreatus* strains. To verify genetic diversity of Korea native strains using RAPD, we amplified DNA fragments from the total cellular DNA of 8 mushroom strains with 9 different random primers, revealed 345 distinct DNA fragments ranging from 200 to 4000 bp. Analysis of the DNA fragment pattern shown that the 8 *P. ostreatus* strains could be categorized into three subgroups and two native strains were suggested that new strain isolated in Korea
Keywords : Pleurotus ostreatus, Genetic diversity, ITS sequence, RAPD fingerprinting, Edible mushroom

I030

Improvement of Intestinal Microflora by Probiotic Kefir Administration in Mice Model

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Kefir is a multispecies probiotic consisting of lactic and acetic acid bacteria as well as yeast. The present study investigated the modulatory effect of kefir on the gut microflora profiles of mice using a group-specific quantitative real-time PCR. At the end of 3-week oral kefir administration, the number of *Firmicutes*, *Proteobacteria*, and *Enterobacteriaceae* were significantly decreased and the number of *Bacteroidetes, Lactobacillus and Lactococcus,* and total yeast was significantly increased in the kefir group compared to control group ($p < 0.05$). Remarkably, Kefir consumption significantly suppressed the proliferation of the opportunistic pathogen *Enterobacteriaceae* compared to saline administration, with increased proportion of *Lactobacillus* and *Lactococcus* to total bacteria, suggesting Lactobacilli – *Enterobacteriaceae* antagonism. In addition, Kefir consumption reduced *Firmicutes*/*Bacteroidetes* (F/B) ratio ($p < 0.05$) suggesting oral administration of kefir could be beneficial in the treatment of obesity.
Keywords : kefir, probiotics, improvement, intestinal microbiota, qPCR

I031

Survival of *Vibrio parahaemolyticus* in *Ganjang-Gejang* and *Yangnyeom- Gejang* at Cold Storage Condition

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Vibrio parahaemolyticus (*V. parahaemolyticus*) is a curved, rod-shaped, gram-negative halophillic bacterium related to gastrointestinal illness in humans. Food-borne illness related to *V. parahaemolyticus* occur mainly by consumption of seafood. *Ganjang gejang* and *Yangnyeom gejang* are Korean traditional foods, which are made of whole crab seasoned with soybean sauce and red pepper powder. Because of using raw whole crab and adequate salt concentration, several food poisoning outbreaks related to these foods have been reported annually which are mainly associated with *V. parahaemolyticus*. The objective of this study is to investigate survivability of *V. parahaemolyticus* in *Ganjang gejang* and *Yangnyeom gejang* at cold storage condition (4°C). Ganjang-gejang and Yangnyeom-gejang are purchased at retail market located in Seoul and were divided into three groups and weighed 200g, respectively. Each group was given three levels of inoculum; high, middle, low dose of *V. parahaemolyticus.V. parahaemolyticus* in both gejang was counted by culture methods at five different time points; 30 minutes after inoculation, 1, 2, 4, and 7 days of storage at 4°C. The average number of *V. parahaemolyticus* in both *gejang* was decreased gradually in all groups. Because the reduction of *V. parahaemolyticus* in both *gejang* is too slow to ensure the safety at consuming point, more attentions were needed to lower the initial contamination level of V. parahaemolyticus in both *gejang*.
Keywords : Vibrio parahaemolyticus, Survival, Gejang, Crab, Cold storage

J001

Sensitivity of *Sclerotinia homoeocarpa* Isolates to to iprodione, propiconazole, and hexaconazole from Golf Course in the Southern Province of Korea

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Dollar spot disease caused by *Sclerotinia homoeocarpa* is an important disease of Kentucky bluegrass (*Poa pratensis*). The use of fungicides for dollar spot control has led to the development of isolates of *S. homoeocarpa* insensitive to systemic fungicides. In vitro fungicide sensitivity assays using five discriminatory concentrations of iprodione, propiconazole, and hexaconazole were developed to screen 60 isolates *S. homoeocarpa* collected from 12 golf courses in southern province of Korea. Mean 50% effective concentration (EC_{50}) values was estimated based on relative mycelial growth of *S. homoeocarpa* on potato dextrose agar (PDA) versus PDA amended with the five discriminatory concentration of each fungicide. The repeated use of these three fungicide in golf course may not lead to the development of isolates of *S. homoeocarpa* insensitive to three fungicides, including iprodione, propiconazole, and hexaconazole. The sensitivity of fungicides to *S. homoeocarpa* were mean EC_{50} value of 0.218 ㎍ a.i. $ml^{-1}$of iprodione, 0.003 ㎍ a.i. $ml^{-1}$of propiconazol, and 0.005 ㎍ a.i. ml^{-1} of hexaconazole, respectively. There was no correlation among EC_{50} values for iprodione, propiconazole, and hexaconazole indicating no cross- resistance relationships with each other. Results of this study confirm led to sensitive isolates of three fungicides in southern province of Korea.
Keywords : Discriminatory concentrations, Effective concentration, Sensitivity, Cross-resistance

J002

Surveillance of mosquito-borne flaviviruses in South Korean

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Animal and Plant Quarantine Agency

We conducted a surveillance of mosquito-borne flaviviruses targeting mosquitoes and dead wild birds. Samples consisting of total s of 1,092 mosquitoes and 393 dead wild birds, which were collected across the country from 2012 to 2013. Samples were tested by a multiplex real-time reverse transcriptase PCR developed previously for detection of medically important mosquitoes-borne flaviviruses including JEV, WNV, SLEV, YFV, and DENV. All samples were negative for WNV, SLEV, YFV, and DENV. JEV, which is endemic in South Korea, was only detected JEV in mosquitoes (4/1,092). The study continues to support the view that South korea is free from WNV, SLEV, YFV and DENV. However, the possibility of introduction of new medically important mosquito-borne flaviviruses into South Korea to increase due to climate change and increase in international trade. Therefore, it is should do to surveillance for continuously monitoring of being mosquito-borne flaviviruses and quickly identifying new medically important mosquito-borne flaviviruses in South Korea.
Keywords : mosquito-borne flaviviruses, JEV, WNV, SLEV, YFV

J003

Establishment of real time RT-PCR method for Middle East respiratory syndrome coronavirus in Korea

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Coronaviruses infect and cause disease in a wide variety of species, including bats, birds, cats, dogs, pigs, mice, horses and humans. In 2012, new coronavirus was found to cause sporadic cases of severe acute respiratory infection in Saudi Arabia. The newly discovered virus was recently named the Middle East Respiratory Syndrome-coronavirus (MERS-CoV). Recently, MERS-CoV was detected in dromedary camels, so it could be a potential reservoir for MERS-CoV. In this study, we established real time RT-PCR method detected for MERS-CoV for prevention of introduction. All RT-PCR assays recommended by WHO. Positive control material for the upE assay and the 1A assay was provided by European Virus Archive (EVA). All primer and probe was synthesized by Bioneer and sensitivity was evaluated using positive control. To evaluate the specificity, Transmissible gastric enteritis virus, Porcine epidemic diarrhea virus, Bovine coronavirus, Canine coronavirus was used. For MERS-CoV diagnosis, two real time RT-PCR assays targeted for UpE and 1A gene was established. The former is used for screening and the latter is confirmation assay. All assay was detected 10^0copies/ §¡and there is no cross reaction with other animal coronavirus. This method will be useful for the quarantine of animal such as camels.
Keywords : MERS, RT-PCR

J004

Serosurveillance of Rift Valley Fever Virus from Goats And Cattle in Korea

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Rift Valley Fever Virus (RVFV) is a member of the genus *Phlebovirus* in family *Bunyaviridae*. RVFV is a mosquito-borne viral disease affecting both livestock and humans RVFV was first identified in Kenya in 1931 and reported endemic in Africa but has recently spread to Arabian Peninsula. There is great concern that the disease will spread to worldwide such as Europe, Asia and Americas. Possibility of RVFV introduction was increasing as climate change and globalization of trade in animals and animal products. For this reason, serosurveillance was conducted from goats and cattle for prevention of RVFV introduction. The study area is divided into 9 areas. Animal was random selected in goats and cattle from the local Veterinary Service. Blood samples were collected and sera stored at -20°C until use. Sera samples were tested for anti RVFV antibodies using ID Screen RVF Competition Multi-species (ID-Vet, Montpellier, France). A total of 875 animal sera were collected in 2013. All animal samples were negative for antibodies against RVFV. This study result is suggested that the disease is not present in Korea. This surveillance is to be carried out continuously and the need to establish early warning system including the vector surveillance such as mosquitoes.
Keywords : RVF, serosurveillance

J005

Postantibiotic Effects and Postantibiotic Sub-MIC Effects of Chlorhexidine on Oral Microorganisms

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Chlorhexidine is one of the most widely used biocides in antiseptic products. Postantibiotic effect (PAE) is difined as the length of time that bacterial growth is suppressed following brief exposure to an antibiotic. In this study, PAE, postantibiotic sub-MIC (PASME) and sub MIC effects (SME) of chlorhexidine on oral microorganisms were investigated. The PAE was induced by 10x MIC of chlorhexidine for 1min and chlorhexidine was eliminated by washing. The PASME were studied by addition of 0.1, 0.2 and 0.3x MICs during the postantibiotic phase of the bacteria, and the SME was studied by exposing bacteria to chlorhexidine at the sub MIC only. For *Streptococcus gordonii*, the mean PAE was 0.1h, and the mean PA-SMEs were 0.25h (0.1x MIC), 0.45h (0.2x MIC), 0.75h (0.3x MIC), and the mean SMEs were 0.08h (0.1x MIC), 0.4h (0.2x MIC), 0.7h (0.3x MIC). For *Streptococcus mutans*, the mean PAE was 0.9h, and the mean PASMEs were 1.9h (0.1x MIC), 2.7h (0.2x MIC), 3.45h (0.3x MIC), and the mean SMEs were 1.0h (0.1x MIC), 1.6h (0.2x MIC), 1.85h (0.3x MIC). For *Lactobacillus acidophilus*, the mean PAE was 0.35h, and the mean PA-SMEs were 0.85h (0.1x MIC), 2.15h (0.2x MIC), 3.7h (0.3x MIC), and the mean SMEs were 0.4h (0.1x MIC), 0.9h (0.2x MIC), 2.25h (0.3x MIC). The present study illustrates the existence of PAE, PA-SME and SME for chlorhexidine against oral microorganisms, thereby extending the pharmacodynamics advantages of chlorhexidine.
Keywords : Chlorhexidine, Postantibiotic effect, Oral, Bacteria

J006

Synergistic Antibacterial Efficacies of the Combination of Chlorhexidine Digluconate or Protamine Sulfate with the Extracts of *Laminaria japonica* or *Rosmarinus officinalis* against *Streptococcus mutans*

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Chlorhexidine digluconate is generally used to inhibit oral bacteria and the formation of dental plaque. Protamine sulfate is polycationic protein which exerts antibacterial activity by changing cell wall of the bacteria. Extracts of *Laminaria japonica* and *Rosmarinus officinalis* are known to possess antimicrobial activities against oral pathogens. The purpose of this study was to investigate the synergistic effect of chlorhexidine digluconate and protamine sulfate in inhibitory activity of extracts of *L. japonica* and *R. officinalis* against *Streptococcus mutans* which is one of the major etiological agents for dental caries. The minimal inhibitory concentrations (MICs) of chlorhexidine digluconate, protamine sulfate, extracts of *L. japonica* and *R. officinalis* were determined by the broth dilution method. The synergistic effect between chlorhexidine digluconate or protamine sulfate and extracts of *L. japonica* or *R. officinalis* were determined by the fraction inhibitory concentration index (FIC). When chlorhexidine or protamine was used in combination with extracts of *R. officinalis* and *L. japonica*, the synergistic effects of inhibitory activities of these antibacterial agents were observed. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0025532).
Keywords : Antibacterial agents, Combination, Synergy, Streptococci

J007

Effects of Sub Minimal Inhibitory Concentration of Metronidazole and Penicillin on Morphology of *Aggregatibacter actinomycetemcomitans*

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Minimal inhibitory concentration (MIC) is the lowest concentration of antibiotics that inhibits the visible growth of a microorganism. It has been reported that sub-MIC of antibiotics may result in morphological alterations along with biochemical and physiological changes in bacteria. The purpose of this study was to examine morphological changes of *Aggregatibacter actinomycetemcomitans* after treatment with sub-MIC antibiotics. The bacterial morphology was observed with scanning electron microscope after incubating with sub-MIC of metronidazole and penicillin. Antibiotics inhibited bacterial division and long filaments were formed. The length of *A. actinomycetemcomitans* was increased after incubation with sub-MIC metronidazole and penicillin. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0025532).
Keywords : *A. actinomycetemcomitans*, Sub-MIC, morphology, metronidazole, penicillin

J008

Effect of Sub Minimal Inhibitory Concentration Antibiotics on Morphology of *Streptococcus mutans* and *Lactobacillus acidophilus*

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Sub-minimal inhibitory concentrations (MIC) of antibiotics have been reported to affect bacterial morphology and biochemical characteristics. The purpose of this study was to examine the morphological change of dental caries-related oral bacteria after treatment with sub-MIC antibiotics. *Streptococcus mutans* and *Lactobacillus acidophilus* were used in this study. The MIC for amoxicillin and doxycycline were examined by broth dilution method. *S. mutans* showed increased length after incubation with amoxicillin and increased number of bacteria in a chain after incubation with doxycycline. The length of *L. acidophilus* was decreased after incubation with amoxicillin and doxycycline. In this study, we observed that sub-MIC amoxicillin and doxycycline can induce the morphological changes in *S. mutans* and *L. acidophilus*. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0025532).
Keywords : *Streptococcus mutans*, *Lactobacillus acidophilus*, Antibiotics, Sub-MIC, Morphology

J009

Novel Diagnostic Method for the Improvement of Detection Limit; Convergence of Oligonucleotide-Linked Gold Nanoparticle and Immunosorbent Assay

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As a small amount of high-risk pathogen, such as Yersinia pestis (*Y. pestis*), Bacillus anthracis (*B. anthracis*) and Clostridium botulinum (*C. botulinum*) can make a potential threat by deliberate infection in a short period of time, more sensitive method which can detect in early stage of accident is essentially requested. Therefore, we developed the novel diagnostic method based on the convergent technology combining gold nanoparticles and immunosorbent technique to increase the sensitivity. The signal generated from enzyme-linked immunosorbent assay (ELISA) was replaced with amplified fluorescence signal of gold nanoparticle (GNP) complex-conjugated with oligonucleotides and antibodies. Thus, extremely low quantity of the pathogens in mixed specimen could be detected with high specificity. This designed technology was named 'gold nanoparticle-oligonucleotide-linked immunosorbent assay (GNP-OLISA)'. Using this method, we detected 492.16±26.25 CFU/mL of Y. pestis, 874.84±190.38 CFU/mL of B. anthracis spore, and 3.03±0.84 pg/mL of C. botulinum neurotoxin type A from PBS-diluted samples. Detection sensitivities were 11, 61, and 881 times higher than the corresponding ELISA results, respectively. The highly sensitive nano-sensing method is expected to be applied for multiplex detection of pathogens keeping both sensitivity and specificity near future, and it would be applied for early detection system for bioterrorism preparedness.

Keywords : high-risk pathogen, limit of detection, gold nanoparticle, OLISA, ELISA

J010

Establishment of Stable Methods for Long-Term Preservation of Viral Pathogen Resource

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Stable and well-characterized viral pathogen resource is essential for vaccine, diagnostic and therapeutic reagent development. For the Nagoya Protocol, which will enter into force in November 2014, the importance of viral pathogen resource has been increased. For properly use of viral pathogenic resource, it is necessary to establish stable long-term preservation method without change of biological properties. Therefore, it is important to determine suitable temperature and efficiency protectants of resource. To establish stable method for long term preservation, we designed the study which the panels of two viruses – adenovirus (capsid virus with DNA genome) and measles virus (envelop virus with RNA genome) - were stored with protectant (45% FBS or 25% sucrose) at different storage temperature (-20°C or -70°C) for 6, 12, 18, 21 and 24months. According to this time schedule, we are comparing the recovery ability and genetic stability using by TCID50 assay and nucleotide sequencing, respectively. We analyzed the efficiency of two protectants by an acceleration test which incubated the panels with each protectant at 37°C for 2hours or at 56°C for 30min. After then, we compared the recovery ability. The sucrose was more efficient than FBS in all viruses. We expect that these data will give a guideline for long-term preservation of virus pathogen resource.[This study was supported by the intramural research fund No.2013-NG47002-00 of KNIH]

Keywords : viral pathogen resource, long term preservation, protectant, Measles virus, Adenovirus

J011

Effect of Microorganism on Growth in Crop

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We investigated effect of microorganism for growth development in crop. To accomplish this, samples were 4 species with IKD 08, KACC91281, KACC91282, KACC91282. The effect of microbial 4 strains was similar to the control in emergence ratio of cucumber. At the growing seedling, stem diameter of cucumber was greater in the microbes group than the control, and the field experiment of cucumber was similar to the growing seedling experiment. The yield of cucumber fruit in microorganism treatment was higher than the control. Thus, the microorganisms of 4 species accelerated the growth of cucumber, and it could increase the yield.

Keywords : microorganism, growth, cucumber

J012

Isolation and characterization of Cell Bound Agar-Degrading Enzyme Producing Bacteria from Southern Coast of South Korea

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We identified and characterized four agar decomposable bacteria and enzymes isolated from the sea water pool around rocks on the southern coast of South Korea. Although, 4 chosen microrganisms were identified as *Agarivorans albus* related species based on 16S rDNA sequence analysis, but microbial properties such as morphology, growth pattern and agar degrading activities were a little different. Enzymes produced by 4 isolates hydrolyzed agar, agarose and low-melting agarose, but little or absent abilities with other polysaccharides such as alginate, carrageenan and laminarin. 4 hydrolytic activities were steadily observed from 20¡É to 50¡É, but rapidly reduced activities were obtained at the 60¡É and no degrading activities were recorded over the 60¡É. With zymogram analysis, molecular weights were estimated and revealed approximately 36 kDa, 50 kDa and 98 kDa, respectively from 4 bacteria. On the gel, more than 3 agar-degrading enzymes were separated and exhibited, and about 50 kDa enzymes of 4 bacteria were indicated strong activities. Generally, agar-hydrolytic enzymes produced from *Agarivorans* and related species were reported as extracellular protein, but enzymes in this investigation were recorded only cell bound or non-soluble cytoplasmic compound. Furthermore, final cleaved agar products were identified as galactose level by thin layer chromatography (TLC). These results and properties make 4 microrganisms to be attractive materials for the industrial application.

Keywords : Agar-degrading, Agarivorans, Cell-bound enzyme

J013

Molecular Epidemiology of Norovirus-associated Outbreaks of Gastroenteritis in South Korea, 2012-13

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Human norovirus (NoV) is major causative agents in nonbacterial acute gastroenteritis and NoV-associated outbreaks have occurred in Korea, continuously. So it is important to analyze molecular epidemiological investigated and understand genotype distribution in Korea. In this reason, we investigated NoV-associated outbreak and analyzed genotypic distribution in Korea, 2012-13.We collected 2,785 of stool specimens from total 122 outbreak cases in this period. Semi-nested RT-PCR was performed to detect of NoVs for analyzing the distribution of genotype of NoV-associated outbreaks. And then, we performed automatic sequencing and genotype determination using amplified products based on web based reporting system 'EnterNet'. Twelve genotypes were belonged to GI and eleven genotypes to GII group, involved in outbreak. GII-4 (41.43%) was most predominant strain in NoV-associated outbreaks, which is compatible with the result of genotype distribution for sporadic cases investigated by acute diarrhea laboratory surveillance. NoV-associated outbreaks occurred continuously in Korea and it is need to analyze molecular epidemiological features to monitor outbreaks. NoV-associated outbreaks consisted of various genotypes, and GII-4 is the most prevalent strain.
Keywords : Norovirus, outbreaks, GII-4

J014

Observation of Phage-Antibiotic Synergy in a Wide Variety of Combinations in Bacterial Host, Bacteriophages, and Antibiotics

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When phages are infected to bacteria cultured with sublethal dose of antibiotics, sizes of phage plaques significantly increase. This phenomenon is known as phage-antibiotic synergy (PAS). We extended observation of PAS to a wide variety of bacteria-phage pairs with 4 different classes of antibiotics. PAS was observed in both Gram-positive and Gram-negative bacteria. Cell wall synthesis inhibitors and DNA metabolism inhibitors generally induced PAS. Some protein synthesis inhibitors induced PAS, while others did not. The use of sub-lethal dose of ampicillin, cefotaxime, ciprofloxacin, or mitomycin C allowed formation of highly visible plaques of increased sizes when various bacteriophages infected *Escherichia coli, Staphylococcus aureus*, and *Enterococcus feacalis*. *Bacillus cereus* and *Pseudomonas aeruginosa*-specific phages showed increased plaques in the presence of sublethal dose of cefotaxime, ciprofloxacin, or mitomycin C. We also confirmed that cells stressed with β-lactam and quinolone antibiotics filamented extensively. Burst size of bacteriophage also increased in the presence of antibiotics. Increase of burst size was also shown in vivo using *Caenorhabditis elegans* as a model animal.
Keywords : Phage, antibiotics, PAS

J015

Removal of *Staphylococcus aureus* on Various Surfaces Using Bacteriophage SA11

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The control of bacteria resistant to multiple antibiotics is becoming challenging for public health. Phage therapy is an important alternative to antibiotics in the current era of multidrug-resistant pathogens. Of those, methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in human. In this study, we isolated and characterized a novel phage SA11 which infected *S. aureus*. Phage SA11 is a member of siphoviridae family and has an icosahedral head of 111 nm in diameter and a long tail of 205 nm. It is a double strand DNA virus with genome size of 136,326 bp, encoding at least 6 structural proteins. SA11 was shown to infect 7 laboratory strains and 11 antibiotic-resistant strains of *S. aureus*. Its latent period was 20 minutes and burst size was 78.9. *S. aureus* was readily removed from contaminated porcine skin when treated with phage SA11 in a dose-dependent manner. Biofilm previously formed on either polystyrene surface or glass surface was effectively removed when phage SA11 was added. The phage also inhibited formation of biofilm.
Keywords : phage, biofilm

J016

Isolation of *Bacillus atrophaeus* strains with Antifungal Activities from Tidal flat Against *Colletotrichum* sp.

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Colletotrichum acutatum is one of major limiting factors in production of pepper and other important crops worldwide by causing anthracnose disease. Anthracnose diseases are usually managed with application of chemical fungicides. The continued use of synthetic fungicides have been paid attention to provoke problems in terms of food safety, fungicide resistance, and ecosystem destruction. For these reasons, biological control using antagonistic microorganisms has emerged as an alternative for disease control of plant pathogen. Here we report isolation and characterization of bacterial strains isolated from mudflat because antifungal activities of mudflat-isolated bacteria have been less studied compared to other cases. In this study, a total of 420 bacterial strains were isolated from mudflat of the western sea of Korea. Among these bacterial strains, 9 bacterial strains were first grouped based on morphological characteristics. Subsequently 9 bacterial strains were found to have antagonistic activity against *C. acutatum*. The area of the inhibition zone was taken as a measure for antagonistic activity. As a result, the strain LB14 was the most effective among these strains. In addition, antagonistic activities of selected bacteria were evaluated against fungal pathogens *C. acutatum* and *C. gloeosporioides* by measuring suppression of growth, germination and disease severity. The strain LB14 was identified as *Bacillus atrophaeus* using 16S rDNA analysis and Biolog API kit.
Keywords : Biological control, *Bacillus atrophaeus*, Antifungal Activity, Tidal flat, *Colletotrichum*

J017

Rapid Detection Methods of High-risk Pathogen using Real-time PCR and PNA Mediated RT-PCR Clamp

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Bioterror and Biological Warfare agents are the deliberate release of viruses, bacteria, or toxins derived from micro-organisms to cause illness or death. These microorganisms are often found in nature. But they can sometimes be made more harmful by increasing their ability to cause disease, spread or resist medical treatment. Bioterrorism is to reduce the damage to biological agents is a essential rapid diagnosis and clinical treatment. This research proposes a diagnosis method of biological agents and modified biological agents using real-time PCR and PNA mediated real time PCR clamp technology. We have established the optimal real-time PCR conditions for diagnosis of *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis* and *small pox*. Novel primers and/or 5'-nuclease detection probes were designed for each pathogen screening of genes selected by the specific genes. PNA probe could inhibit transcription of genes to which it has been targeted, which based on the sequences of mutants type and wild type.
Keywords : Biological Warfare agents, diagnosis, real-time PCR, PNA

J018

Improved method for the isolation of total RNA from *Panax ginseng* C.A. Meyer

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Nowadays, studies of plant transcriptome analysis require high quality and quantity of undegraded RNA. It is, however, not easily accomplished when tissues of plant contain large amounts of polysaccharides and polyphenolic compounds like ginseng leaves and roots. In this study, we developed the protocol for the isolation of total RNA of *Panax ginseng* C.A. Meyer, which makes possible cDNA synthesis and qPCR analysis in this plant species. Guanidinum thiocyanate, CTAB or modified Trizol methods were applied to isolate the integrity of RNA from ginseng roots. Among these methods, high quality of total RNA was obtained with the modified Trizol method. 80 ~ 100 ug/g of total RNA was obtained and the absorbance ratios (A_{260}/A_{280}) were around 2.0. According to this method, RNA extraction of gingseng root is fast and easy-to-use for further analysis. And also, it can be used on a wide range of different species and tissues which contain high polysaccharide content and has problem to isolate total RNAs with traditional method. Furthermore, it is useful for transcriptome analysis in non-model species.
Keywords : Ginseng, Total RNA, Transcriptome

J019

Suppressions of the TC-1 Tumor Cell Growth by Baculoviral Delivering HPV E6/E7 DNA Vaccine

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Previously, we developed human endogenous retrovirus envelope protein-coated non-replicating recombinant baculovirus (AcHERV) for a human papillomavirus (HPV) 16L1 DNA vaccine nanocarrier. Because HPV E6 and E7 are promising tumor antigens in HPV related cervical cancer, we constructed AcHERV-E6/E7. To improve the immunogenicity of E6/E7 gene product, E6/E7 gene was synthesized with codon optimization and fused with sorting signal of the lysosomal-associated membrane protein LAMP-1. AcHERV-(opti)E6/E7, and AcHERV–(opti) E6/E7LAMP-1. C57BL/6 mice were injected I.M with 2×10^7 particles of each DNA vaccine on day 0, day 7 and day 14. After 1 week from first immunization, 1×10^5 TC-1 tumor cells were subcutaneously transplanted into right flank leg. Tumor growth was monitored weekly for 7 weeks. Compare to control group, AcHERV-(opti)E6/E7 treated group showed retardation of tumor growth. AcHERV-(opti)E6/E7LAMP-1 treated group showed the anti-tumor effect. These DNA vaccine generate the highest number cytokine secreting of E6 or E7 specific splenocytes. These results indicate that fusion of LAMP-1 to an E6/E7 gene enhance the potency of HPV DNA vaccine against cervical cancer.
Keywords : HPV, Baculoviral vector

J020

FrdABCD Participate in Persister Cell Formation in *Escherichia coli*

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Bacterial persisters are phenotypic variants that are tolerant even to supralethal concentrations of multiple antibiotics. Persisters showing tolerance against different classes of antibiotics are observed in most microbial species, and have been implicated in chronic and recurrent infections. Furthermore, it is highly probable that persisters are potential reservoir for developing resistance. Recent studies suggest that persisters can be generated through various mechanisms including slow growth rate, active starvation response, and the ubiquitous toxin-antitoxin (TA) systems. However, the mechanisms that underlie persistence still remains elusive. Most earlier insights into persistence have been obtained from gene expression analysis of persister-enriched samples, or through screening of overexpression or knockout libraries. We also attempted to enrich persisters from multi-copy genome fragment library. After a series of screening procedures, 19 plasmids that conferred increased tolerance to ampicillin (Amp) were isolated and sequenced. All plasmids fell into one of the three plasmids (P1, P2 and P3) and commonly contained *ampC* coding for β-lactamase. Introduction of multiple copies of FrdABCD (FRD) via the plasmid pFRD that does not contain *ampC* indeed elevated tolerance to both Ampicillin and Norfloxacin. To investigate a putative mechanism by which antibiotic tolerance is elicited by *FRD* overexpression, the substrate fumarate was supplemented into culture medium.
Keywords : Bacterial persister, Genome fragment library, FrdABCD

J021

Fumarate-mediated Persistence of *Escherichia coli* to Antibiotics

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While genesis of bacterial persisters against various antibiotics has been observed, molecular mechanisms by which bacterial persisters tolerate the antibiotics have been elusive. We found that *Escherichia coli* persisters contain elevated levels of fumarate, suggesting that altered electron fluxes in the electron transport chain (ETC) might be associated with the persister phenotype. Genetic and chemical perturbations of ETC, overexpression library screening, metabolite supplementation assay and even the toxin/antotoxin-related *hipA7* mutation indicated that fumarate is the chemical determinant of *E. coli* persisters. The fumarate-mediated persisters were capable of minimizing the antibiotic-induced hydroxyl radical formation by detouring electrons from ETC using the fumarate as an alternative electron acceptor. Results suggest that succinate dehydrogenase is the persister-forming enzyme and the emergence of persisters, which are the potential source of antibiotic-resistant cells, can be prevented by precluding fumarate accumulation.
Keywords : Persister, Fumarate

J022

Cas9/CRISPR-mediated Resistant Gene Knockout: New Therapeutic Approach for Treatment of Antibiotic-resistant *Escherichia coli*

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Antimicrobial drug development is increasingly lagging behind the evolution of antibiotic resistance. As a result, there is pressing need for new antibacterial therapies that can be readily designed and implemented. In this study, a new antibacterial therapy using CRISPR-Cas9 system was developed. Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/CRISPR-associated (Cas) system are adaptive immune system that silences invading nucleic acid by using RNA-guide endonuclease activity in bacteria and archaea. Targeting of single guide RNA (sgRNA) with Cas9 directs sequence-specific double strands DNA cleavage. Using Cas9 and $sgRNA_{bla}$ cassette, it is shown that cleavage of targeted double-strands DNA resulted in degradation of plasmids containing *bla* gene. Ampicillin-resistant cells could be re-sensitized by expressing Cas9/ $sgRNA_{bla}$, thereby reducing the number of resistant cells. Resistant cells also could be reversed to non-resistant cell using combination therapy that antibiotic plus $reSAfR_{bla}$ phage. The strategy demonstrated in this study enables the continuous use of old antibiotics, which are already developed and the safety issue was cleared, by reverting the resistant cells to sensitive cells.
Keywords : Antibiotic resistance, Cas9/CRISPR

J023

Mutations in the SmoA Gene Bypass the Requirement of SIN for Septation in Aspergillus Nidulans

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Many filamentous ascomycetes like Aspergillus nidulans form mycelia with multinucleate cells. It is largely unknown how septation is regulated in their mycelia. In A. nidulans, the AnMOB1 protein of the septation initiation network (SIN) is essential for septation and conidiation, but is not required for hyphal extension and colony formation. To isolate novel septation regulators, by UV mutagenesis we have isolated suppressor (smo) mutations that restored conidiation and septation when AnMOB1 was not expressed. Among smo mutations, smoA and smoB mutations caused reduced hyphal growth with wavy hyphae in the presence of MOB1. The smoA gene was cloned by DNA-mediated complementation, and it encodes a polypeptide of 652 amino acids which includes a coiled-coil domain. Genes encoding SMOA homologs were detected in genomes of other filamentous fungi like Neurospora crassa. A functional GFP (green fluorescent protein)-AnSMOA fusion protein localized to the nucleus indicating that AnSMOA is nuclear protein. Loss-of-function smo mutations rendered hypersensitivity to low doses of the microtubule-depolymerizing agent benomyl. We conclude that AnSMOA and other SMO proteins likely act as regulators antagonizing the SIN pathway in A. nidulans. This work was supported by the National Science Foundation.
Keywords : *Aspergillus nidulans*, *smoA*, Septation, SIN

J024

Involvement of Antioxidant Defense System in Solvent Tolerance of Pseudomonas sp. strain

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Pseudomonas sp. BCNU 106 and BCNU 171, isolated on the basis of its ability to grow on toluene as sole carbon source, was distinguishable from other *Pseudomonas* strains. *Pseudomonas* sp. BCNU 106 and BCNU 171 were investigated to elucidate the solvent tolerance under specific culture conditions with the presence of solvents and its adaptive mechanisms to those conditions with reference to the antioxidant system. Therefore, the investigation of their antioxidant properties (catalase, superoxide dismutase, glutathione S-transferase and total anti-oxidative capacities) will be useful for further study on toluene-tolerance of bacteria and the defense mechanism of antioxidant enzymes against toluene or other organic solvents. Compared to non toluene-tolerant *P. putida*, toluene-tolerant bacteria had relatively high tolerance to toluene stress, especially *Pseudomonas* sp. BCNU 106. The results demonstrated all toluene-tolerant bacteria possessed a toluene tolerance mechanism that may scavenge reactive oxygen species produced by toluene. (Supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (Contract No 2010-0009141))
Keywords : *Pseudomonas* sp. strains, Solvent-tolerant bacterium, Antioxidant defense, Toluene adaptation

J025

Granulocyte Macrophage Colony Stimulating Factor-Flagellin Fusion Adjuvants for Foot-and-Mouth Disease Virus Commercial Vaccine

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Foot-and-mouth disease (FMD) is a highly infectious and economically devastating disease of cloven-hoofed animals. Although FMD vaccine have been used currently, the disease still affects animal products. To enhance the vaccine efficacy, new vaccination strategies rely on the incorporation of effective adjuvants. Here, this study was utilized Granulocyte-macrophage colony-stimulating factor (GmCSF) and *Salmonella typhimurium* Flagellin 2 (STF2) as adjuvant. GmCSF-STF2 fusion was constructed a recombinant baculovirus, which is connected 2A-linker peptides and encoding adjuvant (Ac-ie1-PERVB-pGmCSF-STF2). pGmCSF and STF2 genes were confirmed gene expression levels by reverse transcription PCR in Porcine Kidney 15 cells. NF-κB, which is induced by Toll-like-receptor 5 (TLR5) signaling pathway, was detected by reverse transcription PCR in Porcine Alveolar Macrophage cells. To investigate the effects as adjuvant, immunized with FMD vaccine (KBNP, O Manisa, A Malaysia97, Asia 1 shamir strain) in mice. The immunological effects of the Ac-ie1-PERVB-pGmCSF-STF2 were determined specific FMDV by ELISA, ELISPOT, and CTL assay. GmCSF-STF2 fusion showed higher IgG titer, INF- γ secretion, and CTL assay than that of FMD vaccine only. Extending these result, pGmCSF-STF2 could stimulate both humoral and cell-mediated immune response as an adjuvant for FMD vaccine. In conclusion, STF2 and GmCSF could be useful for potential vaccine adjuvant against pathogen.

Keywords : FMDV adjuvant, salmonella, STF2, Gm-CSF

J026

Epidemiology of Human Enterovirus71 infection in Republic of Korea, 2006-2012

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Enterovirus (EV) 71 is the main pathogen associated with hand, foot, and mouth disease (HFMD) and may lead to outbreaks of the disease with severe mortality in children. In recent years in the Asia-Pacific region has experienced more frequent EV71-associated HFMD epidemics with high incidence of severe neurotropic complications and fatality rates. This study is the first report to document the EV71 epidemics from 2006 to 2012 in republic of Korea. Specimens and clinical information were collected from patients suspected of having EV infection during 2006–2012. EV71 isolates were examined by RT-PCR amplifying the VP1 region and sequencing. VP1 sequences for the EV71 isolates were compared with foreign strains. We confirmed EV71 with 591 cases in EVs with 3,689 cases from 10,699 patients who suspected with EVs infection.. The mean age was 3.6 years ± 4.5 years and median age was 3 year old. The major clinical manifestations of EV71-positive patients was HFMD (n=350, 59.2%), and 165 (27.9%) cases of HFMD with central nervous system complications. EV71 genetic groups designated three distinct clusters A, B(1~5), C(1~5) with some regional sub-clustering. Based on this classification, almost Korea strains belonged to C subgroup with China, Japan, and Vietnam strains. There are currently no treatments specific to human EV71 infections. This study provides may assist with evaluating the need to research and develop treatments for infections caused by virulent human EV71 infection.

Keywords : Hand foot and mouth disease, Human enterovirus 71.

J027

Development of Competative ELISA for Serodiagnosis of African Swine Fever Virus

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ASF is caused by African swine fever virus(ASFV) and is the only member of the family Asfaviridae, and a fatal hemorrhagic disease in domestic pigs and European wild boar and currently no effective vaccine. The virion is composed of up to 54 proteins, some of which have been formerly identified. The identity of other components remains obscure. Many putative nonstructural proteins of the virus are uncharacterized. So we found the unknown protein K205R homology in a variety of ASFV isolates. We targeted to K205R of ASF for serodiagonsis of ASF, and synthesized a K205R (Georgia 2007/1 strain, NCBI No. FR682468). The sequence of inserted into pRSET A and produced a recombinant protein in E.coli. For production of monoclonal antibody (Mab) for K205R, purified recombinant K205R protein were inoculated two Balb/c female mice of 8 weeks age, twice, 2 weeks interval. A total of 35 antibodies were produced. Two Mabs which were IgG3 kappa, have a good reactivity with capripox virus then selected as a antibody for using a competative ELISA (c-ELISA). For the development of c-ELISA, the puirfied recombinant K205R protein was used as an antigen, and seclected two antibodies for coating and detecting antibody. The optimal concentration of all reagent adopted for the c-ELISA were predetermined by checkerboard titration. The c-ELISA were evlauated to negative serum and positive serum against ASF. Compared to comerical ELISA kit, sensitivity and specificity of developted c-ELISA were similar.

Keywords : ASFV, c-ELSIA, K205R

J028

Development of Rapid Assay Method using PCR-Based Marker for the Detection of Plant Quarantine Pathogen, *Phyllosticta capitalensis*

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Phyllosticta is known as the causal plant pathogenic fungus of leaf spot and various fruit disease in a wide range of host plants. Among them, *P. capitalensis* was defined as the regulated pest by plant quarantine's law in Korea. Therefore, we aim to develop tools as the *P. capitalensis*- specific primers (PC-IF/ER, PC-IF/IR, PC-IF2/IR and PC-TF3/IR) based on elongation factor 1-alpha and ITS region of the *P. capitalensis* for detection on seeds, fruits and trees for quarantine and phytosanitary measures. As a consequence, a rapid technique for detection of *P. capitalensis* has been well developed using polymerase chain reaction (PCR). The PC-IF/IR primer set having 489-bp target size has been chosen from 4 different primer sets. This primer set is specific for *P. capitalensis* that is not showed the target size band for *Phoma* spp. and *Phyllosticta* spp. Also, we plan to detect the fungus from seeds, leaves and root tissues infected with *P. capitalensis* in a humidity condition. Therefore, this *P. capitalensis* PCR detection method can be usefully applied in a rapid and accurate inspection tool of *P. capitalensis* diagnosis for the plant quarantine purpose.

Keywords : PCR, Plant quarantine, *Phyllosticta capitalensis*

J029

Antimicrobial Activity of Benzyl Alcohol against Bacteria and Yeast of Clinical Importance

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Benzyl alcohol is an aromatic alcohol with a mild pleasant odor and found in essential oils such as Ylang Ylang and Jasmin. Benzyl alcohol is also found in plant fruits such as cocoa, apricot, cranberries and Peru Balsam, and in foods such as mushroom and honey. Benzyl alcohol is used in various cosmetic formulations as preservative, fragrance and solvent. To assess antimicrobial activity of benzyl alcohol, the sizes of inhibition zone by disc diffusion, the minimum inhibitory concentrations (MIC) and minimum inhibitory doses (MID) by gaseous contact were determined against 4 Gram-positive and 10 Gram-negative bacteria and the yeast Malassezia furfur. The sizes of inhibition zone ranged from 14.3 to 28.0 and 23.7 to 37.0 mm for Gram-positive and Gram-negative bacteria, respectively. The sizes of inhibition zone for M. furfur was 31.3 mm. MIC of benzyl alcohol ranged from 4 to 8 and 2 to 4 mg/mL for Gram-positive and Gram-negative bacteria, respectively. MIC for M. furfur was 2 mg/mL. MID ranged from 50 to >200 and 25 to 100 mg/L for Gram-positive and Gram-negative bacteria, respectively. MID for M. furfur was 25 mg/L.
Keywords : Benzyl Alcohol, Antimicrobial Activity, Bacteria, Yeast

J030

Antimicrobial Activity of Mandelonitrile against Bacteria and Yeasts of Clinical Importance

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Mandelonitrile is an aromatic compound found in the pits of some fruits such as apricot and bitter almond. Mandelonitrile is used to prepare bitter almond water. Millipede Apheloria corrugate and tiger beetle Megacephala virginica use mandelonitrile as a defense mechanism. To evaluate the potential of mandelonitrile as an antimicrobial agent, the sizes of inhibition zone by disc diffusion, the minimum inhibitory concentrations (MIC) and minimum inhibitory doses (MID) by gaseous contact were determined against 4 Gram-positive and 10 Gram-negative bacteria and yeasts Malassezia furfur and Candia albicans. In the disc diffusion test, mandelonitrile showed maximum activity against Staphylococcus aureus, S. epidermidis, Escherichia coli, Pseudomonas aeruginosa, Shigella sonnei, Salmonella typhimurium and the yeasts (total inhibition of growth). In contrast, no zone of inhibition was observed against Enterococcus faecalis, Enterobacter aerogenes and Citrobacter freundii. MIC ranged from 0.25 to 4, 0.5 to 2 and 0.25 mg/mL for Gram-positive, Gram-negative bacteria and yeasts, respectively. MID ranged from 6.3 to 25 and 3.1 to 12.5 and 0.78 to 3.1 mg/L for Gram-positive, Gram-negative bacteria and yeasts, respectively. Mandelonitrile has potential usage as an air disinfectant, preservative and antimicrobial agent.
Keywords : Mandelonitrile, Antimicrobial Activity, Bacteria, Yeast

J031

Curing of the Mycovirus (LeV) Resulted in Improved Vegetative Growth of an Edible Mushroom *Lentinula edodes*

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This study attempted to cure the edible mushroom *Lentinula edodes* strain FMRI0339 of the *L. edodes* mycovirus (LeV) in order to obtain an isogenic virus-free fungal strain as well as a virus-infected strain for comparison. Mycelial fragmentation, followed by being spread on a plate with serial dilutions resulted in a virus-free colony. Viral absence was confirmed with gel electrophoresis after dsRNA-specific virus purification, Northern blot analysis, and RT-PCR. Interestingly, the viral titer of LeV varied depending on the culture condition. The titer from the plate culture showed at least a 20-fold higher concentration than that grown in the liquid culture. However, the reduced virus titer in the liquid culture was recovered by transferring the mycelia to a plate containing the same medium. In addition, oxygen-depleted culture conditions resulted in a significant decrease of viral concentration, but not to the extent seen in the submerged liquid culture. Although no discernable phenotypic changes in colony morphology were observed, virus-cured strains showed significantly higher growth rates and mycelial mass than virus-infected strains. In addition, we are currently exploring effects of LeV on fruiting body formation and mushroom yield. The fruiting body formation yield of virus-free *L. edodes* was significantly larger than virus-infected *L. edodes*. These results indicate that LeV infection has a deleterious effect on mycelial growth and fruiting body formation.
Keywords : *Lentinula edodes*, dsRNA, Isogenic strain, Mycovirus

J032

Biological function of the mycovirus PoV in the edible mushroom *Pleurotus ostreatus*

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PoV virus was found in *Pleurotus ostreatus* Halla strain, a bottle cultivated commercial strains of oyster mushroom in Korea. In this study, we attempt to cure the edible mushroom *P. ostreatus* Halla strain of the *P. ostreatus* mycovirus (PoV) in order to obtain an isogenic virus-free fungal strain as well as virus-infected strain towards contrast. Mycelial fragmentation, followed by being spread on a plate with nylon mesh filtrations resulted in a virus-free colony. Virus curing was verified with gel electrophoresis after dsRNA-specific virus purification and Northern blot analysis. For the Northern blot analysis of dsRNA was performed a hybridization with RDRP probe. The growth rate and mycelial dry weight of virus-infected *P. ostreatus* strain were compared to two virus-free isogenic strains using 11 different media. The virus-cured strain was observed higher growth rate than virus-infected strain on culture media czapex-dox, ME, sawdust, V8, and YMG media. In addition, we are currently exploring effects of PoV on fruiting body formation and mushroom yield. The fruiting body formation yield of virus-free *P. ostreatus* strain was significantly larger than virus-infected *P. ostreatus* strain. These results indicate that the presence of PoV affect the mycelial growth and fruting body formation of the *P. ostreatus*.
Keywords : *Pleurotus ostreatus*, Isogenic strain, dsRNA, PoV

J033

Determination of the Dose Response Curve for the UV Sensitivity of HPC in Drinking Water

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Ultraviolet disinfection has been used commercially as an effective method for inactivating waterborne pathogens. As this system becomes more popular, more manufacturers are becoming aware of NSF/ANSI Standard 55. This Standard separates UV systems into two distinct classes. The Class A UV systems must deliver a minimum UV dose of 40mJ/cm2. And Class B systems are required to deliver of 16mJ/cm2.The purpose of this study is to determine the UV sensitivity of the heterotrophic plate counts (HPC) in drinking water. It can be useful for monitoring of operational UV dose delivered by the UV disinfection system. The dose response characteristics of the HPC is determined by collimated beam assay. The dose response curve determined that an effective dose of 40mJ/cm^2 was necessary to achieve 3-log inactivation of HPC. And 1.2-log inactivation of HPC was achieved with a UV dose of about 16mJ/cm^2

Keywords : Ultraviolet disinfection, HPC

J034

Antifungal Activities of Actinobacteria against the *Malassezia* species

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Malassezia spp. *are* yeasts and members of the human skin flora, in particular the head, neck, chest, upper arms, back, abdomen and face. *Malassezia* spp. are also considered to be etiological agents in superficial skin diseases, such as pityriasis versicolor, seborrhoeic dermatitis, and *Malassezia* folliculitis, dandruff and infrequently cause systemic disease associated with lipid-rich hyper alimentation fluids. As there is cumulative resistance against antibiotics of many bacteria, the development of new antimicrobial agents for the treatment of skin infections is needed. We have isolated the actinobacteria that inhibiting the growth of *Malassezia sympodialis*, *Malassezia furfur*, and *Malassezia solooffiae*. Among 350 isolates strain T1506, T327 and T23 had more clear inhibition zone. This result suggests that these strains of actinobacteria may produce antibiotics to treat the skin diseases and hair dandruff through makeup creams and hair shampoos. Further studies are needed and should focus on the assessment of these strains that also exhibit activity against potential skin pathogens and will indeed persist on the skin in vivo and be active there.

Keywords : Malassezia, antifungal activity, Actinobacteria, skin disease, dandruff

J035

Non-Sapoonin Contents of Fresh, White and Red Ginseng of Various Varietals from Different Region in Korea

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Korean ginseng, Panax ginseng C. A. Meyer, has useful biofunctional properties. In this study, different varietals of 6-year-old fresh, white, and red ginsengs were obtained from 4 different regions (Inje, Geumsan, Jinan, and Punggi) in Korea. Five varietals were Chunpoong, Yunpoong, Gumpoong, K-1, and Hybrid. Non-saponin contents of each ginseng powder, such as acidic polysaccharide, crude polyacetylene, and total polyphenol, were analyzed. Acidic polysaccharide and crude polyacetylene contents of fresh, white and red ginsengs were different. Acidic polysacchriade of red ginseng was high, 4.76 ㎎/㎖, compared to that of fresh and white ginsengs, 1.36 ㎎/㎖ and 1.86 ㎎/㎖, respectively. Average of crude polyacetylene in five varietals were 27.33% in fresh ginseng, 22.71% in white ginseng, and 28.97% in red ginseng. The contents of non-saponin in most samples were, however, not significantly different between regions. The contents of ginsenoside of each sample are analyzed in progress.

Keywords : ginseng, non-saponin

J036

Functional Soil Metagenome Mining for Novel Gentamicin Resistance Genes

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Many infectious diseases that once killed people can now be treated effectively with antibiotics. However, certain bacteria have become resistant to commonly used most antibiotics. Antibiotic resistance occurs when the bacteria are "resistant" and continue to multiply in the presence of therapeutic levels of an antibiotics. Bacteria may also become resistant by a genetic mutation or by acquiring resistance from another bacterium. Here, we screened soil metagenome library for isolating gentamicin resistance related genes from environmental samples, mostly soil. Gentamicin is a bactericidal aminoglycoside antibiotic that works by irreversibly binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis. Gentamicin resistance bacteria are problematic in hospitals because has been used extensively. From large screening, we selected four clones that are resistant on 10 ug/ml gentamicin application on agar medium and constructed shot-gun libraries. The sequencing of the shot-gun clones revealed 5 unique genes that encode novel protein variants of four protein families including RNA lagases T4 rnlA, mechanosensitive ion channel proteins, aminopeptidases, and 1-acyl-sn-glycerol-3-phosphate transferases. Our results indicate that the soil metagenome can provide an important resource for the gentamicin resistance and the molecular target to overcome the drug resistance to gentamicin.

Keywords : Metagenome, Superbacteria, gentamicin

J037

Antimicrobial Activity of Korean and Chinese *Crataegus fructus*

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The *Crataegus fructus* has been used as medicinal and food source in Korea and China. This study was conducted to compare the antimicrobial activity of Korean and Chinese *Crataegus fructus*. The Korean and Chinese *Crataegus fructus* were extracted with methanol. Antimicrobial activity of extracts were tested against pathogenic bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus gordonii*, *Streptococcus mutans and Streptococcus sanguinis*) by paper disc methods. The methanol extracts of Korean and Chinese *Crataegus fructus* showed antimicrobial activity against all tested strains. The methanol extracts of Chinese *Crataegus fructus* more than Korean *Crataegus fructus* showed strong antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis and Streptococcus sanguinis* with minimum inhibitory concentration (MIC) range of 0.5-1 mg/ml. Especially, the methanol extracts of Chinese *Crataegus fructus* was inhibited the biofilm formation of *Staphylococcus aureus* at a concentration of 0.8 mg/ml. These results suggest that extracts of *Crataegus fructus* can be developed as a potent antimicrobial agent.
Keywords : Antimicrobial activity, Chinese *Crataegus fructus*, Korean *Crataegus fructus*

J038

Functional Soil Metagenome Mining for Triggering Induced Systemic Resistance in Tobacco

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Plants in environments are exposed to diverse microorganisms that would affect to plants. Previous studies have been discovered beneficial microbes resulting in improvement of plant defenses and its specialized bacteria are known as plant growth-promoting rhizobacteria leading to enhancing plant productivity and eliciting plant immunity against plant pathogens. However, there is limitation on the research because the study of bacterial metabolism is based solely on cultivated organisms. To overcome the pitfall, metagenomics that is an innovative technique to use diverse environmental samples was developed and employed. Recent development of metagenomic approaches has opened the window to be offered the discovery of novel genes and pathways. In the current study, the 605 metagenome pools were screened and a single clone was selected in order to test the capability to elicit induced resistance against *Pectobacterium carotovorum* subsp. *carotovorum* and *Pseudomonas syringae* pv. tabaci treated into tobacco seedling *in vitro*. The in-depth investigation on searching determinant to elicit induced resistance revealed unknown gene, predicted as around 2kb. The selected single clone was enough to be acquired induced resistance capacity and expression of the induced resistance marker genes, *pathogenesis-related* (PR) *1a*, *1b*, and *1c*. Our results suggest that metagenome has a strong capacity for potential biological control and becomes a valuable resource for plant defense system.
Keywords : Soil Functional Metagenome, ISR

J039

Isolation and Characteristics of Photosynthetic Bacteria from Soils

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Photosynthetic bacteria are found widely distributed in various habitats. These bacteria have been isolated and selected for applications in the areas of agriculture because they are metabolically versatile organisms. To develop biofertilizer, photosynthetic bacteria were isolated and investigated functional analysis. The photosynthetic bacteria were isolated from various soil samples which were collected paddy field, greenhouse, riverbeds, and pond. Approximately 87 isolates of photosynthetic bacteria were isolated by enrichment culture with paraffin wax overlay method. These isolates were identified as 2 genera (*Rhodobacter* and *Rhodopseudomons*) and 5 species by 16S rDNA sequence analysis. These isolates were evaluated in vitro for the antifungal activity against 9 species of plant pathogenic fungi. All isolates could not inhibited growth of tested plant pathogenic fungi. The germination and root elongation assay was performed with cucumber seed to estimate effects of seed treatment with 5 isolates which selected by species. There were no differences in germination of cucumber seeds due to bacteria, but the length of bacteria treated root was shorter than the control. The growth promoting assay was performed with cucumber seedlings. Photosynthetic bacteria were treated 3 times at every week to seedlings, and plant length was measured after 30 days cultivation. The length of the plant which was treated bacteria increased by 23.6 - 34.7% compared with untreated control.
Keywords : photosynthetic bacteria, biofertilizer, plant growth promoting, cucumber

J040

Anti-bacterial Derived Extract of Spent Mushroom(Hericium erinaceus) Compost

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Bacterial wilt caused by Ralstonia solanacearum is one of the most devastating soil-borne diseases of plants worldwide and affects many important crop species. R. solanacearum has been investigated both biochemically and genetically and recognized as a model system for the analysis of pathogenicity. Chemical control has become less effective due to the development of pathogen resistance beside the potentially undesirable effects of the fungicides on human, plants and other beneficial organisms. Control could be considered as an alternative of chemical control. Derived extract of spent mushroom(Hericium erinaceus) compost was screened for antibacterial activity against phytopathogenic bacteria. The Derived extract of spent mushroom(Hericium erinaceus) compost showed antibacterial activity against R. solanacearum in vitro. Also, this extract suppressed the disease development of Bacterial wilt on tomato in pot test.
Keywords : Derived Extract, Mushroom, Bacterial wilt, Control

J041

Metagenomic Comparison of Oral Microbiome between Dogs and Owners by Next Generation Sequencing

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Oral microbiome which is closely connected with various diseases and the pathogenic oral bacteria can transfer with close physical contact is importantly consider in public health problem. Although the dog is the most common companion animal, oral microbiome composition and relationship between dog and human oral microbiome is in begging step. Comparison of oral microbiome between dogs and owners exhibits characterization of dog oral microbiome, information about potential pathogenic bacteria which harbor in dog oral cavity as well as etiological insight of periodontopathic bacteria. In this study, 16s gene pyrosequencing was applied to examine ten oral samples of dogs and their owners to compare their microbiome similarity and zoonotic pathogens. Pyrosequencing revealed 246 operational taxonomic units in the 10 samples, representing 57 genera from 8 bacterial phyla. Oral microbiome composition of dog and the owner were significantly different. The similarity of oral microbiome between dog and their owner was influenced by oral to oral contact. The suspected genera which transfer from dog to human were four genera: *Neisseria shayeganii*, *Porphyromonas canigingivalis, Tannerella forsythia* and *Streptococcus minor.* Since, potentially zoonotic and periodontopathic bacteria were found in dog oral microbiome, control dog oral microbiome transmission is crucial point in public health.
Keywords : high-throughput pyrosequencing, oral microbiome, periodontopathic bacteria, canine, 16s rRNA

J042

The Occurrences of Cyanobacteria, Actinomycetes and geosmin in Drinking Water Resource, Korea

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In 2012, a massive geosmin had been occurred in Paldang reservoir which is drinking water resource of Seoul. We investigated cyanobacterial, geosmin and actinomycetes for identifying of geosmin source in July and August. In total, 68 water samples were collected in two sampling sites(Paldang and Sambong), and were used to concentrate and analyze the correlation. The highest cyanobacteria and geosmin were concentrated as 11, 568 cell/ ml and 3,157 ng/L, respectively in Paldang. In Sambong, cyanobacteria was detected up to 24,722 cell/ml and geosmin analyzed with 4,384 ng/L. Cyanobacterial have been found to positively correlate with geosmin (R^2=0.85, $p<0.0001$) and actinomycetes was showed the negatively correlation with geosmin. In additionally, actinomycetes are associated with increased turbidity. Various water quality parameters such as temperature, pH and dissolved oxygen, temperature were found to affect the cyanobacteria in Paldang reservoirs. These results indicated the main source of geosmin was cyanobacterial in Paldang reservoirs, which may be provided useful information to manage the off-favor of drinking water resource.
Keywords : *Cyanobacteria*, Geosmin, *Actinomycetes*, Paldang, Drinking water resource

J043

Baculovirus based GP5 and M DNA Nanocarrier for Porcine Reproductive and Respiratory Syndrome Virus Vaccine

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Porcine reproductive and respiratory syndrome virus (PRRSV) causes an economically devastating of porcine industry, by reproductive failure in pregnant sows and by respiratory tract illness in piglets. PRRSV belongs to *Arteriviridae* family. The GP5 and M proteins are the key immunogenic proteins of PRRSV. In this study, we constructed a baculovirus based Recombinant DNA vaccine (AcPERV-C5/C6). SPF 6-weeks BALB/c mice and SPF 5-weeks miniature pigs were immunized intramuscular AcPERV-C5/C6 and commercial PRRSV killed vaccine. All of the groups were immunized 3 weeks interval, two times injection. Serum samples were collected at 3 and 5 weeks after post-immunization. There results showed that AcPERV-C5/C6 can significantly enhance PRRSV-specific antibody, PRRSV-specific neutralizing antibody, IFN- γlevel rather than those of commercial PRRSV killed vaccine. Therefore, this study suggests that AcPERV-C5/C6 DNA Vaccine can be a potential efficient prophylactic vaccine candidate.
Keywords : PRRSV, Baculovirus, DNA vaccine, Immunization, Immune response

J044

First record of genus *Buchwaldoboletus* (Boletaceae, Basidiomycota) in Korea: *B.* cf. *lignicola*

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During a researches of Korean mushroom flora in 2014, we collected two boletoid specimens (KA14-0711 and KA14-0907) which are growing on felled tree (*Pinus koraiensis*) in Gwangneung Forest, Korea. Both specimens were identified as *Buchwaldoboletus* cf. *lignicola* based on ecological and morphological characteristics including macro- and micro-scopic characters. The main characters of specimens are the yellow-brown pileus with appressed tomentum, lack of veil, blueing instantly when bruised and golden-yellow rhizomorphs at the base of stipe. Interestingly, genus *Buchwaldoboletus* is the uniquely saprophytic and lignicolous fungi in the Boletaceae. Here we discribed and reported this species for the first time in Korea.
Keywords : *Buchwaldoboletus*, Boletaceae, Boletoid, Morphology, Gwangneung Forest

J045

The Surveillance of Imported Infectious Diseases in the South West Coastal Area of Korea by Yeosu National Quarantine Station

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Depending on the travel destination, international travelers may be exposed to a number of endemic infectious diseases such as malaria, influenza and dengue fever. In spite of many studies from the 2009 pandemic influenza, the rapid spread of contagious diseases was also illustrated in the middle east respiratory syndrome outbreak of 2013 and ebola virus disease outbreak in west Africa recently. In Korea, thirteen quarantine stations located at major ports of entry and land border crossings where international travelers arrive. In order to prevent the spread of communicable diseases, quarantine officers are obligated to inspect incoming conveyances, cargos and passengers according to the Quarantine Act and International Health Regulations 2005 by World Health Organization. Furthermore, Yeosu national quarantine station is the major inspection center of south west coastal area of Korea and diagnoses the biological and environmental specimens from quarantine inspection. As a result of test from 2011 to 2013, the 4,416 positive samples were detected from 27,338 quarantine inspection specimens.
Keywords : imported infectious diseases, quarantine, communicable diseases, *Vibrio* species

J046

A Comparison of the Phyllophere Microbial Community and Disease Incidence Changes between Drought Resistant Transgenic Rice and Non-transgenic Rice

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We surveyed number of phyllosphere microbe populations and disease incidence, comparing drought resistance transgenic rice (Agb0103) with non-transgenic rice (Ilmi) during 2011-2014. Number of phyllosphere microbe populations such as fungi or bacteria was determined by counting colony which cultured on PDA or LB media while polymerase chain reaction with denaturing gel electrophoresis (PCR-DGGE) analysis supported those data. Disease incidence survey was conducted focused on major disease; Rice blast, Brown spot, Bakanae, Sheat blight and False smut. Also fungal pathogen occurring Sheat blight and Brown spot disease were isolated each of transgenic and non-transgenic rice, and then inoculated to healthy rice. As the results, there were no differences between transgenic and non-transgenic rice for the phyllosphere microbe colony, PCR-DGGE analysis, disease incidence and pathogenicity test. Meanwhile upweighted pair-group method with arithmetic average (UPGMA) dendrogram based on PCR-DGGE analysis revealed that the community of bacteria and fungi organized by each month, and not organized by rice variety. Although drought resistant transgenic rice was genetically modified, there was no difference compare to non-transgenic rice in natural condition. Therefore, it is assumed that transgenic rice could not influence the phyllosphere microbe populations and environmental conditions.
Keywords : Disease incidence, PCR-DGGE, Phyllosphere microbe, Transgenic rice

J047

Antimicrobial Activity of Weakly Acidic Electrolyzed Water against Strict Anaerobic Pathogens

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The purpose of this study was to evaluate the bactericidal activity of weakly acidic electrolyzed water (WAEW) generated by a small-scale electrolysis device manufactured by Spacelink Corp. against very diverse aerobic and anaerobic microorganisms including strict anaerobic pathogens. The following microorganisms have been used for evaluating the antibacterial activity of WAEW; Propionibacterium acnes, Clostridium perfringens Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus, Streptococcus mutans, Pseudomonas aeruginosa, Streptococcus pneumoniae, Serratia marcescens, Lactobacillus acidophilus, Campylobacter jejuni, Campylobacter coli, Candida albicans, Malassezia furfur, Aspergilus flavus, Vibrio parahaemolyticus and Trichophyton rubrum. Each cell suspension (10^9 and 10^8 CFU/mL) was added to 19 mL WAEW (40 and 80 ppm, pH 5.5) and after 30 seconds the treated suspensions were spread on each agar plate and the viable colonies were counted. Several Gram-positive bacteria with 10^9 CFU/mL such as Staphylococcus aureus and Bacillus cereus still survived with 80 ppm WAEW. However, no colonies were detected from all the tested microorganisms of 10^8 CFU/mL regardless of bacterial cell wall type or concentration of WAEW. These results indicated that the cell wall type of bacteria and concentration of the cells could be critical for the antimicrobial activity of WAEW.
Keywords : electrolyzed water, pathogens, Antimicrobial activity

J048

Effect of Protectants on Survivability of Freeze-dried Human Pathogens during Long-term Storage

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Preservation of useful microorganisms in culture collection requires successful long-term storage and reactivation techniques. However, some species like *Vibrio and Streptococcus* often fail to long-term preservation, resulting in loss of them. The aim of this study is therefore to develop a simple and effective method for long-term preservation of human pathogens such as *S.pneumoniae*, *N.gonorrhoeae*, *H. influenzae* and *Vibrio pathogens*. Bacterial cells purely cultured were harvested at the early exponential stage, suspended in serum, sucrose, trehalose, skim milk and/or inositol solution as a protectant, and aliquoted in sterilized ampoules. After frozen and dried at -80°C under vacuum, the ampoules were stored for 2 weeks at 37°C for accelerated test. Freeze-dried bacterium was observed under the electronic microscope and their survivability was determined by flow cytometry and colony count methods. The data from four species commonly showed great viability (10^8 CFU ml^{-1}) when cultured in phosphate buffer saline (pH7.4) after preserved in 5% inositol or 10% sucrose solution. In addition, we are currently conducting proteomic analysis of long-term preserved *Vibrio* cells to find out a protective mechanism of inositol to their flagella and outer membrane proteins as the further study. These suggest that saccharide could be a good candidate as a cryo- and lyo-protectant for human pathogens and PBS positively helps them to reactivate and proliferate.
Keywords : Human pathogen, Long-term storage, Protectant, Inositol, Sucrose

J049

Selection of Entomopathogenic Fungi for Dual Control of both Aphid and Plant Fungal Disease

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During crop cultivation, crop was infested several insect pests and/or plant diseases. Chemical control has been major control method, but development of resistance by pest or disease is demanding alternative control methods. Entomopathogenic fungi is one of alternative control agents. Narrow host range is one of disadvantages using mycopesticides using fungi. To solve this problem we conducted bioassay to select entomopathogenic fungi having high control efficacy against both insect pest, especially cotton aphid and green peach aphid, and plant fungal disease. We cultivated 11 isolates of entomopathogenic fungi on three different media. Virulence of the fungi differed from isolate and cultured media. Some isolates showed high pathogenicity in all culture media to aphids and some isolates had different control effect from different culture media. These isolates were tested antagonistic effect to 8 plant fungal diseases. Few entomopathogenic fungi showed week control effect to fungal plant disease on crop leaf.
Keywords : entomopathogenic fungi, biological control, cotton aphid, green peach aphid, plant fungal disease

J050

Aphid Control with Culture Filtrate of Entomopathogenic Fungi *Beauveria bassiana*

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Aphids are one of the most destructive pests in crop production such as pepper, cucumber, and egg plants. The importance of entomopathogenic fungi as alternative pest control agents is increasing. Conidia of entomopathogenic fungi are influenced by environmental conditions such as temperature and relative humidity and caused slow and fluctuation of mortality. These factors are preventing wider application and use of these biocontrol agents. We selected one entomopathogenic fungal isolate which showed high mortality against green peach aphid with liquid culture filtrate. The culture filtrate separated through gel filtration with different chloroform: methanol concentration. 5 different fractions were sprayed onto cucumber leaf infested with cotton aphid. Mortality of cotton aphid was highest with chloroform: methanol = 50:1 followed 30:1 but fractions from 70:1, 90:1 and 100:1 had no control efficacy. In future we will analyses the fraction having high mortality to find active ingredient causing aphicidal.
Keywords : entomopathogenic fungi, culture filtrate, aphicidal

J051

Inhibition of Gmmaherpesviruses by Natural Phytochemicals from *Robinia pseudoacacia L.*

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Despite the importance of human gammaherpesviruses including Epstein-Barr virus (EBV) as the first discovered human cancer virus, few antiviral drugs are developed to efficiently control viral replication. Here we tested the antiviral activity of natural phytochemicals from *Robinia pseudoacacia* against gammaherpesviruses. *R. pseudoacacia*, also known as the black locust, is a major honey plant that is widely distributed around the world. Our previous results demonstrated that the total extracts and various fractions of *R. pseudoacacia* exhibited antiviral activity against gammaherpesviruses. Here we tested whether natural phytochemicals present in *R. pseudoacacia* efficiently controled viral lytic replication. Reactivation of human gammaherpesviruses as well as *de novo* infection of murine gammaherpesvirus 68 (MHV-68) was inhibited by a subset of tested phytochemicals in a dose-dependent manner. Distinct inhibitory mechanisms were shown from two single compounds among flavonoids in *R. pseudoacacia*. These results suggest that the natural phytochemicals may provide a lead structure to develop an antiviral drug against gammaherpesviruses.
Keywords : Gammaherpesvirus, Antiviral activity, Phytochemical

J052

Antiviral Activity of Crude Extracts and Organic Solvent Fractions of *Ligustrum lucidum* and *Robinia pseudoacacia* against Gammaherpesviruses

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Human gammaherpesviruses including Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) are important pathogens as they persist in the host and cause various malignancies. However, few antiviral drugs are available to efficiently control gammaherpesvirus replication. Here we screened a number of plant extracts and identified the antiviral activity of extracts from *Ligustrum lucidum* and *Robinia pseudoacacia* against gammaherpesviruses. Because of its tolerance of air pollution and poor soils, *L. lucidium* is highly used as a street tree and become a popular landscape plant in many countries. *R. pseudoacacia* is also widely distributed in various parts of the world as a major honey plant. Our results showed that extracts of *L. lucidum* and *R. pseudoacacia* efficiently inhibited TPA-induced viral lytic replication in EBV- or KSHV-infected cells as well as *de novo* infection of murine gammaherpesvirus 68. Various fractions of *L. lucidum* and *R. pseudoacacia* showed antiviral activity against gammaherpesviruses to different degrees. Taken together, these results suggest that bioactive substances from *L. lucidum* and *R. pseudoacacia* may exhibit potent antiviral activity against gammaherpesviruses.
Keywords : Gammaherpesvirus, Antiviral activiry, Plant extract

J053

Characteristic of a New Variety *Lentinula edodes*, 'Nongjin-go'

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'Nongjin-go' is a new breed strain of *Lentinula edodes,* saw-dust bag variety. It is a cross combination of dikaryon ASI 3305mut and monokaryon L5-16. We crossbred them in 2011 and verified productive capacity from 2012 to 2013 in Rural Development Administration. Optimum temperature of mycelial growth is 30 celsius and it of fruit-body primodium formation is range from 15 to 23 celsius. It is mid-high temperature adaptable *Lentinula edodes*. Fruit-body is platy-hemisphere, light brown and centralizing. And bast is formed around edge of pilei. Yield productions per period is regular than 'Sanjo701'. Plastic bag culture medium is 1.5 kilogram and culture periods are 90~100 days. As its browning in culture is a little slow, Light and ventilation is needed a lot in light-culturing. Humidity is controlled properly for its color in fruit-body growing. Tested culture medium is consisted of 80% Oak-Tree saw-dust and 20% rice-bran

Keywords : *Lentinula edodes*, new variety, saw-dust bag, Di-Mono cross, mid-high temperature

J054

Center for Fungal Genetic Resources (CFGR): Housing Plant Pathogenic Fungi for Educational and Research Purposes

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Fungi are eukaryotic organisms, growing in a wide range of habitats. Fungi are significantly important in a variety of ways. They play an essential role in the decomposition of organic matter. They have been used as a source of food, and agents for fermentation of food products and for the production of various antibiotics and enzymes that are used in a field of research, industry, medicine, etc. In contrary, impact of many fungi on animals and plants is economically and socially detrimental. For example, *Magnaporthe oryzae* causes the most destructive disease, "rice blast". Annual yield loss of rice by rice blast is equivalent to rice that could feed about 60 million people. The Center for Fungal Genetic Resources (CFGR) was established to collect, maintain and distribute genetic resources mainly from plant pathogenic fungi, which are important for both educational and research purposes. This will contribute to development of new strategies for management of crop diseases and of new components for improvement of our lives. CFGR possesses important fungal species; a total of 42,000 isolates from 54 species of fungi including 20,902 T-DNA transformants of rice blast fungus and anthracnose fungus. In addition to the biological materials, CFGR has developed user-friendly databases to maintain genetic information of fungal stocks and help to solve questions about fungal pathogenicity, population genetics, development, and evolution. Also, CFGR seeks strategies for sustainable and scientific plant quarantine to better protect our ecosystem from invasive microorganisms.

Keyword : Plant pathogen, Fungi, Mutant, Plant quarantine, Pathogenicity genes, Genetic resource

J055

Korean Metagenome Bank for Exploiting Microbial Diversity

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Microorganisms have played important roles in biotechnology and bioindustry for long times. The recent use of molecular ecological methods and environmental DNA (eDNA) has changed our knowledge of microbial diversity dramatically and provided rapid access to genes of yet-uncultured microorganisms. Application of molecular ecological studies has shown that the majority (99 %) of microorganisms present in the nature are under uncultivation. Many attempts to improve the recovery of microorganisms and their genes from the environmental samples have recently been achieved. Metagenomic approach that recovers the environmental DNA without the limitations of culture-dependent methods and constructs DNA libraries in suitable cloning vectors and host strains have been utilized for retrieving novel and useful genes. Korean Metagenome Bank (KMGB), a member of Korea National Research Resource center (KNRRC), has opened with the goal for the collection and distribution of metagenome (eDNA) and metagenomic library. The aims of the Korean Metagenome Bank are to contribute to the development of biotechnology by providing the metagenomic resources into various researches and to perform a national mission for maintaining the metagenomes as future biological resources

Keyword : Metagenome, library, environmental DNA

J056

Bacteriophagebank

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Bacteriophages are viruses growing on bacterial hosts. They are antagonistic to bacteria and first reported by Frederick Twort and Felix d'Herelle in 1915 and 1917, respectively. They are found in sea, air, land and even foods. It is assumed that 10^{30} to 10^{32} phages exist on earth and they play a role in maintenance of biological balance. Recently, new applications for phages are increasingly reported. As they are a part of useful biological resources, there are increasing demands for securing these resources. In response to these demands, the bacteriophage bank was established in 2010. The bank collects phages from environments as well as from working groups worldwide. Currently, 600 different phages are stocked. The host bacteria include E. coli, Salmonella enteritidis, Pseudomonas aeruginosa, Listeria monocytogenes, Acinetobacter, Camphylobacter jejuni, Enterococcus faecium, Enterococcus faecalis, Cronobacter sakazakii, Seratia marcescens and Staphylococcus aureus. The number of stock is growing continuously. The bank also serves as a distributor for the collected phages. (www.phagebank.or.kr)

Keyword : Bacteriophage, 600 different phages, useful biological resources

J057

Korea Bank for Pathogenic Viruses

Ki-Joon Song[1]
[1] *Korea Bank for Pathogenic viruses*

Korea Bank for Pathogenic Viruses(KBPV) has been established in 2005 as a repository agent for the collection, management and distribution of the various pathogenic viruses that are essential to use for researches in biomedical sciences. The Institution operates in collaboration with The Institute for Viral Disease at Department of Microbiology, College of Medicine, Korea University, founded in 1973. The bank has unique viral collections such as Hantaan, Seoul, Muju, Soochong, and imjin the etiologic agents of hemorrhagic fever with renal syndrome. To date, total of more than 43,000 materials(~100,000vials) from human and animal have been collected and maintained. We have provided a highly collaborative environment for researchers in various fields by providing valuable viral resources including consulting service. We also provide the educational program related to pathogenic viruses including biosafety training. Requestors of such agents are required to register with KBPV and to supply details of their laboratory facilities and safety management. More details about KBPV can be found at ; http://kbpv.knrrc.or.kr

Keyword : Pathogenic Viruses, biosafety, viral disease, genetic information, antibody

J058

Plant Virus GenBank

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Plant Virus GenBank (PVGB) is a nonprofit semi-governmental organization, one of the Korea National Research Resources Collections (KNRRC) for special research materials Banks program financially supported by the Ministry of Education, Science & Technology (MEST) dedicated to collection, identification, characterization, preservation, research development, distribution and deposition of plant virus research biomaterials established since 1999. PVGB is one of substructure of Korea National Microbiological Research Resources Collections (KNMRRC). PVGB retains a number of accessions and a wide range of collections of Plant Virus Biomaterials useful for Plant Virology and Biotech-related research areas. PVGB has moved to its current status on November in 2000 and has modern facilities and infrastructures for supporting broad research fields as well as Plant Virology Community. PVGB has been recognized as a member of World Federation for Culture Collection & World Data Center for Microorganisms (WFCC- WDCM) and ISBER since April of 2001 and June of 2007, respectively. Main objectives and contents of PVGB can be categorized as 7 topics as follow ; collection and development of Plant Virus Research Biomaterials such as infectious plant virus culture, plant viral cDNA clone, plant virus antiserum, biologically active full-length cDNA clone, viral cDNA library, virus-induced plant cDNA library, and diagnostic primers, preservation of Plant Virus Research Biomaterials, Distribution of Plant Virus Research Biomaterials to worldwide researchers to support their research fields and Safe Deposit from virologists, Development of New Plant Virology Techniques, i.e., molecular taxonomy of plant viruses, infectious cDNA clones, molecular indexing of virus variation, screening of virus resistance, virus-resistant transgenic plants, and risk assessment for living modified (LM) virus and LM plant systems, collection and support of Research Information

Keyword : Plant Virus, GenBank, Resource center

J059

Lichen as a Novel Bioresources in Korea

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Lichens are symbiotic organisms composed of a fungus (mycobiont) and an alga (photobiont). They produce characteristic secondary metabolites, lichen substances, which seldom occur in other organisms. Lichen and their metabolites have many biological activities. In spite of the wide spectrum of biological activities shown by the lichens, they have long been neglected by mycologists and overlooked by agrochemical industry because of its slow growth in nature and difficulties in the artificial cultivation of organisms. Use of lichen-forming fungi can overcome the disadvantage of natural lichen extracts for industrialization of their metabolites because of their much faster growth and larger production of the metabolites in culture than the natural thalli. Korean Lichen and Allied Bioresources Center focuses on isolation, maintenance and distribution of lichen bioresources to research groups in universities, national institutes and industrial sectors. It also screens their biological activities, and investigates cultural conditions for large production of lichen substances. Chemical library of some lichen extracts is also available from the center.

Keyword : lichen, lichen-forming fungi, photobiont

J060

Korean Collection for Oral Microbiology

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It has been known that about 700 species of oral bacteria inhabit the human oral cavity. Of them, 350 species have been cultured. The oral bacteria are the major causative agents of systemic diseases such as cardiovascular diseases as well as oral diseases, periodontitis and dental caries. However, the causative bacterial species for oral diseases have not been known because the dental diseases are occurred by the multiple infections. In addition, the prevalence of the oral bacterial species is different by the geographic location of the host and individual. It is very important to obtain the oral bacteria from Koreans for pathogenesis studies related to oral infectious diseases. The purpose of Korean Collection for Oral Microbiology is to obtain the oral clinical strains and their genetic resources, such as 16S rDNA, species-specific PCR or qRT-PCR primers, and genome sequences, for offering them to the researchers.

Keyword : oral bacteria, oral diseases, pathogenesis, resource center

J061

Korea Marine Microalgae Culture Center

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Today microalgae are widely used in research and as educational materials. They are also commercialized in the industries of food, animal feed and environment. Microalgae exhibit a promising potential to be converted into pharmaceutical products and bio-fuel energy. For this reason, there are active, ongoing researches on microalgae with tremendous expectations of scientists. The Korea Marine Microalgae Culture Center (KMMCC) was established with a financial support from National Research Foundation of Korea in 1995. The collection of microalgae has been increasing continuously since 1995, and its number has reached to about 2,100 strains in 2013. The collection mainly consists of marine strains (80%) which are mostly isolated from Korean waters (96%). The major classes of the strains are Bacillariophyceae (54%), Chlorophyceae (18%), Dinophyceae (9%), Cyanophyceae (5%), Prasinophyceae (4%), Eustigmatophyceae (3%), Haptophyceae (2%), etc. With respect to identification of the strains, about 97% and 56% of them are identified at the level of species and genus, respectively. In addition, 3% are still unidentified, and about 51% of the strains are under axenic state. The culture strains of the KMMCC are introduced regarding information on sampling, culture, biological and chemical characteristics of each strain. The initial direction of the KMMCC focused on finding microalgal strains that had a good dietary value for larvae in aquaculture. Such strains used to be supplied to the hatchery. Our recent work, however, has shifted to collecting a wider range of diverse microalgae which are taxonomically different. We also pursue the effective preservation and quality control of the microalgae strains. In accordance with the KMMCC's progression on the strain collection, the demand for the strains in research fields has been expanding from the industry of aquaculture to biotechnology, environmental sciences, engineering, biological oceanography, etc. In Korea, recently, the annual domestic request for the microalgae from academic and commercial organizations has increased up to nearly 190 requests for as many as 400 strains. Making a deposit with newly isolated microalgal strains in the KMMCC by other investigators is always possible if they agree to be open about distributing their strains to other researchers as well. The final decision of the strains to be deposited belongs to the KMMCC. Even though assigning a correct taxonomical position to the strains has always been our primary concern, many difficulties still exist in identifying them.

Keyword : Marine microalgae, Marine bio-industry, Marine biological resource, Marine bio-information, Bio-energy

J062

Culture Collection of Antimicrobial Resistant Microbes

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Today, the increasing clinical abuse of antimicrobials in people and animals, led to a high rate of occurrence of resistant microbes. In addition, drug resistance is easily transferred from one resistant species to another related one in many ways, thereby complicating the issue. Therefore, treatment for disease caused by antimicrobial resistant microbes has emerged as a critical issue worldwide, and development of new drugs that inhibit resistant microbes became an urgent issue of research. As the issue should be dealt across clinical research, regulation, and pharmaceutical development, communication and cooperation between researchers among these areas are necessary. Since Culture Collection of Antimicrobial Resistant Microbes was established in 1999, CCARM has been played a role as a connector among various research fields by providing the antimicrobial resistant microbes with known mechanism and information. CCARM collects, keeps, and preserves the resistant microbes in a systemic manner for constant supply of certified microbes and share the information with researchers in various fields. CCARM has a collection of over 20,000 strains of bacteria and yeast from 87 genera and provides various information including international meeting, newest information related to resistance via homepage and newsletter. CCARM is now increasing the interaction and collaboration between culture collections through national and international network as a member of Clinical Laboratory Standards Institute since 2000, World Federation for Culture Collection & World Data Center for Microorganisms since 2003, International Society of Biological and Environmental Repositories since 2007, Korea National Research Resource Center since 2008, and Biological Repositories since 2009.

Keyword : Antimicrobial Resistant, Research Resource Center, bacteria

J063

Helicobacter pylori Korean Type Culture Collection (HpKTCC) Collects and Distributes Clinical Isolates of *H. pylori*

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H. pylori that colonizes only in human gastric mucosa is one of the most common human pathogens and is the main cause of gastritis, peptic ulcer, and gastric cancer. Despite the clinical and commercial importance of *H. pylori*, many researchers have been blocked to investigate the diagnosis, treatment, and prevention of *H. pylori* infections because of difficulty in obtaining *H. pylori* isolates from patients. We have collected and characterized *H. pylori* isolates obtained from worldwide areas to allow researchers to access a variety of characterized *H. pylori* isolates. characterized *H. pylori* isolates. *H. pylori* KTCC contributes to promote the study for the diagnosis, treatment, and prevention of *H. pylori* infections by providing fundamental research materials to investigators.

Keyword : Helicobacter pylori, strain, virulence gene, gastritis, gastroduodenal ulcer, gastric cancer

J064

Korea Environmental Microorganisms Bank

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#103 Research Center Kyonggi University

Korea Environmental Microorganisms Bank(KEMB) has been established as a microbial and genetic resource center for environmental industries. The KEMB plays an essential role as follows: ①the collection and conservation of native environmental microorganisms and genetic resources, ② the construction of systematic management system for effective conservation and application of microbiological resources for environmental industries, ③the provision fundamental data for ecosystem research and microbial classification, and ④the development of biological treatment system for bioremedation of environmental pollutant and ecosystem restoration. There are about 14,000 strains of bacteria collected from environments, at this time. These collections are classified in accordance with scientific and functional characteristics, respectively. It is considered to promote academic and industrial activities by supplying basic materials for research and industrial applications, which accomplish the ecological recovery through constructing eco-friendly bioremediation system by supplying basic microbial resources.

Keyword : Microorganism, Korea National Environmental Microorganisms Bank (KEMB), Environmental Restoration, Bioremediation of Environmental Pollutant, Recovery of Ecosystem

J065

Korea National Microorganisms Research Resource Center

Se joung Yeom* and Sang Seob Lee
Kyonggi University, Research Center

The Korea National Microbiological Research Resource Center is the core center of the twelve microorganism banks designated by the Ministry of Education, Science and Technology. The KNMRRC supports microorganism banks with necessary guidelines, standards, training for efficient operation of the banks. It also provides with an effective forum to solve common issues of the related banks. The ultimate goal of the KNMRRC is the followings: ① construction of standardized and integrated management system, ②construction of Core center and other organs network, ③ Quality Control(QC) of microbial resources in the member banks, ④conservation of Resources in the member banks and the interrupted banks, ⑤education for professionals in the member banks, ⑥ public Relations for raising people's awareness of the importance of microbiological resources.
Keyword : Korea National Microorganisms Research Resource Center, Bacteria, Virus, Fungi, cDNA library and clone

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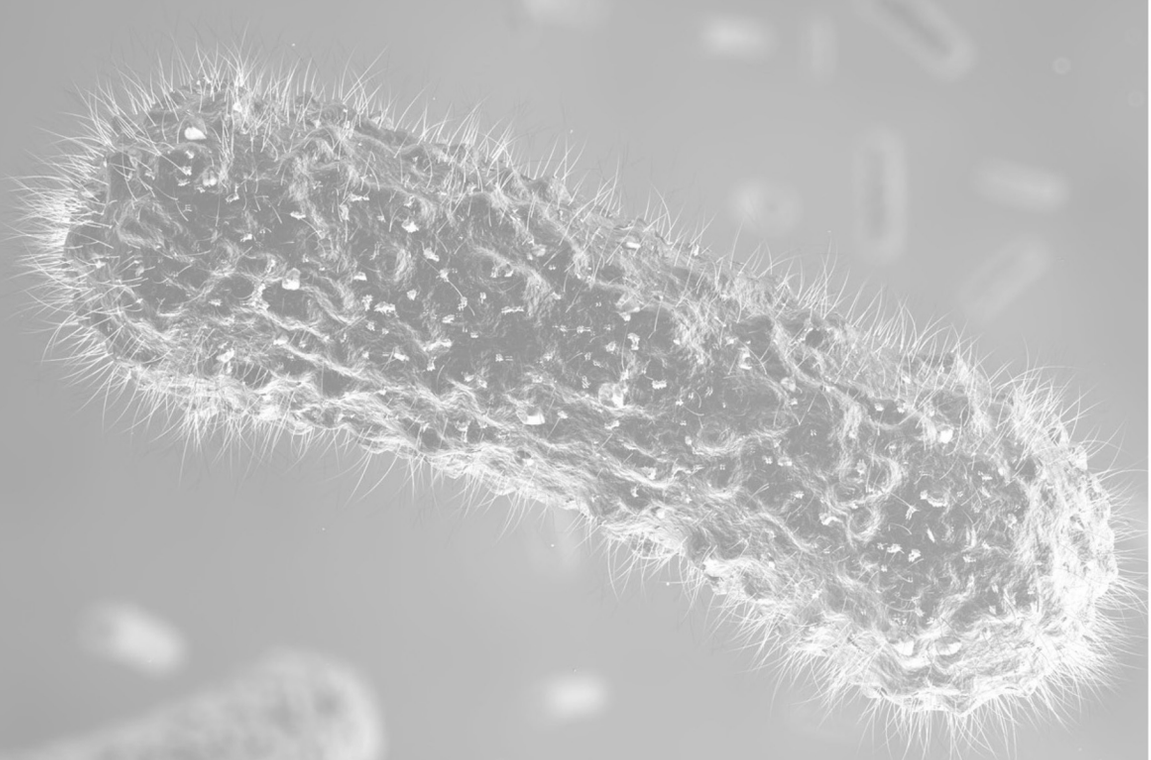

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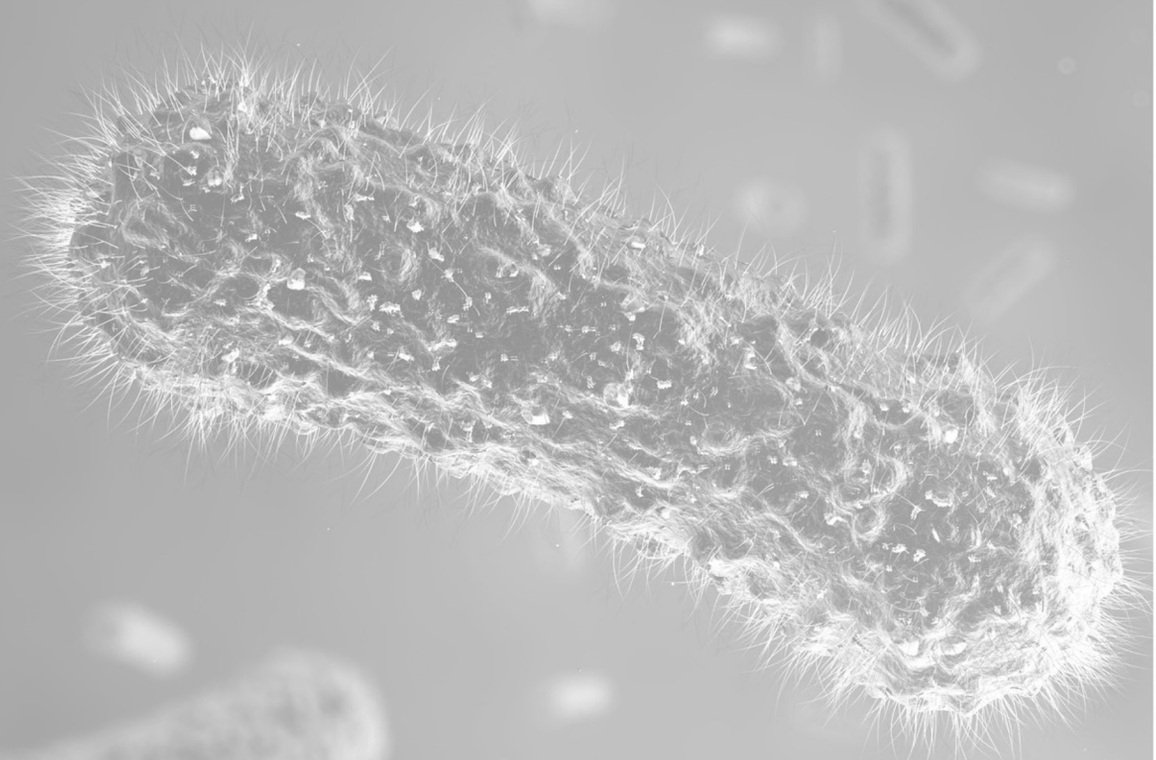

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기타

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신종인플루엔자 범 부처 사업단

인플루엔자 R&D를 적극 지원합니다

인플루엔자에 대한 체계적이고 효율적인 대응 및 해결책을 위해
연구개발을 주도하는 당신의 노력과 열정을 지원합니다.

비전 신종인플루엔자로부터 국민의 안전을 보장할 수 있는 국가 R&D 역량 강화 및 범 부처 협력체계 구축

미션
- 신종인플루엔자 대응대비 국가 R&D 역량 강화 및 범 부처 협력체계 구축
- 신종인플루엔자에 대한 진단, 치료 및 백신기술 개발을 통한 국내 보건의료산업의 기술경쟁력 제고
- 신종인플루엔자에 대한 사회와 국민을 위해 소통 및 위해 관리 마련

Research > Readiness > Response

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범 부처 협력체계를 구축합니다."

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Diagnostics
Public health measures

신종인플루엔자 범 부처사업단
TRANSGOVERNMENTAL ENTERPRISE FOR PANDEMIC INFLUENZA IN KOREA

주관 연구기관
고려대학교 KOREA UNIVERSITY

보건복지부 MINISTRY OF HEALTH & WELFARE
KHIDI 한국보건산업진흥원

한국연구재단
knrrc
국가지정연구소재은행

헬리코박터파이로리분리균주은행

http://hpktcc.knrrc.or.kr

헬리코박터은행

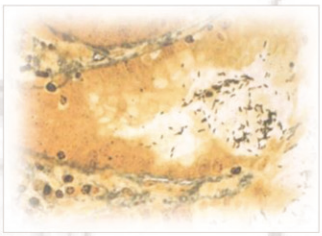
헬리코박터파이로리(*Helicobacter pylori*)는 전세계 인구 절반 이상의 위점막에 감염되어 위염, 위궤양 및 위암을 유발하는 병원성 세균이다. *H. pylori*는 사람의 위점막에서만 서식하기 때문에 세균의 분리·동정이 쉽지 않고 또한 배양이 까다로워 연구에 필요한 *H. pylori* 균주를 대량 확보하기가 어렵다. 이 세균의 임상적, 상업적 중요성에 따라 이를 퇴치하기 위한 다양한 방면의 연구가 필요함에도 이와 같은 임상균주 확보의 어려움은 늘 많은 연구자들의 접근을 어렵게 하는 제약이 되어왔다. 이러한 어려움을 극복하기 위해 본 헬리코박터파이로리분리균주은행(HpKTCC)은 생물학적 및 병리학적 특성을 기준으로 *H. pylori* 임상균주의 분류기준을 확립하고 이러한 기준에 따라 연구자들이 균주 소재 및 정보에 쉽게 접근할 수 있는 연구기반을 구축함으로써 이 세균 감염증의 진단, 치료, 예방에 관한 연구 활성화를 촉진하기 위하여 설립·운영되고 있다.

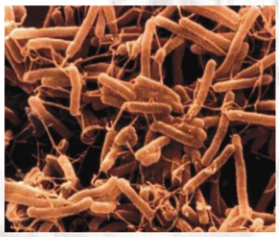
소재 현황

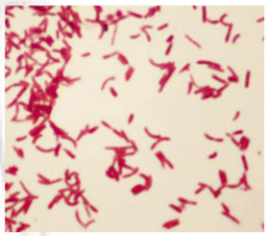
본 소재은행은 국내외에서 분리된 약 1,700주의 *H. pylori*, 기타 병원성 세균 70여종, 각종 세포주 300여종 및 *H. pylori* 균주의 genomic library를 확보하고 있다.

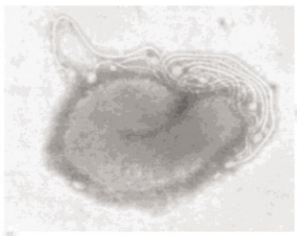
Items	No. of strains	No. of Vials	Remarks
H. pylori strains	1,703	6,316	Liquid nitrogen
Pathogenic bacteria	70	5,492	Lyophilized
Recombinant Plasmid clones		1,643	
Cell line & Hybridoma cell lines	300	900	
H. pylori genomin libraries	50,000		*H. pylori* strain 51 & 52

*Helicobacter pylori*의 성상





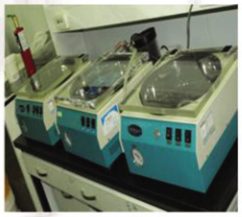




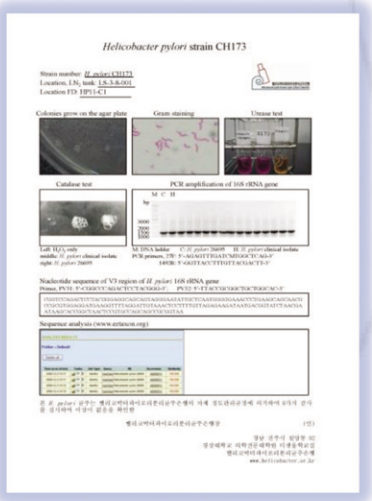
주요 사업

1) *H. pylori* 임상분리균주와 기타 병원성 세균의 수집 또는 수탁을 통한 자원화 및 무상 분양 서비스
2) *H. pylori* 배양 및 관련 실험기법 워크샵 서비스
3) 생리활성 물질 또는 항생물질 감수성 등 산업적 연구의 지원 및 분석대행 서비스
4) 균주의 분류체계 수립을 위한 국내외 임상균주의 미생물학적 특징 또는 병리학적 성상 분석 연구

주요 기자재



균주 정도 관리서



헬리코박터파이로리분리균주은행
Helicobacter pylori Korean Type Culture Collection

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누군가 반드시 가야할 길을 외면하지 않고 묵묵히 걸어온 녹십자가 이제
대한민국을 넘어 세계인의 건강을 지키는 새로운 희망으로 도약하고 있습니다.
이미 세계적으로 인정받은 백신과 혈액제제 등을 개발한 탁월한 R&D 역량을 바탕으로 세포배양,
유전자재조합 등의 차원 높은 솔루션을 더해 세계로 나아가는 녹십자, 만들기는 힘들지만
꼭 필요한 의약품으로 세계인의 건강과 행복을 지키는 믿음직한 이름이 되겠습니다.

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