2006
International Meeting of the Federation of Korean Microbiological Societies

Contents

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Timetable

October 19 (Thursday)

Time \ Place	Guhmoongo Hall			Crystal Hall
	Hall A	Hall B	Hall C	
08:00~09:00	Registration			
09:00~11:00	S1 Lactic Acid Bacteria and Biotechnology	S2 Molecular Microbiology	S3 Molecular Evolution of Viral Infection	S4 Fungal Biosciences and Molecular Mycology I
11:00~11:30	S5 Biomonitoring of Environmental Pollution			
11:30~12:30				Progress Report*
12:30~12:45				
12:45~13:45	Lunch			
13:45~14:00	Ceremony of Awarding Hantaan Prize (Hall C)			
14:00~14:45	Plenary Lecture 1 (Hall C)			
14:45~16:45	S6 New Biomaterials Technology	S7 Genomics and Proteomics	S8 Emergence of New and Variant Viruses	S9 Fungal Biosciences and Molecular Mycology II
16:45~17:00	Forum: Trend & Prospect of Microbial–Industry		KSV GM	KSMy GM
17:00~17:30		MSK GM		
17:30~18:00				
18:00~18:30				
18:30~20:00	Reception (Hall B+C)			

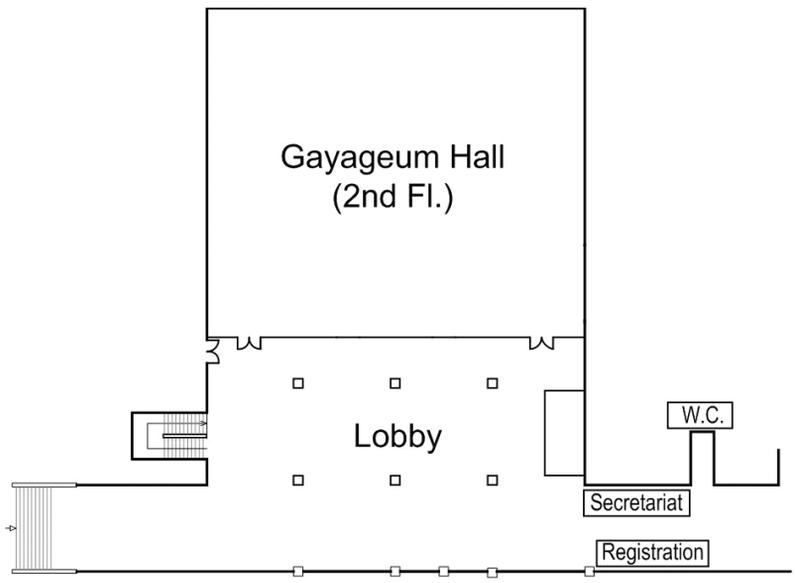
* Progress Report of Task Committee for Strategy Development for Future Microbiological Research in Korea

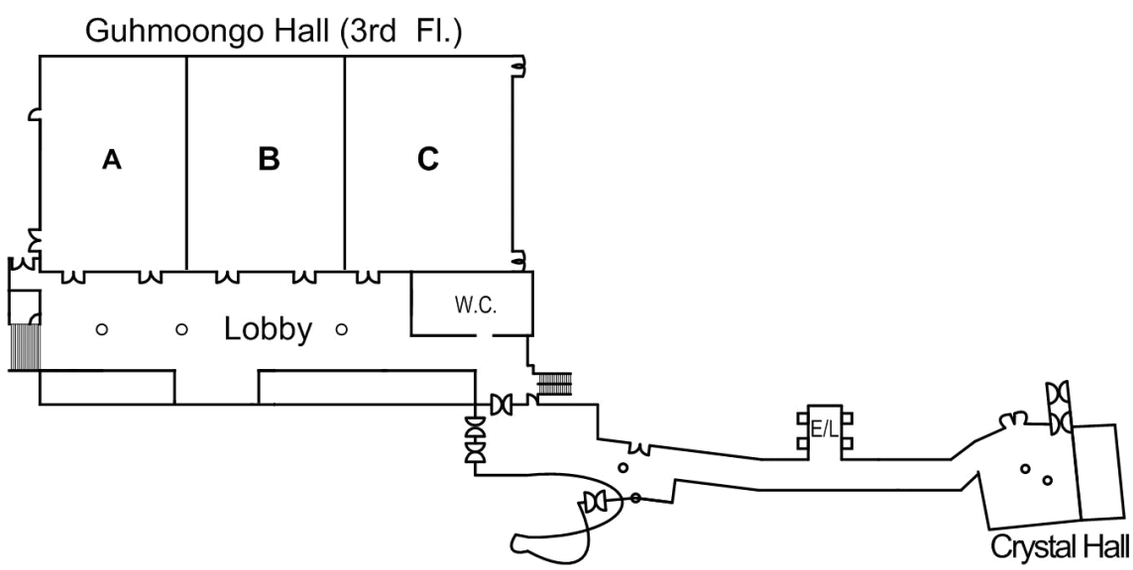
October 20 (Friday)

Time \ Place	Guhmoongo Hall			Crystal Hall
	Hall A	Hall B	Hall C	
08:00~09:00	Registration			
09:00~11:00	S10 Emerging Infectious Disease	S11 Eukaryotic Microorganisms	S12 Epidemiology of Emerging, New and Old Viral Diseases	Colloquium 1
11:00~11:45	Plenary Lecture 2 (Hall C)			
11:45~12:30	Plenary Lecture 3 (Hall C)			
12:30~13:30	Lunch			
13:30~14:30	KSMi GM	S14 Microbial Pathogenesis	S15 Current Opinions on the Research for *Helicobacter pylori* (HpKTCC Symposium)	Colloquium 2
14:30~15:00	S13 Biodefense			
15:00~16:00				Colloquium 3
16:00~17:00		S16 Microbial Diversity		
17:00~18:30				
18:30~19:00	Closing Ceremony (Hall B)			

- KSMi : The Korean Society for Microbiology
- KSV : The Korean Society of Virology
- KSMy : The Korean Society of Mycology
- KMB : The Korean Society for Microbiology and Biotechnology
- MSK : The Microbiological Society of Korea
- GM : General Meeting

Floor Plan





2F	Gayageum Hall	Poster Sessions Exhibition	3F	Guhmoongo Hall	Plenary Lectures Symposia Reception Closing Ceremony
	Lobby	Poster Sessions Exhibition Registration Desk Secretariat		Lobby	Exhibition
				Cystral Hall	Symposia Colloquia

Program Schedule

Plenary Lectures

PL 1

October 19 (Thursday) Guhmoongo Hall C

Chair : Yong-Hyun Lee (Kyungpook National University)

14:00-14:45
Outer Membrane Biogenesis in Gram-Negative Bacteria
Thomas J. Silhavy (Princeton University, USA)

PL 2

October 20 (Friday) Guhmoongo Hall C

Chair : Jin-Won Song (Korea University)

11:00-11:45
Viruses as Markers for Tracing the Peopling of the Pacific
Richard Yanagihara (University of Hawaii at Manoa, USA)

PL 3

October 20 (Friday) Guhmoongo Hall C

Chair : Kwang-Ho Rhee (Gyeongsang National University)

11:45-12:30
Bacterial Exploitation and Subversion of Host Cell Function
Chihiro Sasakawa (The University of Tokyo, Japan)

Ceremony of Awarding Hantaan Prize

Hantaan Prize

October 19 (Thursday) Guhmoongo Hall C

13:45-14:00

Symposia

S1 Lactic Acid Bacteria and Biotechnology

October 19 (Thursday) Guhmoongo Hall A

Chair : Hyong Joo Lee (Seoul National University)

S1-1 **09:00-09:20**
Functional Studies Based on the Probiotics and Their Transforming Activities
Geun Eog Ji (Seoul National University)

S1-2 **09:20-09:40**
Highly Purified LTA from *Lactobacillus plantarum* Reduced the LPS-Induced TNF-α Production via Regulation of Pathogen Recognition Receptors
Han Geun Kim (Kyung Hee University)

S1-3 **09:40-10:00**
Characterization of the Sucrose Phosphorylase Gene Cluster of *Bifidobacterium longum* and Application of the Recombinant Sucrose Phosphorylase for Glycoside Production
Jong-Hoon Lee (Kyonggi University)

Chair : Yong-Ha Park (Yeungnam University)

S1-4 **10:00-10:20**
Regulations on Probiotics in Korea
Ji Yeon Kim (Korea Food and Drug Administration)

S1-5 **10:20-10:40**
The Adhesive Characteristics of *Lactobacillus fermentum* VRI 003 to Peyer's Patches and Its Effect on the Immune Response in Mice
Seok-Seong Kang (Seoul National University)

S2 Molecular Microbiology

October 19 (Thursday) Guhmoongo Hall B

Chair : Chankyu Park (Korea Advanced Institute of Science and Technology)

S2-1 **09:00-09:25**
Aer mediates Tumor-Targeting by *Escherichia coli*
Hyon E. Choy (Chonnam National University)

S2-2 **09:25-09:50**
Differential Expression of Sporulation-Specific Factors in *Streptomyces griseus*
Jangyul Kwak (Korea Research Institute of Bioscience and Biotechnology)

S2-3 **09:50-10:15**
Structure-Based Analyses of the Mlc/Enzyme IIB^{Glc} Complex Mediating Glucose Signaling in *E. coli*
Sun-Shin Cha (Pohang Accelerator Laboratory)

S2-4 **10:15-10:40**
The *Shigella flexneri* Type III Secretion Effector OspG Binds Ubiquitinylated Ubiquitin-Conjugating Enzymes and Prevents $I\kappa B\alpha$ Degradation
Dong Wook Kim (International Vaccine Institute)

S2-5 **10:40-11:05**
Catabolite Repression in *Escherichia coli,* Revisted: Regulation of Adenylyl Cyclase by an Unknown Factor as well as Enzyme IIA^{Glc}
Yeong-Jae Seok (Seoul National University)

S2-6 **11:05-11:30**
Glucose Sensing and Signal Transduction in Yeast
Hisanori Tamaki (Kyoto University, Japan)

S3 Molecular Evolution of Viral Infection

October 19 (Thursday) Guhmoongo Hall C

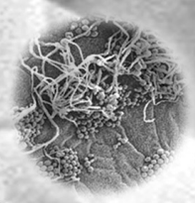
Chair : Ki-Joon Song (Korea University)

S3-1 **09:00-09:30**
Genome Evolution and the Rise of Human Pathogens
Heenam Kim (Korea University)

S3-2 **09:30-10:00**
Recent Genetic Mutation and Changes of H5N1 Avian Influenza Viruses
Jae-Hong Kim (National Veterinary Research & Quarantine Service)

S3-3 **10:00-10:30**
Segmentation of the Nonsegmented RNA Genome from a Paramyxovirus for the Development of Multivalent Vaccines
Man-Seong Park (Mount Sinai School of Medicine, USA)

S3-4 **10:30-11:00**
5' Terminus Deletion of Coxsackievirus B3 Genome and Persistence of Replication Defective Viruses in Murine Cardiomyocytes and Mouse Heart
Kisoon Kim (Korean National Institute of Health)



S4 Fungal Biosciences and Molecular Mycology I

October 19 (Thursday) Crystal Hall

Chair : Min-Woong Lee (Dongguk University)

S4-1 **09:00-09:30**
The Biodiversity of Wood Destroying Fungi in the Central Europe and Their Possible Application in Biotechnology
Libor Jankovský (Mendel University of Agriculture and Forestry in Brno, Czech Republic)

S4-2 **09:30-10:00**
Polyphasic Taxonomy of *Aspergillus* Section *Fumigati* and Its Teleomorph, *Neosartorya*
Seung-Beom Hong (National Institute of Agricultural Biotechnology)

Chair : Seung Hun Yu (Chungnam National University)

S4-3 **10:00-10:30**
Phytophthora Diseases in Forest: Histocytological Study of Port-Orford Cedar Root Disease
Eunsung Oh (Korea Forest Research Institute)

S4-4 **10:30-11:00**
Determining Fungal Diversity on Mountain Pine Beetle and Its Infested Lodgepole Pine Using Cultural and Molecular Methods
Young Woon Lim (Seoul National University)

S5 Biomonitoring of Environmental Pollution

October 19 (Thursday) Guhmoongo Hall A

Chair : Kyu-Ho Lee (Hankuk University of Foreign Studies)

S5-1 **11:00-11:20**
BIO-Defense System (ABADIS)
Wen-Chul Park (Bioneer Corporation)

S5-2 **11:20-11:40**
Application of Biosensor Based on the Nitrate Reductase (NaR) to Portable Total Nitrogen (TN) Analysis System
Moo Hoon Kim (Samsung Engineering)

Chair : Hee-Mock Oh (Korea Research Institute of Bioscience and Biotechnology)

S5-3 **11:40-12:00**
Biochip/Biosensor-Based Detection of Environmental Pathogens
Min-Gon Kim (Korea Research Institute of Bioscience and Biotechnology)

S5-4 **12:00-12:20**
Integrative Community Analysis and Water Quality Assessment in Streams across Different Levels of Pollution
Tae-Soo Chon (Pusan National University)

S5-5 **12:20-12:40**
Prediction Model for Cyanobacterial Bloom in Daechung Reservoir
Chi-Yong Ahn (Korea Research Institute of Bioscience and Biotechnology)

S6 New Biomaterials Technology

October 19 (Thursday) Guhmoongo Hall A

Chair : Moon-Hee Sung (Kookmin University)

S6-1 **14:45-15:05**
Characteristic of Poly-γGlutamic Acid Producing Strain *Bacillus subtilis* (Chungkookjang) and Mass Production of Poly-γGlutamic Acid
Chung Park (BioLeaders Corporation)

S6-2 **15:05-15:25**
A Platform Technology for the Production of Massive Recombinant Proteins in the Post-Genome Era
Jung-Hoon Sohn (Korea Research Institute of Bioscience and Biotechnology)

S6-3 **15:25-15:45**
T7 Helicase Recovers dsDNA Unwinding Ability by DNA Synthesis
Yong-Joo Jeong (Kookmin University)

Chair : Yong Chul Shin (Gyeongsang National University)

S6-4 **15:45-16:05**
Organization and Regulation of *arg* and *his* Operons in *Corynebacterium glutamicum*
Myeong-Sok Lee (Sookmyung Women's University)

S6-5 **16:05-16:25**
Development of Bio-Related Functional Materials Based on Poly(γGlutamic Acid)
Hiroshi Uyama (Osaka University, Japan)

S7 Genomics and Proteomics

October 19 (Thursday) Guhmoongo Hall B

Chair : Hyen Sam Kang (Seoul National University)

S7-1 **14:45-15:10**
Omics Approach in Biology: Interactomics
Jong Bhak (Korea Research Institute of Bioscience and Biotechnology)

S7-2 **15:10-15:35**
Genetic Insight into Microbial Degradation of Aromatic Compounds
Jong-Chan Chae (Rutgers University, USA)

S7-3 **15:35-16:00**
Genome Based Systematic Approaches for Development of Succinic Acid Producing *Zymomonas mobilis* ZM4
Hyonyong Chong (Macrogen Inc.)

S7-4 **16:00-16:25**
Protein Expression Profiles of Curdlan-Producing *Agrobacterium* sp. under pH-Controlled Batch Fermentation
Yang-Hoon Kim (Chungbuk National University)

S7-5 **16:25-16:50**
Complete Sequence and Comparative Genome Analysis of *Leuconostoc citreum*, a Key Player in Kimchi Fermentation
Haeyoung Jeong (Korea Research Institute of Bioscience and Biotechnology)

S8 Emergence of New and Variant Viruses

October 19 (Thursday) Guhmoongo Hall C

Chair : Jooshil Lee (Korean National Institute of Health)

S8-1 **14:45-15:15**
The Avian Influenza Virus Gene Pool from Domestic and Aquatic Birds in Korea and Assessment of Their Pathogenic Potentials
Young-Ki Choi (Chungbuk National University)

S8-2 **15:15-15:45**
Current Understanding of Emerging Flaviviruses: from Basic Research to Vaccine Development
Young-Min Lee (Chungbuk National University)

Chair : Jai Myung Yang (Sogang University)

S8-3 **15:45-16:15**
Molecular Epidemiology and Immune Response of Noroviruses
GwangPyo Ko (Seoul National University)

S8-4 **16:15-16:45**
Limited Genetic Variation of HIV-1 Korean Clade B Transmitted in Korea
Sung Soon Kim (Korean National Institute of Health)

S9 Fungal Biosciences and Molecular Mycology II

October 19 (Thursday) Crystal Hall

Chair : Jae-Seoun Hur (Sunchon National Universty)

S9-1 **14:45-15:15**
Lichen-Derived Culture and Its Application
Yoshikazu Yamamoto (Akita Prefectural University, Japan)

S9-2 **15:15-15:45**
The Lichen Flora of Oases of Continental Antarctic, and the Ecological Adaptations of Antarctic Lichens
Mikhail P. Andreev (Komarov Botanical Institute of the Russian Academy of Sciences, Russia)

Chair : Tae-Soo Lee (Incheon University)

S9-3 **15:45-16:15**
Gβγ-Mediated Signaling Pathway for Growth, Developmental Control and Toxin Biosynthesis in *Aspergillus nidulans*
Jeong-Ah Seo (University of Wisconsin-Madison, USA)

S9-4 **16:15-16:45**
Analysis of Genomic Structure of an Aflatoxin Biosynthesis Homologous Gene Cluster in *Aspergillus oryzae* RIB Strains
Yun-Hae Lee (Mushroom Research Institute, GyeongGi-Do Agricultural Research and Extension Services)

S10 Emerging Infectious Disease

October 20 (Friday) Guhmoongo Hall A

Chair : Jooshil Lee (Korean National Institute of Health)

S10-1 **09:00-09:30**
Molecular Characterization of *Brucella abortus* of Human Isolates in Korea
Mi-Yeoun Park (Korean National Institute of Health)

S10-2 **09:30-10:00**
Characterization of Immune Responses to SARS-CoV Spike Induced by DNA Vaccine and/or Recombinant Adenovirus
ManKi Song (International Vaccine Institute)

Chair : Haryoung Poo (Korea Research Institute of Bioscience and Biotechnology)

S10-3 **10:00-10:30**
Mucosal Immunization with Surface-Displayed SARS Coronavirus Spike Protein on *Lactobacillus casei* Induces Neutralization Activity in Mice
Chul-Joong Kim (Chungnam National University)

S10-4 **10:30-11:00**
Evolution of H9N2 Avian Influenza Viruses and Ongoing Active Surveillance Activities in Korea
Youn-Jeong Lee (National Veterinary Research & Quarantine Service)

S11 Eukaryotic Microorganisms

October 20 (Friday) Guhmoongo Hall B

Chair : Hee-Moon Park (Chungnam National University)

S11-1 **09:00-09:30**
Genome-Wide Analysis of Transcriptional Responses to Secretion Stress in the Methylotrophic Yeast *Hansenula polymorpha*
Hyun Ah Kang (Korea Research institute of Bioscience and Biotechnology)

S11-2 **09:30-10:00**
Anti-Oxidative Enzymes and Cell Proliferation in Fission Yeast *Schizosaccharomyces pombe*
Jung-Hye Roe (Seoul National University)

Chair: Hyoung Tae Choi (Kangwon National University)

S11-3 **10:00-10:30**
Analysis of the Transcriptome During Asexual and Sexual Development in *Aspergillus nidulans*
Pil Jae Maeng (Chungnam National University)

S11-4 **10:30-11:00**
Siderophore-Iron Uptake in *Fusarium graminearum:* Production of Siderophores and Regulation of Iron Utilization by FgSit1, a Putative Ferrichrome Transporter in *F. graminearum*
Cheol-Won Yun (Korea University)

S12 Epidemiology of Emerging, New and Old Viral Diseases

October 20 (Friday) Guhmoongo Hall C

Chair : Soo Hwan An (Kyungpook National University)

S12-1 **09:00-09:30**
Emerging and Re-Emerging Animal Viral Diseases
Mo Salman (Colorado State University, USA)

S12-2 **09:30-10:00**
The Epidemiology of Bovine Viral Diarrhea Virus
Hana Van Campen (Colorado State University, USA)

S12-3 **10:00-10:30**
Epidemiology of Ovine Herpesvirus Type 2 in Sheep, Cattle, and Bison
Robert J. Callan (Colorado State University, USA)

S12-4 **10:30-11:00**
Approaches to Eradicate Classical Swine Fever in Pigs in Korea
Byoung-Han Kim (National Veterinary Research & Quarantine Service)

S13 Biodefense

October 20 (Friday) Guhmoongo Hall A

Chair : Hee-Bok Oh (Korean National Institute of Health)

S13-1 **14:30-15:00**
Korea's Present State and Perspective on Biosafety
Cheon-Kwon Yoo (Korean National Institute of Health)

S13-2 **15:00-15:30**
Smallpox Vaccine: *Yesterday, Today and Tomorrow*
Doo-Sung Cheon (Korean National Institute of Health)

S13-3 **15:30-16:00**
Countermeasures against Botulinum Neurotoxins: Detection, Diagnostics, and Therapeutics
Na-Ri Shin (Korean National Institute of Health)

Chair : Sang In Chung (Chung-Ang University)

S13-4 **16:00-16:30**
Genome-Wide Screening of *Bacillus anthracis*-Specific Chromosomal Markers
Kijeong Kim (Chung-Ang University)

S13-5 **16:30-17:00**
The Study on *Bacillus anthracis* Protein Associated with Sporulation-Germination and Surviving in the Host
Chulmin Park (The Catholic University of Korea)

S14 Microbial Pathogenesis

October 20 (Friday) Guhmoongo Hall B

Chair : You-Hee Cho (Sogang University)

S14-1 **13:30-14:00**
Calcium Dependent Repression of *Streptococcus pneumoniae* Survival
Dong-Kwon Rhee (Sungkyunkwan University)

S14-2 **14:00-14:30**
Vibrio vulnificus RtxA, a Key Virulence Factor, Mediates Contact Dependent Cytotoxicity
Joon Haeng Rhee (Chonnam National University)

S14-3 **14:30-15:00**
Regulation of *Salmonella* Virulence Functions
Eduardo A. Groisman (Washington University, USA)

Chair : Sang Ho Choi (Seoul National University)

S14-4 **15:00-15:30**
Quorum Sensing and Group Activity: The Role of Orphan Quorum Sensing Regulator, QscR in *Pseudomonas aeruginosa*
Joon-Hee Lee (Pusan National University)

S14-5 **15:30-16:00**
Quorum Sensing of *Burkholderia glumae*: The Causative Agent of Bacterial Rice Grain Rot
Ingyu Hwang (Seoul National University)

S15 Current Opinions on the Research for *Helicobacter pylori* (HpKTCC Symposium)

October 20 (Friday) Guhmoongo Hall C

- **Hosted by :** *Helicobacter pylori* Korean Type Culture Collection
- **Sponsored by :** Korea Science and Engineering Foundation

Chair : Myung-Je Cho (Gyeongsang National University)

S15-1 **13:30-14:00**
Unexpected Invasiveness of *Helicobacter pylori* into Lamina Propria of Human Gastric Mucosa
Hee-Shang Youn (Gyeongsang National Universiy)

S15-2 **14:00-14:30**
Supramolecular Assembly and Acid Resistance of *Helicobacter pylori* Urease
Nam-Chul Ha (Pusan National University)

S15-3 **14:30-15:00**
Fur- and Growth Fhase-Dependent Regulation of *H. pylori* Protein Expression
Na Gyong Lee (Sejong University)

S15-4 **15:00-15:30**
Quantitative Effect of *luxS* Gene Inactivation on the Fitness of *Helicobacter pylori*
Woo-Kon Lee (Gyeongsang National University)

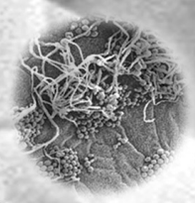
S16 Microbial Diversity
Exploring Unseen Life: From Virus to Fungi

October 20 (Friday) Guhmoongo Hall B

Chair : Ki Seong Joh (Hankuk University of Foreign Studies)

S16-1 **16:00-16:30**
Human Pathogenic Enteric Viruses in the Aquatic Environments of Korea
Sang-Jong Kim (Seoul National University)

S16-2 **16:30-17:00**
Microbial Diversity: Progress and Potential
Chang-Jin Kim (Korea Research institute of Bioscience and Biotechnology)



S16-3 **17:00-17:30**
Diversity of Pumping Rhodopsins in Maxwell Bay, Antarctica
Kwang-Hwan Jung (Sogang University)

S16-4 **17:30-18:00**
Fungal Diversity and Discovery of Bioactive Metabolites
Hyang Burm Lee (Chonnam National University)

S16-5 **18:00-18:30**
Genomic Heterogeneity and Geographic Structure in Endemic Genotypes of Fluorescent *Pseudomonas*
Jae-Chang Cho (Hankuk University of Foreign Studies)

Colloquia

C1 Microbial Diversity in Ecosystem and Host Pathology Induced by Microbes

October 20 (Friday) Crystal Hall

Chair : Je Chul Lee (Kyungpook National University)

C1-1 **09:00-09:15**
Fungal Diversity of the Islands on the Yellow Sea of Korea
Changmu Kim (Seoul National University)

C1-2 **09:15-09:30**
Taxonomic Study on Lichen Genus *Hypogymnia* in China
Xinli Wei (Chinese Academy of Sciences, China)

C1-3 **09:30-09:45**
Phenotypic and Genotypic Diversity of Fluorescent Pseudomonads Isolated from Rice Rhizosphere Soil
Niraikulam Ayyadurai (Korea Ocean Research & Development Institute)

09:45-10:00 Break

C1-4 **10:00-10:15**
Clinicopathological and Immunological Characteristics of Thoroughbred Horses with Streptococcal Infectious Upper Respiratory Diseases
Hye Cheong Koo (Seoul National University)

C1-5 **10:15-10:30**
Regulation of Tumor Necrosis Factor-Alpha Gene Expression and Signal Transduction during *Orientia tsutsugamushi* Infection
Ji-Hyun Yun (Cheju National University)

C1-6 **10:30-10:45**
Dissecting the Mechanism of Lipid Rafts Involvement in Mouse Hepatitis Virus Replication
Keum Sook Choi (Korea Food & Drug Administration)

C2 Diverse Approaches to Understand Human Pathogen

October 20 (Friday) Crystal Hall

Chair : Heuiran Lee (University of Ulsan)

C2-1 **13:30-13:45**
Distribution of Waterborne Enteric Viruses in Korea Surface Water
Gyucheol Lee (Korea Water Resources Corporation)

C2-2 **13:45-14:00**
Mass Spectrometric Studies of Virus Structure and Dynamics
Sebyung Kang (University of Alabama at Birmingham)

C2-3 **14:00-14:15**
Regulation of Hepatitis C Virus RNA Replication by Phosphorylation of the Viral RNA Polymerase
Seong-Jun Kim (Yonsei University)

14:15-14:30 Break

C2-4 **14:30-14:45**
Comparison of Gene Expression Profile of Prion Protein-Deficient Neuronal Cell Lines and Mice with Wild-Types
Boe-Hyun Kim (Hallym University)

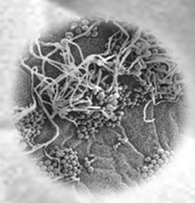
C2-5 **14:45-15:00**
Activation of Mitogen-Activated Protein Kinases in Hamster Brains Infected with 263K Scrapie Agent
Hyun-Pil Lee (Hallym University)

C3 Challenge in Microbial Physiology

October 20 (Friday) Crystal Hall

Chair : Pil Kim (The Catholic University of Korea)

C3-1 **15:00-15:15**
Identification of Phenotypic and Genetic Differences between *Listeria monocytogenes* Strains
So Hyun Kim (Seoul National University)



C3-2 **15:15-15:30**
Toward the Interdisciplinary Research at the Biology
Hui Sun Lee (Sookmyung Women's University)

C3-3 **15:30-15:45**
Coactivation of *Vibrio vulnificus putAP* Operon by cAMP Receptor Protein and PutR through Cooperative Binding to Overlapping Sites
Jeong Hyun Lee (Seoul National University)

15:45-16:00 Break

C3-4 **16:00-16:15**
Escherichia coli Enzyme IIA^{Ntr} Interacts with the K^+ Transporter TrkA to Regulate *ilvBN* Derepression
Chang-Ro Lee (Seoul National University)

C3-5 **16:15-16:30**
Revolutionary Recombinant Hybrid Mussel Adhesive Protein fp-151
Dong Soo Hwang (Pohang University of Science and Technology)

C3-6 **16:30-16:45**
Central Carbohydrate Metabolism in Extremely Thermoacidophilic Archaea: Comparative Studies of Archaeal and Bacterial *D*-gluconate Dehydratases, a Key Enzyme of the Modified Entner-Doudoroff Pathways
Seonghun Kim (Pohang University of Science and Technology)

Plenary Lectures

백지

PL1

Outer Membrane Biogenesis in Gram-Negative Bacteria

Thomas J. Silhavy

Princeton University, USA

The cell envelope of Gram-negative bacteria such as *Escherichia coli* is composed of the inner (cytoplasmic) membrane (IM), the outer membrane (OM), and the peptidoglycan cell wall, which is located in the periplasmic space between the two membranes. The OM functions as a barrier to protect these organisms from toxic agents in their environments. Like other biological membranes the OM is a lipid bilayer, but it is unusual in that the OM bilayer is asymmetric. The inner leaflet is composed of typical phospholipids, but the outer lipid is made of lipopolysaccharide (LPS). Two kinds of proteins are found in the OM. Lipoproteins are inserted into the inner leaflet of the OM by posttranslationally attached lipid moieties. Integral OM proteins are β-barrel proteins (OMPs). In Gram-negative bacteria the OM is essential for the life of the organism.

All of the components of the OM are synthesized in the cytoplasm or the inner leaflet of the IM. They must be transported to, and assembled in the OM in the correct orientation to maintain barrier function during growth. Proteins involved in transporting these components across the IM have been characterized. However, until recently the components required for transit through the periplasm and incorporation into the OM were unknown. Moreover, since these processes occur outside the IM, there is no obvious energy source such as ATP available to drive the assembly reactions. I will describe the genetic and biochemical strategies we have used to identify and characterize the cellular components required for LPS and OMP biogenesis.

We began by studying envelope stress responses reasoning, in analogy with the classic heat shock response, that some of the genes required to repair envelope damage would likely also be required for envelope biogenesis. Amongst the genes identified one of them, *imp*, caught our attention for two reasons. First, it is located in an operon with *surA*, a gene for a well-known periplasmic chaperone. Second, *imp* mutations that disrupt the OM barrier function had been identified previously in the lab. We then demonstrated that *imp* is an essential gene required for OM biogenesis.

Having identified one gene required for OM biogenesis the door was now open for additional genetic and biochemical approaches to identify additional components. We devised a chemical genetic approach to search for suppressors of the *imp* mutations that compromise OM barrier function. In addition, we used biochemical methods to identify proteins that interact directly with the components identified using genetics.

Together these methods have identified two OM protein complexes. One of these complexes contains Imp, an OM β-barrel, and an OM lipoprotein termed RlpB. The other complex contains the OM β-barrel protein YaeT and three lipoproteins, YfiO, YfgL, and NlpB. Both of these β-barrel proteins and two of the lipoproteins, RlpB and YfiO, are essential. Evidence will be presented showing that the Imp complex assembles LPS at the cell surface and that the YaeT complex assembles β-barrel proteins. In addition, I will discuss structure studies with YaeT that suggest how β-barrel assembly might be facilitated.

Keywords: *Escherichia coli*, lipopolysaccharide, β-barrel proteins, permeability barrier YaeT/Imp

PL2

Viruses as Markers for Tracing the Peopling of the Pacific

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Located in the middle of the Pacific Ocean, the Hawaiian archipelago consists of 132 islands, reefs and shoals that extend for over 2,400 km, and lies approximately 4,000 km from both North America and the major islands of Polynesia. For many first-time visitors to Hawaii, it is difficult to comprehend how very small these islands are and how very vast the Pacific is. Hawaii (16,600 sq km) comprises less than 1% of the U.S. landmass (9,000,000 sq km), while the total area of the Pacific Ocean is about 15 times the size of the contiguous 48 states, or 155 million sq km. Moreover, the Pacific Ocean covers about 28% of the Earth's surface and is larger than the total land area of the entire world. That early humans ventured into this vast unknown, guided only by the stars and their unbridled spirit of adventure, is a convincing testament of human courage and their remarkable sense of exploration and discovery. In many ways the peopling of islands elsewhere in the Pacific is no less astonishing.

Viruses can serve as markers to trace these early voyages and to reconstruct the peopling of the Pacific. For example, early and recent movements of human populations have been traced by analyzing the molecular variants of human papillomavirus type 16 (HPV-16). Five phylogenetic branches (two confined to Africans, one to Europeans, one to Asians, and one to indigenous and immigrant populations in the Americas) of HPV-16 variants have been found, suggesting co-evolution of the virus with people of the three major human lineages, namely, Africans, Caucasians and Asians. The degree of genomic diversity of present-day HPV-16 suggests evolution from a precursor genome that may have originated in Africa more than 200,000 years ago. Using HPV-16 as a marker presents logistical challenges, however, because of the need to obtain cervical specimens.

Our previous studies, which led to the discovery of genetically distinct variants of human T-cell lymphotropic virus type I (HTLV-I) in remote Melanesian and Australian Aboriginal populations, having no prior contact with Africans or Japanese, are consistent with the emergence of a common ancestral proto-Australo-Melanesian strain, somewhere in the then Southeast Asian landmass (Sunda) or the offshore islands in Wallacea between Sunda and the Greater Australian continent (Sahul), and its introduction by one of several founder populations, possibly as early as 40,000 years ago when the first Australoids migrated from Sunda to settle Sahul. Several millenia following these early migrations and settlements, Austronesians began to people the smaller islands in Melanesia, beginning approximately 3,000 to 5,000 years ago, as judged by archeological, anthropological and linguistic data. These Melanesian populations lived in total isolation from western influence until the age of global exploration, principally by Spain and Portugal, during the 16th century, when HTLV-I spread to parts of the New World by the African slave trade. Molecular genetic studies of HTLV-I strains from western and central Africa (Central African Republic, Mauritania, Guinea Bissau, Côte d'Ivoire, Zaire), the West Indies (Martinique, Guadeloupe, French Guyana, Haiti, Jamaica) and South America (Brazil, Peru) corroborate

this history.

As with the dissemination of other viruses, the spread of HTLV-I in Pacific populations has been a dynamic process. The absence of uniformity in the geographic distribution of HTLV-I in Oceania, even in populations having decades- or centuries-long cohabitation with carrier groups, implies that HTLV-I is not efficiently transmitted even by the sexual route and that long-term maintenance of the virus within a given population is dependent on complex interactions between environmental factors and social, behavioral and cultural practices. Thus, while high prevalences of infection with HTLV-I variants have been demonstrated in some remote Melanesian and Australian Aboriginal populations, studies conducted on sera collected during the 1960s to 1980s from indigenous populations in Micronesia (Mariana Islands, Caroline Islands), Polynesia (Cook Islands, French Polynesia, Marquesas, Anuta, Tikopia, American and Western Samoa) and other regions in Melanesia (Fiji and New Caledonia) fail to disclose evidence of HTLV-I infection. In this regard, the rapidly declining prevalences of HTLV-I infection in southwestern Japan and the near disappearance of HTLV-I infection among third- and fourth-generation or *sansei* and *yonsei* Japanese Americans in Hawaii speaks to the relative ease with which HTLV-I can be eliminated from populations with unrestricted patterns of movement and marriage and whose hygienic and socio-cultural milieu are compatible with early cessation of breastfeeding.

Thus, the low prevalence of HTLV-I in most populations makes it an unsuitable viral marker for tracing human population movements. A more ubiquitous agent, which produces a subclinical, persistent infection, would be preferable. In this regard, human polyomavirus JC (JCV), which causes a rare neurodegenerative disorder called progressive multifocal leukoencephalopahty, offers several advantages over HPV-16 and HTLV-I in that it causes a persistent infection to a very high degree in all populations, even in small remote groups in the Pacific. Also, the JCV genome is quite small (5.1 kb) making full-length sequencing somewhat trivial. Moreover, although the mode of JCV transmission remains unclear, children become infected from JCV-viuric adults, making specimen collection quite easy. Phylogenetic trees of JCV sequences from the major continental population groups show a trifurcation at the base indicating early division into European, African and Asian branches. Our studies have focused on exploring JCV relationships in the island populations of the western Pacific. Since these islands were settled from the Asian mainland and islands of Southeast Asia, we expected that their virus genotypes might show an Asian connection. We found that Type 2E (Austronesian) and Type 8 (non-Austronesian) were widely distributed in western Pacific populations. A subtype of Type 8, Type 8A, was confined to Papua New Guinea. In general, our findings support the Asian origins of the western Pacific JCV strains, and suggest three broad movements: an ancient one characterized by Type 8A, and then Type 8B, followed much later by migrations carrying Type 2E, which may correlate with the arrival of Austronesian-language speakers, the bearers of the "Lapita" cultural complex (~3,500 to 5,000 years ago), and relatively recent movements carrying largely Type 7A (south China) strains directly from the West.

Interestingly, the molecular evolution of three very different viruses (HPV-16, HTLV-I and JCV) provides strikingly similar patterns consistent with the movement of human populations. Sequence and phylogenetic analyses of HPV-16 from Pacific Islander populations, currently underway, might further complement studies using HTLV-I and JCV as viral markers to trace the peopling of the Pacific.

Keywords: virus, molecular evolution

PL3

Bacterial Exploitation and Subversion of Host Cell Function

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Pathogenic bacteria have evolved mechanisms of infection of various host cells, where bacteria can colonize and multiply within their niches by defending against the host innate immune systems. *Shigella* are highly adapted human pathogens, that cause bacillary dysentery (shigellosis), a disease provoking a severe inflammatory diarrhea. Shigellosis has long been ranked as a major leading infectious killer in developing countries, where 1-0.5 million people, those are mostly under 5 years, die of the disease every year. Since *Shigella* can efficiently transmit person-to-person via the fecal-oral route even by ingesting with a small number of bacteria such as 10-100, the prevention of shigellosis under low sanitary condition is not easy. *Shigella* infection of and colonization within the colonic epithelium are achieved through the multiple infectious steps, where the numerous bacterial factors and host cell functions are involved. *Shigella* infection of the intestinal epithelium is initiated by the bacterial entry into the M cells. Once the pathogen has reached the underlying M cells, bacteria infect the resident macrophages and multiply within the cytoplasm, where *Shigella* release lipopolysaccharide which causes rapid macrophage cell death. Meanwhile, the bacteria enter surrounding enterocytes from the basolateral surface by inducing phagocytic event via the formation of large-scale membrane ruffles. Within the cytoplasm, *Shigella* multiply and move intra- and intercellularly by inducing actin polymerization at one end of bacterium. In this stage, *Shigella* release peptidoglycan (PGN) into the host cytoplasm, where PGN binds Nod1, thus leading to strong inflammatory responses via the activation of NF-κB. Therefore, the predominant pathogenic feature of *Shigella* is the ability to invade various host cells and multiply within the cells.

Shigella (and *Salmonella*) use a special mechanism to invade epithelial cells, called 'the trigger mechanism of entry', which is characterized by macropinocytic and phagocytic events that allow cells to trap several bacterial cells simultaneously. When *Shigella* come into contact with epithelial cells, the type III secretion system is stimulated and delivers effectors into the host cells and the space around the bacterium. The bacterial effectors are capable of modulating various host cellular functions and signal transduction pathways engaged in remodeling the surface architecture of the epithelial cells required for bacterial entry, and also down regulating host innate immune system and escaping from autophagy.

In my talk, I would like to highlight our recent topics related to the ability of *Shigella* to exploit the host cellular functions required for promoting the infectious process, and also the ability to subvert the host innate defense systems elicited by bacterial infection. We believe that the bacterial infectious tactics will be a paradigm of other mucosal bacterial pathogens.

Keywords: bacterial pathogenesis, infection, invasion, bacterial motility, innate immunity, autophagy, *Shigella*, type III secretion system

Symposia

백지

S1-1

Functional Studies Based on the Probiotics and Their Transforming Activities

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Probiotics such as *Bifidobacterium* and *Lactobacillus* exert beneficial functions for the maintenance of the balanced intestinal microflora of the host. The beneficial effects of the probiotics are known to be strain-specific. Development of probiotic strains with the activities such as allergy-suppression, inhibition of helicobacteria and rotavirus, tumor suppression, improvement of lactose tolerance, and reduction of serum cholesterol levels are being actively pursued. Probiotics can also be used for the production of bio-materials such as CLA (conjugated linoleic acid), GABA (gamma amino butyric acid), lactic acid, and various bacteriocins. Another aspect of the use of probiotics include the improvement of the biological functions of the natural compounds by their transforming activities. Transformation of the platycodin saponins from *Platycodon grandiflorum* A. DC (Campanulaceae) reduced the cytotoxicity on V79-4 (Chinese hamster lung fibroblasts) and the hemolytic toxicity, while the nitrite-scavenging activity was increased and the sensory scores responsible for the pungency, bitterness and after-tastes were improved. Fenugreek diosgenin saponins from *Trignoella foenum-graecum* (Fenugreek) were transformed by crude microbial enzymes apparently resulting in the conversion of furostanol saponin glycosides into deglycosylated spirostanol saponin forms. Transformed FSE inhibited alpha-glucosidase and pancreatic alpha-amylase activities higher than FSE at the concentration of 0.1 mg/ml. The cytotoxic effects of the various traditional medicinal plant extracts transformed by enzymes from several probiotic strains on the colon cancer cell line HT-29 were investigated. Cytotoxic activities of the transformed plant extracts were measured by MTT assay, LDH assay, and BrdU incorporation assays. Several transformed plant extracts from such as *Angelicae Sinensis* Radix, *Ginkgo biloba, Actinidia chinensis, Zizyphus jujuba* with the crude enzymes from *B. bifidum* BGN4 and *Bifidobacterium* sp. SH5 showed significantly higher cytotoxic effects on HT-29 colon cancer cells compared with pre-transformed extracts. The transformed extracts from *Angelica gigas Nakai*, *Cordyceps militaris*, *Liriope platyphylla Wang. et Tang* showed inhibitory effects on compound 48/80-induced histamine release.

Keywords: probiotics, transformation, structure, function

S1-2

Highly Purified LTA from *Lactobacillus plantarum* Reduced the LPS-Induced TNF-α Production via Regulation of Pathogen Recognition Receptors

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Introduction

Bacterial infections are a major cause of severe systemic complications such as septic shock, which is characterized by refractory hypotension and eventually multiorgan failure and death. The production of cytokines, such as TNF-α and IL-1, initiated by bacterial components, such as LPS, LTA and PGN, can lead to the development of septic shock and multiorgan system disfuction. Therefore, many studies have attempted to elucidate the molecular pathways leading to an inflammatory response in order to abrogate them during clinical sepsis.

Materials and Methods

Preparation of highly purified LTA from *Lb. plantrum*: Highly purified LTAs from *Lb. plantarum* (pLTA) was prepared by butanol extraction followed by purification using DEAE-sepharose following octyl-sepharose chromatography as described previously (Morath *et al.,* 2001).

ELISA assay: The TNF-α ELISA was performed using monoclonal mouse IgG1, clone #28401 for capture and the biotinylated human TNF-α specific polyclonal goat IgG followed by streptavidin HRP for detection. ELISA was developed using o-phenylenediamine as a substrate, and OD was determined at a wavelength of 450 nm using 550 nm reference wavelength.

Results and Discussion

LPS-induced TNF-α production is induced by a synergistic effect of LPS and endotoxin proteins through PRR signaling cascades that involve TLR2, TLR4, CD14, NOD1, and NOD2. In our experiments, pretreatment of pLTA inhibited LPS-induced TNF-α production. pLTA pretreatment down-regulated LPS-induced PRRs and up-regulated IRAK-M, a negative regulator of TLR signaling. pLTA pretreatment also inhibited the phosphorylation of MAP kinases, and down-regulated NF-κB activation. Most of all, it reduced LPS-induced LITAF production, which resulted in the direct reduction of TNF-α production. These results suggested that pLTA pretreatment can reduce some risks associated with inflammatory disease such as septic shock.

Keywords: *Lactobacillus plantarum*, Lipoteichoic acid, Tolerance, Sepsis

S1-3

Characterization of the Sucrose Phosphorylase Gene Cluster of *Bifidobacterium longum* and Application of the Recombinant Sucrose Phosphorylase for Glycoside Production

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Bifidobacteria, the major members of normal intestinal microflora in the human, are considered to play an important role in the health of the host. In addition to the health-promoting activities, bifidobacteria are known to produce hydrolytic enzymes for some oligosaccharides, such as α-galactosidase, β-galactosidase, β-xylosidase, α-glucosidase, and β-glucosidase, which are not digestible by the human. Some of these enzymes are also found to possess a transferase activity. Due to the fact that bifidobacteria are claimed to be safe for the human, the production of oligosaccharide-hydrolyzing enzymes in bifidobacteria aiming at food-related industrial applications is highly promising.

A DNA fragment, which complemented the growth of *E. coli* both on M9 medium containing raffinose and on LB medium containing ampicillin, IPTG and 5-bromo-4-chloro-3-indoxyl-α-D-galactoside, was isolated from the genomic library of *Bifidobacterium longum* SJ32, which was digested with *Eco*RI. The nucleotide sequence of 8.6-kb *Eco*RI fragment contained five open reading frames including the gene cluster for sucrose utilization such as a sucrose phosphorylase (ScrP), a sucrose transporter (ScrT), and a GalR-LacI-type transcriptional regulator (ScrR) identified by amino acid homology. Each gene showed over 94% amino acid homology among various *B. longum* strains. Genomic organization of the gene cluster is the same as those of other strains of *B. longum* but different from that of *B. lactis*. In spite of high homology of each gene among *B. longum* strains, the difference of flanking sequences of the gene cluster between strains SJ32 and NCC2705 insinuates the horizontal transfer of *scrPTR* between *B. longum* strains.

The sucrose phosphorylase gene of *B. longum* SJ32 was amplified by PCR and the amplified DNA fragment was transferred to *E. coli* JM109. A 56-kDa protein was synthesized in *E. coli* and partially purified by DEAE-ion exchange chromatography. The partially purified enzyme was not reactive for melibiose, melezitoze and raffinose, but reactive for sucrose. The enzyme appeared to have transglucosylation activity, in addition to the hydrolytic activity.

Transglycosylation from sucrose to phenolic compounds by the recombinant sucrose phosphorylase from *B. longum* was studied. HPLC analysis revealed that the enzyme transferred glucosyl residue of sucrose to 1,2-dihydroxybenzene, 1,4-dihydroxybenzene, 1,2,3-trihydroxybenzene, and 2-hydroxybenzyl alcohol. The enzyme could transfer the glucosyl moiety of sucrose to phenolic compounds which have phenolic OH or alcoholic (hydroxymethyl) OH group.

Transglycosylation from sucrose to L-ascorbic acid by the recombinant sucrose phosphorylase from *B. longum* was studied in order to apply for the production of the stable L-ascorbic acid derivative. A novel major product which has almost same retention time with 2-*O*-α-D-glucopyranosyl-L-ascorbic acid (AA-2G) was detected in HPLC chromatogram and the product was confirmed as AA-2G by HPLC-MS/MS analysis. The enzyme can split sucrose and transglucosylated L-ascorbic acid to the chemically stable L-ascorbic acid derivative, AA-2G.

Recently, the optimal production of a recombinant sucrose phosphorylase and application of the glucosyl transferase activity for the production of variety of flavonoid compounds are in process.

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Keywords: *Bifidobacterium longum*, Sucrose phosphorylase, Ascorbic acid, Transglycosylation

S1-4

Regulations on Probiotics in Korea

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Introduction

Probiotics are defined as live microbial food ingredients that are beneficial to health. A wide range of bacteria such as lactobacilli and bifidobacteria tend to predominate in the probiotic food sector. In Korea, we regulate probiotics as Health/functional foods(HHFs) as well as conventional foods. In Health/functional food, probiotics regarded as generic HHFs. However, the strains, which are not included in the generic, could be evaluated as a product-specific HHFs. In this seminar, I would like to introduce about Korea regulations.

Generic Probiotics

Several probiotics are listed in Health/functional food code and its functional claim as follows; “helps maintain the balance of the intestinal flora”, “helps you stay regular”.

Product-Specific Probiotics

Identity

It was recognized that it is necessary to known the genus and species of the probiotic strain. The current state of evidence suggests that probiotic effects are strain specific. Specification of the bacteria must be established using the most current, valid methodology. It is recommended that a combination of phenotypic and genetic tests be used.

Safety Consideration

Historically, lactobacilli and bifidobacteria associated with food have been considered to be safe. However, probiotics may theoretically be responsible for four types of side-effects: systemic infection, deleterious metabolic activities, excessive immune stimulation in susceptible individuals, gene transfer.

Efficacy

In some cases, animal models exist to provide substantiation of in vitro effects and determination of probiotic mechanism. Where appropriate, we encourage use of these prior to human trials. Probiotics have been tested for an impact on a variety of clinical conditions. Generally in the form of randomized, double blind, placebo-controlled design, measure efficacy compared with placebo.

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Keywords: Probiotics, regulation, Health/functional food, safety, efficacy

S1-5

The Adhesive Characteristics of *Lactobacillus fermentum* VRI 003 to Peyer's Patches and Its Effect on the Immune Response in Mice

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Lactobacilli used as probiotics are selected for their capacity to adhere to the gastrointestinal tract and stimulate the host's immune system. In the present study, the mechanism of adhesion of *Lactobacillus fermentum* VRI 003 to Peyer's patches and the effect of the adherence on triggering mucosal and systemic immune responses were studied. *L. fermentum* VRI 003 adhered to Peyer's patches and the adhesion of *L. fermentum* VRI 003 was strongly inhibited in the presence of D-mannose and methyl-α-D-mannoside. *L. fermentum* VRI 003 was shown to strongly attach to mannose immobilized on a surface using BSA, suggesting that *L. fermentum* VRI 003 specifically adhered to mannose-containing molecule(s). Pretreatment of *L. fermentum* VRI 003 with proteinase K and trypsin decreased the adhesive capacity and bacterial surface extracts diminished adhesion of *L. fermentum* VRI 003 indicating that cell surface proteins are involved in adhesion to Peyer's patches. IgG, IgG1 and IgG2a subclasses in serum of BALB/c mice given lactobacilli for 4 weeks were investigated. Oral administration of *L. fermentum* VRI 003 enhanced the induction of lactobacilli-specific total IgG, IgG1, and IgG2a compared to the control group and another control group of mice given *L. fermentum* LMG 8896. It was observed that *L. fermentum* VRI 003 and *L. fermentum* LMG 8896 increased the level of IgA in the supernatants of Peyer's patch whole-organ culture following oral administration. Additionally, tolerance to acidic pH and growth with bile acid were tested. *L. fermentum* VRI 003 was able to resist pH 2 for 4 h and grow 0.3 % and 0.6 % bile concentrations for 8 h. *L. fermentum* VRI 003 showed antagonistic activity against indicator bacteria including *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes, Clostridium difficile* and *C. perfigens*. Therefore, this study may have important implications for the adhesive ability of probiotics to trigger mucosal and systemic immune responses.

Keywords: Probiotics, Lactobacilli, Adhesion, Immune response

S2-1

Aer mediates Tumor-Targeting by *Escherichia coli*

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Use of microbial system has attributed to the recent advances in gene therapy application especially for its tumor-specific accumulation and proliferation. In an attempt to elucidate the underlying mechanism, we monitored spatial and temporal migration of the light-generating *E. coli* injected into tumor-bearing mice using *in vivo* optical imaging system. This was possible by engineering a plasmid, Asd^+ pLux, which carried *lux* cassette to generate luciferase-catalyzed light and also *asd* gene encoding aspartate β-semialdehyde dehydrogenase to stably maintain the plasmid in the absence of external selection. It was observed that *E. coli* could target and proliferate a broad range of tumors at different stages. It was found that the *E. coli* were selectively accumulated in the various tumor tissues grafted into mice, up to ~10^8 colony forming unit (CFU)/g of tissue, while none in other organs after 5 days post-inoculation (dpi). We suggest here that targeting tumor tissue by *E. coli* is by the mechanism of aerotactic response based on the following evidence: i) kinetics of tumor targeting by those mutant defective in flagella synthesis and aerotaxis (Aer^-) were about the same – less than 1% of wild type bacteria was accumulated. ii) kinetics of tumor targeting by the mutant carrying deletion of *cheA* or *cheW* were as defective as Aer^-, but not that by the mutant carrying deletion in *cheR*, or *cheB*. Methyl-accepting receptor-mediated chemotactic behaviors are dependent on two specific enzymes, CheR (methyltransferase) and CheB (methylesterase). But, not the aerotactic response mediated by Aer, which lacks conventional methylation site motifs. The Aer presumably detects oxygen-related cellular redox changes with its FAD prosthetic group. Whereas, the CheA-CheW signaling to communicate with the flagella motors is needed for aerotaxis as well as for methyl-accepting receptor-mediated behaviors. The above observation, therefore, is consistent with the postulation that aerotaxis is responsible for the targeting tumor by *E. coli*. Aer mediates response to rapidly oxiaizable substrates including glucose. This correlates with the model that Aer sense redox status within cell. Glucose content is known to be higher in the rapidly growing tumor cells than the rest of normal cells that should generate concentration gradient. It is thought that the E. coli senses the glucose gradient and migrates toward tumor tissue. Once arrive in the tumor tissue, *E. coli* survived due to its immunocompromised environment lacking infiltration of immune cells and expressing immunosupressive cytokines and inhibitors of complementation activation. In this permissive environment, bacteria would proliferate on the nutrients provided by destroyed tumor cells especially at the interface of the necrotic region

Set aside the mechanism, here we have successfully visualized the genetically engineered bioluminescent *E. coli* in various tumor lesions using non-invasive *in vivo* bioluminescence imaging technique. The *E. coli*, selectively guided to tumor tissue, could therefore be developed to a means to deliver gene-based anti-cancer agents specifically to tumors. In addition, such *E. coli* system could also be developed as a molecular imaging probe for its selectivity for tumor and amplification within the tumor (Massoud and Gambhir, 2003).

Keywords: gene therapy, tumor detection, *Escherichia coli*, chemotaxis, *in vivo* imaging system

S2-2

Differential Expression of Sporulation-Specific Factors in *Streptomyces griseus*

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To understand the regulation of the septation machinery during sporulation in *Streptomyces griseus*, we investigated the expression of sporulation-specific factors, the *eshA* and *pbpC* genes. Genetic complementation experiments and sequence determination demonnstrated that a group of Class III developmental mutants, SKK1003, 1004, and 1005, which accumulate the *eshA* transcript and protein much earlier than the wild-type strain, are *adpA* mutants. Since *adpA* encodes a key transcription regulator required for aerial mycelium formation and streptomycin production, we accomplished gel mobility shift assay using a recombinant AdpA. The AdpA bound to the upstream regulatory region of *eshA* containing two putative AdpA-binding sites in a concentration-dependent manner. These results demonstrated that AdpA activates the transcription of *eshA* by direct binding to the upstream regulatory regions. Out of six penicillin-binding proteins (PBPs), an 85 kDa PBP that preferentially binds to fluorescein-tagged β-lactams accumulated during submerged sporulation. The 85 kDa PBP was prevented from binding to Flu-ACA by cefoxitin that inhibits sporulation septum formation. The *pbpC*-disrupted strain was not defective in sporulation septation, implying that another sporulation septum-specific PBP is present in *S. griseus*. Computer analyses revealed the *pbpC* gene was clustered within developmental genes *mreB*, *mreC*, *mreD* (murein formation genes) and *sfr* (member of *SpoVE/FtsW/RodA* family) identified in other *Streptomyces*. S1 nuclease protection assays revealed that four *pbpC* transcripts accumulated at the highest levels after 16 hr of submerged sporulation, but not during vegetative growth. The transcripts were present much earlier and more abundantly in a group of nonsporulating mutants, *adpA* and *bldA* that prematurely form their sporulation septa from vegetative mycelia. These observations indicated that the *pbpC* gene encoding the 85 kDa PBP plays a role in forming sporulation septa.

Keywords: *Streptomyces griseus*, *eshA*, *pbpC*, *AdpA*, septum, Sporulation

S2-3

Structure-Based Analyses of the Mlc/Enzyme IIB^{Glc} Complex Mediating Glucose Signaling in *E. coli*

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In *Escherichia coli*, glucose-dependent transcriptional induction of genes encoding a variety of sugar-metabolizing enzymes and transport systems is mediated by the phosphorylation state-dependent interaction of membrane-bound enzyme IICB^{Glc} (EIICB^{Glc}) with the global repressor Mlc. Here, we report the crystal structure of a tetrameric Mlc in a complex with four molecules of Enzyme IIB^{Glc} (EIIB), the cytoplasmic domain of EIICB^{Glc}. Each monomer of Mlc has one bound EIIB molecule, indicating the 1:1 stoichiometry. The detailed view of the interface, along with the high resolution structure of EIIB containing a sulfate ion at the phosphorylation site, suggests that the phosphorylation-induced van der Waal's repulsion and disturbance of polar intermolecular interactions impede complex formation. Furthermore, we reveal that Mlc possesses a built-in flexibility for the structural adaptation to its target DNA and the limited flexibility of the Mlc structure resulting from the fixation of four ends of Mlc through binding to EIICB^{Glc} in the membrane is the underpinning mechanism for the loss of its DNA binding ability.

Keywords: enzyme IICB^{Glc}, glucose signaling, Mlc, protein-protein interaction, transcription regulation

S2-4

The *Shigella flexneri* Type III Secretion Effector OspG Binds Ubiquitinylated Ubiquitin-Conjugating Enzymes and Prevents IκBα Degradation

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Bacteria of *Shigella* spp. are responsible for shigellosis in humans, a disease characterized by the destruction of the colonic epithelium that is responsible for 1 million deaths per year (Kotloff et al., 1999). These bacteria use a type III secretion (TTS) system to enter epithelial cells and trigger apoptosis in macrophages (Cossart & Sansonetti, 2004). TTS systems are widely spread among Gram-negative pathogens and comprise (i) a secretion apparatus that spans the bacterial envelope, (ii) translocators that transit through the TTS apparatus and insert into the membrane of the host cell to form a pore, (iii) effectors that transit through the TTS apparatus and the translocator pore to be injected into the cell cytoplasm where they interfere with a variety of cellular functions, (iv) molecular chaperones and (v) specific transcription regulators (Hueck, 1998). The *S. flexneri* TTS system is encoded by a 200-kb virulence plasmid and includes ≈ 20 potential effectors (Buchrieser et al., 2000). The TTS apparatus is assembled during growth of *S. flexneri* in broth and is activated upon contact of bacteria with epithelial cells (Menard et al., 1994). Transcription of a set of genes encoding effectors is specifically induced in conditions of secretion and is controlled by MxiE, a transcription activator of the AraC family (Mavris et al., 2002).

The ubiquitination pathway results in the covalent attachment of the 76-residue ubiquitin to target proteins in three sequential steps performed by one ubiquitin-activating enzyme (E1), a limited number of ubiquitin-conjugating enzymes (E2, Ubc) and a large number of ubiquitin-ligating enzymes (E3) respectively (Pickart, 2001). Each E3 recognizes a set of substrates and cooperates with one or a few E2s. The E3 complex $SCF^{\beta\text{-}TrCP}$ promoting ubiquitination of phospho-IκBα consists of 5 proteins, the scaffold protein Cullin1, the adaptor protein Skp1, the RING domain protein Roc1, the F box protein β-TrCP that interacts with phospho-IκBα and the E2 UbcH5b (Zheng et al., 2002).

We present the functional analysis of the *S. flexneri* effector OspG, a 196-residue substrate of the TTS apparatus whose expression is regulated by secretion activity (Buchrieser et al., 2000). A two-hybrid screen in yeast identified ubiquitin conjugating enzymes (Ubc, E2) as potential partners of interaction of OspG and *in vitro* studies showed that OspG binds ubiquitinylated E2s (Ub-E2s) and is endowed with autophosphorylation activity. Transfection experiments indicated that OspG prevents degradation of phospho-I κBα and activation of an NF-κB regulated promoter. Characterization of the phenotype of an *ospG* mutant using both *in vitro* and *in vivo* models of infection confirmed that OspG is involved in the down-regulation of the host innate response induced by invasive bacteria.

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Keywords: Shigella flexneri, ubiquitin, innate immunity

S2-5

Catabolite Repression in *Escherichia coli*, Revisted: Regulation of Adenylyl Cyclase by an Unknown Factor as well as Enzyme IIA^{Glc}

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A widely accepted model for catabolite repression posits that phospho-enzyme IIA^{Glc} of the bacterial phosphotransferase system (PTS) activates adenylyl cyclase (AC) activity. For this reason enzyme IIA^{Glc} was formerly called as Crr standing for catabolite repression resistance. Attempts over many years to observe such regulatory properties of AC *in vitro* had been unsuccessful. To further study the regulation, AC was produced fused to the transmembrane segments of the serine chemoreceptor, Tsr. Cells harboring Tsr-AC and normal AC, expressed from the *cya* promoter on a low copy-number vector, exhibit similar behavior with respect to elevation of cAMP levels resulting from deletion of *crp*, expressing the catabolite regulatory protein. Membrane-bound Tsr-AC exhibits activity comparable to the native form of AC. Tsr-AC binds enzyme IIA^{Glc} specifically, regardless of its phosphorylation state, but not the two general PTS proteins, enzyme I and HPr; enzyme IIA^{Glc} binding is localized to the C-terminal region of AC. Binding to membranes of either dephospho- or phospho-enzyme IIA^{Glc} has no effect on AC activity. However, in the presence of an *E. coli* extract, P-enzyme IIA^{Glc}, but not dephospho-enzyme IIA^{Glc}, stimulates AC activity. The regulatory factor in the crude extract was not affected by RNase but lost its inhibitory activity by heat treatment or filtration using a 10K Centriprep filter. Based on these findings of a direct interaction of enzyme IIA^{Glc} with AC, but activity regulation only in the presence of *E. coli* extract, a revised model for AC activity regulation is proposed: CRP regulates expression of a protein inhibitor whose activity is neutralized by P-enzyme IIA^{Glc}, but not by dephospho-enzyme IIA^{Glc}.

Keywords: adenylyl cyclase, catabolite repression, CRP, Crr, protein-protein interaction

S2-6

Glucose Sensing and Signal Transduction in Yeast

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Yeast *Saccharomyces cerevisiae* cells grow well in the presence of plentiful nutrients while they arrest the cell cycle upon nutrient deprivation. Nutrient starvation triggers the meiotic pathway in the diploid cells and they form spores to survive the severe conditions. Thus, yeast cells sense nutrient levels and decide to initiate cell growth, cell cycle arrest or differentiation accordingly. Glucose, a preferred carbon source, has been shown to regulate intracellular cAMP level, which affects the cell growth and/or differentiation. But how cells monitor the glucose and transduce signals is a fundamental, unanswered question.

We have isolated the gene *GPR1* encoding a G protein coupled receptor using two-hybrid system with heterotrimeric G protein α subunit Gpa2p as a bait. *GPR1* was required for glucose dependent cAMP spike indicating Gpr1p was a glucose receptor that regulates cellular cAMP level. We also found that *GPR1* was required for pseudohyphal development as well as invasive growth in *S. cer*evisiae. Moreover, Gpr1 was shown to regulate glucose dependent large cell size. This signal transduction system was shown to be conserved in many other yeast strains including *Schizosaccharomyces pombe* and *Candida albicans*.

In this lecture, the meaning of glucose signaling through Gpr1p will be discussed.

Keywords: Signal transduction, GPCR, glucose, yeast

S3-1

Genome Evolution and the Rise of Human Pathogens

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Bacteria that belong to the genus *Burkholderia* are ubiquitous in the nature, living freely in the environments or associated with plants or animals. They are known as soil saprophytes, plant growth promoters, antagonists and pathogens to diverse life forms. Even in the sea, a significant level of *Burkholderia* was revealed by the recent meta-genomic expedition to the Sargaso Sea lead by Craig Venter. The enormous adaptability and diversity exhibited by the bacteria of this group are believed to have resulted from the rich repertoire of gene contents (genome size ~4 to 9 mb) carried by flexible chromosomes, which greatly facilitated genome modifications. These bacteria truly provide a valuable opportunity to study genome evolution associated with adaptation to various environments. A number of completed genomes of *Burkholderia*, although biased to pathogens, are currently available. Analysis of these genomes will help understand the evolution and mechanisms of their diversified virulence, and will ultimately lead to the development of effective countermeasures against the important human health hazards and potential bio-warfare agents, represented by *Burkholderia cepacia* complex, *Burkholderia mallei* and *Burkholderia pseudomallei*.

Keywords: Genome, Evolution, Pathogen, Burkholderia

S3-2

Recent Genetic Mutation and Changes of H5N1 Avian Influenza Viruses

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Highly pathogenic avian influenza (HPAI) caused by H5N1 subtype circulating in poultry and wild birds in Southeast Asian countries has been of great concern globally because of pandemic influenza (PI) potential for human since the first human infections with H5N1 avian influenza virus (AIV) occurred in Hong Kong in 1997. The disease resurged in Southeast Asian countries in late 2003 and, thereafter, spread to Russia, the Middle East, Europe, and Africa in 2006. Currently (as of August, 2006), a total of 12 countries in Asia have been affected with H5N1 HPAI, 5 in Middle East, 25 in Europe and 8 in Africa, since 2006. More than 200 million birds were culled as a consequence of HPAI outbreaks. H5N1 AIVs already caused the deaths of 141 of 241 infected persons in 10 countries, as of 5 September, 2006, corresponding to a death rate over than 50% for known infections. And, recently, H5N1 HPAI became endemic long enough to evolve distinct phylogenetic signatures in some area of Asian countries (Indonesia, China, Thailand, Vietnam, etc), while new strains have emerged in the area and other places. Consequently, multiple genetic lineages of the virus are thought to be co-circulating in ducks and geese (considered as 'Trojan horse' of HPAI viruses because of infection with no apparent clinical signs) in the area.

Influenza A viruses which consists of 8 segmented RNA genes (PB2, PB1, PA, HA, NP, NA, M and NS) are inherently unstable, therefore, can be readily mutated through genetic shift (genetic reassortment) and genetic drift (point mutation) due to genetic reassortment among RNA segments of different subtypes of AIVs and lacking a genetic proof-reading mechanism for the correction of transcription errors in the process of RNA virus replication cycle, respectively. H5N1 viruses isolated from the current outbreaks have been reported to reveal some genetic mutations especially in HA, NA, M, PB2 and NS genes. These genetic variation was acquired mainly through mutations in the RNA polymerase (Lys627 in PB2) gene, insertions in the HA gene, and deletions in the NA gene (stalk region) and nonstructural NA gene (Asp92 in NS1).

The hemagglutinin gene sequences of most of the H5N1 AIVs since 2003 separated into two distinct clades. Clade I includes human and bird isolates from Vietnam, Thailand and Cambodia and bird isolates from Laos and Malaysia. Clade 2 viruses were first identified in bird isolates from China, Indonesia, Japan and South Korea in 2003 and 2004, before spreading westwards to the Middle East, Europe and Africa during 2005-2006. Some of the clade 2 viruses have been primarily responsible for human H5N1 infections that have occurred during late 2005 and 2006, according to WHO. And genetic analysis has identified 6 subclades of clade 2, three (subclades 1, 2 and 3) of which have distinct geographic distribution and have been implicated in human infections: Subclades 1 (Indonesia), Subclades 2 (Middle East, Europe and Africa) and Subclades 3 (China). Another research report demonstrated that Korean and Japanese H5N1 isolates were of the same origin identified as a genotype V which was distinctively different from other genotypes, while most of H5N1 viruses from other countries belonged to dominant genotype Z.

Reciprocal cross-reactions in hemagglutination inhibition (HI) tests demonstrated antigenic similarity of HAs within the same genetic clade of H5N1 and distinguished representatives of different clades, with one exception. Based on the genetic clade and serological cross-reactivity, H5N1 viruses representative of subclade 1 and subclade 2 of clade II, as well as clade I virus, were recently selected by WHO for preparation of modified reassortant vaccine viruses using reverse genetics.

In pathogenicity of the viruses, recent H5N1 AIVs have become progressively more lethal in experimentally infected chickens and mice, and also stronger, surviving several days longer in environmental condition. And they have acquired the unprecedented capability to cause high mortality and neurological signs in several species. The viruses are known to be not yet fully adapted to poultry and to be continuing to evolve. Since the first detection of the precursor of the H5N1 AIV in Guangdong, China, in 1996, host range of the viruses has been expanded from domestic and aquatic birds to human, many kinds of non-aquatic birds and other mammalian species, including pigs (without clinical sign in Indonesia), cats and tigers. To date, no human cases have been linked to exposures to wild birds. Close contact with infected poultry and other domestic birds remains the most important source of human infections.

Such an expanding host range and high pathogenicity of the H5N1 AIVs due to continuous mutation will be likely to pose a significant threat of new PI potential in future. Therefore, global system for early detection, warning and control of PI should be well established to contain and eliminate the disease earlier at source.

Keywords: avian influenza, AIV, HPAI, H5N1, genetic mutation, pathogenicity

S3-3

Segmentation of the Nonsegmented RNA Genome from a Paramyxovirus for the Development of Multivalent Vaccines

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Paramyxoviruses are members of the order *Mononegavirales,* which have nonsegmented RNA genome. Many attempts have been made to develop vaccines using recombinant paramyxoviruses as vectors, but their nonsegmented genomes have not been amenable to multiple or large insertions for the development of multivalent vaccine. The division of nonsegmented RNA genomes would allow the creation of additional transcriptional units for insertion of multiple foreign genes. We have thus generated recombinant Newcastle disease viruses possessing segmented RNA genomes by using reverse genetics system. Authentic 3'- and 5'-non-coding termini were added to each segment. Reporter genes (such as green fluorescent protein or red fluorescent protein) were inserted into each segment in order to easily detect the replication and transcription of the segment in infected cells. We were able to detect the expression of both reporter genes in the same cell infected with recombinant virus containing two segmented genome. This virus grew well and segmented genomes in the virus were stable over multiple passages. These results suggest that nonsegmented RNA genome can be divided into segments which harbour additional foreign genes. It is hoped that the generation of paramyxoviruses possessing multiple foreign genes can lead the way to the development of efficient multivalent vaccines and/or gene therapy vectors. In addition, the segmentation strategy for nonsegmented RNA genome may be advantageous in terms of viral multiplication because shortened segmented genome can be replicated more efficiently than a longer one. Therefore, division of the nonsegmented RNA genome may give us insight into the evolution of negative-strand RNA viruses since they share common features (a similar gene order [3'-core proteins-glycoproteins-polymerase-5'], a single 3' promoter, and helical nucleocapsids) in their genome organization and replication strategy.

Keywords: Paramyxoviruses, Vaccine, Evolution, Reverse genetics system, nonsegmented RNA genome

S3-4

5' Terminus Deletion of Coxsackievirus B3 Genome and Persistence of Replication Defective Viruses in Murine Cardiomyocytes and Mouse Heart

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Adult human enteroviral heart disease is often associated with the detection of enteroviral RNA in cardiac muscle tissue in the absence of isolation of infectious virus particles. Passage of coxsackievirus B3 (CVB3) in adult murine cardiomyocytes produced CVB3 that was noncytolytic in HeLa cells. Detectable but noncytopathic CVB3 was also isolated from hearts of mice inoculated with CVB3. Sequence analysis revealed five classes of CVB3 genomes with 5' termini containing 7, 12, 17, 30, and 49 nucleotide deletions. Structural changes (assayed by chemical modification) in cloned, terminally deleted 5'-nontranslated regions were confined to the cloverleaf domain and localized within the region of the deletion, leaving key functional elements of the RNA intact. Transfection of CVB3 cDNA clones with the 5'-terminal deletions into HeLa cells generated noncytolytic virus (CVB3/TD) which was neutralized by anti-CVB3 serum. Encapsidated negative-strand viral RNA was detected using CsCl-purified CVB3/TD virions, although no negative-strand virion RNA was detected in similarly treated parental CVB3 virions. The viral protein VPg was detected on CVB3/TD virion RNA molecules which terminate in 5_ CG or 5_ AG. Detection of viral RNA and determination of relative $TCID_{50}$ ($rTCID_{50}$) in mouse hearts from 1 week to over 5 months postinoculation with CVB3/TD demonstrated that CVB3/TD virus strains replicate and persist in vivo. Taken together, evolution of a novel defective enterovirus quasispecies *in vivo* provides a mechanism by which to explain long-term viral persistence in human cardiomyopathies in the absence of cytopathic virus.

Keywords: Coxsackievirus B3, 5'-terminal deletion, virus replication, long-term persistence, cardiomyopathy

S4-1

The Biodiversity of Wood Destroying Fungi in the Central Europe and Their Possible Application in Biotechnology

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Wood rotting fungi represent significant component of forest. They play a crucial active role in processes of wood degradation, especially in the degradation of the most important constituents of wood – cellulose and lignin. These fungi are the only eukaryotic organisms producing lignolytic enzymes able to destroy lignin. Besides the above mentioned enzymes. Another compounds potentially utilizable in medicine have been found in various wood rotting fungi. So that why nowadays many researchers are interested in exploring of the useful compounds produced by wood destroying fungi, mainly polysaccharides (glucans), polypeptides and also terpens, alkaloids and other. Wood rotting fungi are very important organisms also from the point of view of biodiversity. Although some of wood destroying fungi are considered as important pathogens of woody plants, many of wood destroying fungi are the most endangered organisms at all. The reason of it is decreasing quantity both of retained dead wood as a substrate and niche of these organisms in managed forests, in spite of conservation of some types of forest ecosystems in protected areas within programs of dead wood and trees conservation. Some of wood rotting fungi are on the Red list in some of the European countries.

For the conservation of biodiversity is a fundamental condition to retain a sufficient volume of wood which is kept in forests for natural processes, including a wood decay made by wood destroying fungi. Important condition there is even quantity and quality of rotten wood, like a kind of timber, diameter and length of logs, position of fallen down stems etc. The diversity of dead wood is required condition for conservation of biodiversity of many organisms, including fungi. Above all The stems of big diameter are important.

The ratio of dead wood is estimated as 7% from the total stand volume in the contemporary managed forests in the Czech Republic. In Europe ranged quantity of dead wood in forests are from 1% - 12% in managed forests. The volume of dead wood is significantly important in the natural type forests, where the ratio between dead wood and total stand volume is 9 – 50%, depending on stand development. The volume of dead wood under conditions of natural reserves is 50–220 m^3 per ha. At this moment is conservation of some wood destroying fungi depending on the strategy of nature protection in the Czech Republic and also in other central European countries.

Besides wood rotting fungi are involved species from Agaricales like *Armillaria* spp., *Kuehneromyces* spp., *Hypholoma* spp., *Pholiota* spp., *Lentinus* spp. etc. Most of wood rotting fungi belonging to Aphyllophorales, especially bracket fungi from genera *Phellinus* spp., *Inonotus* spp., *Trametes* spp., *Daedalea* spp. etc. There are only few wood rotting species from sac fungi Ascomycetes, like *Ustulina* spp., *Hypoxylon* spp., *Camarops* spp. etc. From the point of view of wood decay and also its further application in biotechnologies have the crucial importance a type of decay, respectively if this fungus belong to white or brown rot fungus. In the nature predominate white rot fungi both the same from the point of view of quantity of species and the volume of decayed wood. Only in some special cases predominate in wood decay the brown rot fungi,

optionally predominate one or two species like in the case of mountains spruce stands, disrupted by bark beetles or other mortality stress factors. Brown rot fungi prevail also in wood decay in buildings like *Merulius lacrymans, Coniophora puteana* or in decay of trimmed wood, including impregnated wood, like *Lentinus lepideus*, *Gloeophyllum sepiarium*, *Deadalea quercina* etc.

The mycofloristic research in different nature reserves areas in the Czech republic shows, that wood rotting fungi share 60-80% of species which where noted in the plots within several year of research. The rotten wood is besides the soil one of the richest niche in forests at all.

Some of wood destroying fungi were successfully tested in biotechnologies for decomposition of waste material, like organic pollutants, oils, petroleum derivates, plastic materials, phenolic compounds etc. The high price and technological difficulties make recently wide applications in industry and environmentology impossible.

Mushrooms have been long considered having a medicinal value, especially in Asia. Myco-pharmacological investigations of bioactive metabolites and medicinal properties of mushrooms play an important role in development of new biotech products and biopharmaceuticals. Especially wood rotting fungi are studied for purpose of production pharmaceutically active compounds, and so new species and strains of wood rotting fungi are explored.

The wood rooting fungi are in Central Europe intensively studied group from the point of view of taxonomy, ecology, bionomy and pathology. Especially groups of polypores and some agaricales are quite known well. In these groups there are some of fungi which are interesting for their potential use in biotechnologies and biopharmacy.

From Agaricales there is a very interesting group honey fungus Armillaria, which 5 annulate species (*A. ostoyae, A. gallica, A. cepistipes, A. borealis, A.mellea*) are very common in Central Europe. Exannulate *A. tabescens* is thermophilic species, growing only in some areas, *A. ectypa* is very rare species, in the Czech republic known only from a few findings in peat bogs. In some secondary spruce stands there are *Armillaria* spp. Which are the important pathogens of root system. Honey mushrooms are the favourite edible fungi collected in Czech forests.

Another interesting group from polypores (bracket fungi) is *Ganoderma* species. In Europe there are three species producing perennial fruit bodies: *G. applanatum*, *G. adspersum and G. pfeiferii*, other species produce annual basidiocarps. *G. lucidum*, *G. resinosum* are connected with broadleaved species, *G. carnosum* and *G. valesiacum* prefer coniferous hosts. *G. lucidum* and related species are used for the production of pharmaceutical active compounds. Central European species and also strains produce the same compounds as cultivated strains of *G. lucidum,* there are variation of concentration searched compounds only. Especially cultivated G. *resinosum* produce mushrooms of excellent quality. The fructifications of cultivated *G. resinosum* are high similarity to *G. lucidum* with its enormous production of spores.

For biotechnologies there are also very interesting two genera of white rotting fungi, *Phellinus* s. l. and *Inonotus* s. l., playing the crucial role in forest ecosystems of northern hemisphere. Some of them, e.g. *Inonotus obliquus* and *Phellinus linteus*, are used for anti-carcinogenic purpose in Eastern Asia and northern part of Russia. In case of the two genera the knowledge of biochemical features of various species is unequal and some European species (e.g. *Inonotus radiatus, Phellinus conchatus, P. hartigii, P. lundellii*) have not been sufficiently studied yet. There are 40 species altogether of *Inonotus* s.l. and *Phellinus* s.l. species are currently recognized in Central Europe: *Inonotus andersonii, I. cuticularis, I. dryadeus, I. dryophilus, I. hastifer, I. hispidus, I. leporinus I. nidus-pici, I. nodulosus, I. obliquus, I. radiatus, I. rheades, I. ulmicola, I. tomentosus, I. triqueter; Phellinus alni, P. cinereus, P. cavicola, P. chrysoloma, P. conchatus, P. contiguus, P. ferrugineofuscus, P. ferruginosus, P. hartigii, P. igniarius, P. laevigatus, P. lundellii, P. mediterraneus* (known as of *Fomitiporia mediterranea*), *P. nigrolimitatus, P. pilatii, P. pini, P. populicola, P. pouzarii, P. pseudopunctatus, P. punctatus, P. rhamni, P. robustus, P. torulosus, P. tremulae, P. tuberculosus, P. viticola.*

Also some other wood rotting fungi like *Meripilus giganteus, Grifola frondosa, Perenniporia fraxinea*, were already tested in biotechnologies, are in Central Europe mentioned like pathogens of root system of woody plants. Also products from *Sparassis crispa*, *S. brevipes*, S. nemecii could be interesting for production of some useful compounds, eg. Antibiotics and preserved basidiocarps in nature. Also monkey head species growing in Europe like *Hericium alpestre* and *H. coralloides* could be very interesting for further application studies.

Compendium of Central European species with possible application in mycotechnologies and mycopharmacy could be much longer. Most of mentioned species are well available for isolation and introduction; some of them are endangered species as *Aurantioporus croceus* due to the absence of suitable substrates.

Keywords: Biodiversity, Wood rotting fungi, Biotechnologies, Fungi conservation

S4-2

Polyphasic Taxonomy of *Aspergillus* Section *Fumigati* and Its Teleomorph, *Neosartorya*

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Aspergillus section *Fumigati* (AsF) (teleomorph, *Neosartorya* Malloch & Cain) is a medically and agriculturally important group, but its species differentiation concept has not been established. The study was performed to establish concept of species differentiation in AsF and to clarify the taxonomic position of known AsF species. About 350 strains of AsF were isolated from arable soil in Korea or introduced from Centraalbureau voor Schimmelcultures (CBS) and analyzed with phenotypic characters including macro-, micro-morphology, growth temperature regimes and extrolite patterns, and genotypic characters including multi-locus sequence typing (MLST) of partial β-tubulin, calmodulin and actin genes and RAPD-PCR. In polyphasic taxonomy of *Aspergillus fumigatus* and related species, strains previously considered as *A. fumigatus* were divided into five groups, *A. fumigatus* sensu stricto, *A. lentulus*, *A. viridinutans* complex, *A. fumigatiaffinis* and *A. novofumigatus*. MLST and growth temperature regimes could be critical determinants for the delineation of the *A. fumigatus* sensu stricto species. *A. lentulus* was well distinguished from *A. fumigatus*. *Aspergillus fumigatiaffinis* sp. nov. and *Aspergillus novofumigatus* sp. nov. were proposed as novel species. 147 strains of *Aspergillus fumigatus* sensu lato previously identified on the basis of morphology were re-identified as *A. fumigatus* sensu stricto (141, 95.9 %), *A. lentulus* (3, 2.0 %), *A. viridinutans* species complex (1, 0.7%), *Neosartorya udagawae* (1, 0.7%), and *N.* cf. *nishimurae* (1, 0.7%). Strains of *Neosartorya spinosa*, *N. glabra* and related species were reclassified. Strains of *N. glabra* sensu lato were divided into *N. glabra* sensu stricto, *N. laciniosa, N. coreana* and undetermined species. *Neosartorya laciniosa* sp. nov. and *Neosartorya coreana* sp. nov. were proposed as new to science, but *N. botucatensis, N. paulistensis* and *N. takaki* were reduced to synonyms with *N. spinosa.* In the other clades, *N. delicata, N. primulina* and *N. otanii* were proposed to be synonymized with *N. tatenoi, N quadricincta* and *N. fennelliae*, respectively. The four new species, *Neosartorya denticulata* sp. nov., *Neosartorya assulata* sp. nov., *Neosartorya galapagensis* sp. nov. and *Aspergillus turcosus* sp. nov. were described and illustrated. The other known species in *Aspergillus* section *Fumigati* were reviewed on the basis of polyphasic taxonomy. Consequently, it was suggested that out of 40 species, 29 species be accepted, 4 species be candidates for acceptance, but the species need more molecular analyses, and 7 species be rejected. MLST of partial β-tubulin, calmodulin and actin genes, RAPD-PCR, growth temperature regimes and extrolite patterns were critical tools for the delimitation of AsF species, although morphological characters were also important to describe the species.

Keywords: Polyphasic Taxonomy, *Aspergillus* section *Fumigati*, *Neosartorya*, *Aspergillus fumigatus, Neosartorya coreana*

S4-3

Phytophthora Diseases in Forest: Histocytological Study of Port-Orford Cedar Root Disease

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The approximately 80 known species of *Phytophthora* are all destructive plant pathogens, causing rots of roots, stems, leaves, and fruits of a huge range of agriculturally and ornamentally important plants. Port-Orford-cedar (*Chamaecyparis lawsoniana*, POC) is a native conifer in southwest Oregon and northern California. In 1923, a root disease was first reported on POC in nurseries and landscape plantings caused by *Phytophthora lateralis*. Since about 1954 the disease has been spreading along roads and streams in forests where POC grows wild. A few tree families have been shown to have resistance and selected for study of resistance mechanism. Since no research has been done yet for why and how the tree families are resistant against *P. lateralis*, this study was designed to help understand the mechanisms of resistance to *P. lateralis* in individual POCs. Cytological observations by means of microscopy indicated that 1) encystment, penetration, and colonization of *P. lateralis* were the same on both susceptible(SP) and resistant POCs(RP) but their frequencies in resistant seedlings were lower than in susceptible seedlings. 2) after 24 hr infection on root, frequency of initial infection, penetration, and colonization was different between SP and RP but mode was same. 3) inter- and intracellular hyphae were present in cambial, parenchyma, and sieve cells in functional phloem of stems. 4) Slow growth of pathogen in resistant POC cells is caused by general resistance responses, such as increased cell wall thickness, wall apposition, and deposition of electron dense particles.

Therefore, this study provided evidence for resistance mechanisms based on induced physical (barrier) and mechanical defense of POC to *P. lateralis*. Although no rapid cell death by inducing lignification and phenolic compounds as a hypersensitive response was documented, this study provides evidence for general resistance mechanisms in POC to *P. lateralis* and reveals the relationship between POC and *P. lateralis*.

Keywords: Phytophthora lateralis, Resistance

S4-4

Determining Fungal Diversity on Mountain Pine Beetle and Its Infested Lodgepole Pine Using Cultural and Molecular Methods

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Lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) is an economically and ecologically important resource of Western Canada and the Northwestern United States. It accounts for 50% of the total growing stock and 25% of the total volume of timber harvested in interior British Columbia, Canada, which is used in construction, for pulpwood, and for railway ties and poles. Although the mountain pine beetle (MPB, *Dendroctonus ponderosae* Hopkins) is natural components of lodgepole pine ecosystems, it is a serious threat for mature lodgepole and several other pine species. Recent moderate winters have caused lower larval mortality, resulting in an epidemic that is currently spreading across British Columbia and causing extensive economic and environmental damage to lodgepole pine forests.

The MPB carry a diversity of fungi on their body surface and symbiotic fungi in mycangia. Within a tree, the MPB remains in the region just under the bark, mining the phloem and reproducing, while the fungi, mainly staining fungi, propagate in beetle galleries in the phloem and into the underlying sapwood. The beetles benefit because the staining fungi lower the wood moisture content and may produce an environment more favorable for the beetle brood and some of the fungal associates are potential nutrient sources for the next beetle generation. In trees that have sustained high levels of attack by MPB, the sapwood is stained by fungi within a few weeks. As there is no appreciable loss in most strength properties, only a small decrease in toughness, stained wood can be used in most applications where appearance is not an important factor. However, MPB-killed trees that are left uncut for extended periods are susceptible to fungal decay that will further reduce their value in markets that are otherwise open to stained wood.

In order to evaluate fungal diversity from the exoskeleton of the MPB and its infested lodgepole pine trees, the cultural and molecular methods were used. All nine fungal taxa were isolated from the MPB. Identification was based on cultural morphology and high sequence similarities of the internal transcribed spacer (ITS) and large subunit ribosomal DNA (LSU rDNA) region to sequences of known fungi. The isolates from the cultural method represented 7 ascomycetes and 2 basidiomycetes. Fungal ITS regions were amplified from DNA directly extracted from the beetle surface. The PCR products were cloned and 125 clones were classified by their restriction pattern with *Hae*III and *Rsa*I. A total of 15 RFLP types were identified and subsequently sequenced. Sequence analysis of the RFLP types showed that 14 ascomycetes and 1 basidiomycetes were represented in the clone libraries. We found that yeast and non-staining filamentous Euascomycetes fungi were detected efficiently using a molecular approach, while the major sapstaining fungi and decay fungi were best detected using cultural methods. The infested lodgepole pine trees were mainly colonized by *Ophiostoma clavigerum* (Robinson-Jeffrey & Davidson) Harrington, *Ophiostoma montium* (Rumbold) von Arx, *Ophiostoma nigrocarpum* (Davidson) De Hoog, *Ophiostoma minutum* (Olchow. & Reid) Hausner, and unknown *Leptographium* species. Among basidiomycetous fungi, decay fungi were rarely present in green trees but were isolated more frequently in red and grey trees. The frequency and the type of decay fungi isolated varied between harvesting sites.

Keywords: fungal diversity, ITS, lodgepole pine, mountain pine beetle

S5-1

BIO-Defense System (ABADIS) (Automatic Biological Agent Detection & Identification System)

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Since “9.11” incident, there have been no safety zones from silent threat of bio-terrorism as well as armed terrorism. Bio-terrorism is released through the air, water or by food to contaminate random people, and it is beyond the capabilities of existing disaster management systems.

The intentional use of any microorganism, virus, infectious substance, or biological product that may be engineered as a result of biotechnology, or any naturally occurring or bio-engineered component of any such microorganism, virus, infectious substance, or biological product could cause death, disease, or other biological malfunction in a human beings, animals, plants, or another living organisms in order to influence the conduct of government or to intimidate or coerce a civilian population.

Terrorists may use biological agents because the production is not difficult, inexpensive to construct, and is an easily concealed position. There are so many the bio-terrorism agents, but it is transmitted from person to person easily, and the highest risk and toxic to the public is Bacillus anthracis, Yersinia pestis, Smallpox.

Biological agents are effective in very low does. Biological agent detection system need to exhibit high sensitivity, high selectivity, high speed or response. Early detection makes it possible to prevent the damages by early response measures such as injecting vaccines.

The Bio-Defense system includes the IBAD(Interim Biological Agent Detector), BIDS(Biological Integrated Detection System), ABADIS(Automatic Biological Agent Detection & Identification System).

ABADIS is usually operated in cities, airports, and government and public agencies. Every international event takes a place, and ABADIS is installed in the particular places to collect the samples and detect Pathogen, Protein and Toxin to take an action accordingly. It has a positive pressurized air condition system freedom from contamination and rapid detection & broad identification characteristics.

ABADIS used in Korea-Japan world cup 2002, Asian Game in Busan, Universiad in Daegu, APEC conference in Busan.

Keyword: Bio-Defense, ABADIS, rapid detection, broad identification

S5-2

Application of Biosensor Based on the Nitrate Reductase (NaR) to Portable Total Nitrogen (TN) Analysis System

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We developed a biosensor based on nitrate reductase (NaR), which is composed of working, counter and reference electrode. NaR can be isolated from a denitrifying bacterium *Pseudomonas* sp. or purchased from manufacturer. The NaR was immobilized on working electrode of sensor strip by polymer entrapment and then dried in the air or in a refrigerator. Counter electrode was not modified, and reference electrode was composed of Ag/AgCl. This system can detect nitrate concentrations at the level below 1 μg/L (9.8 nM), which is as precise as ion chromatography(IC). However, application areas of the biosensor are limited to the nitrate analysis of water. To extend the application spectrum of the biosensor, we developed an electrochemical nitrification system for conversion of organic nitrogen, ammonium and nitrite to nitrate. Nitrification could be reached up to 95% of ammonium and 75% of organic N by using the electrochemical nitrification system. By combination of the biosensor and the nitrification system, it is possible that all kinds of nitrogen compounds including organic nitrogen, ammonium, nitrite and total nitrogen(TN) may be separately analyzed in both laboratory and field.

Keywords: Biosensor, Nitrate reductase, Electrochemical nitrification, Total nitrogen analysis, Sensor strip

S5-3

Biochip / Biosensor-Based Detection of Environmental Pathogens

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Pathogens in environmental samples are traditionally detected by a culture method, which requires at least 3-4 days to identify pathogens. Alternative detection methods, such as PCR, biochip, and biosensor, have been developed [1, 2]. Both the biosensor and biochip technologies have a same principle that specific capture probes such as antibodies and nucleic acids are used. While the biosensor technology enables to detect pathogens rapidly, the DNA microarray technology, which is a representative biochip, provides a way to investigate differential gene expression profiling for thousands of genes in a single experiment. In this presentation, the application of biosensor and DNA microarray in environmental monitoring will be reviewed, and research results carried out in our laboratory will be introduced.

To detect bacterial pathogens, 16s rRNA was analyzed using a surface plasmon resonance (SPR) biosensor and an oligonucleotide microarray. The 16s rRNA has been used as a genetic marker for identifying organism, and can be analyzed directly without PCR amplification due to relatively high copy number [3]. However, the direct detection of 16s rRNA shows sensitivity limitation compared with PCR-based assays. In this study, the signal enhancing method for high sensitive direct detection of bacterial 16S rRNA using peptide nucleic acid (PNA) probes was developed. The amount of hybridization was monitored by the SPR biosensor, which enables detection of molecular interactions on surface in response to changes in the index of refraction [4]. Biotinylated PNA probes, which were designed to be complementary to 16s rRNA gene sequences, were immobilized on avidin surface, and then *E. coli* 16s rRNA was hybridized at room temperature. As a result, the amount of *E. coli* 16s rRNA hybridization on PNA probes was about two-fold higher than oligonucleotide probes. In further experiments, signal amplification was possible utilizing the different backbone structures between PNA and DNA. To amplify the interaction of PNA and 16s rRNA, surface-modified nanoparticles were designed to interact with 16s rRNA selectively. Based on the results of SPR study, a microarray of DNA oligonucleotides and PNAs was fabricated. Hybridized 16s rRNA was detected using fluorescent intercalating materials.

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Keywords: biosensor, biochip, microarray, pathogen, 16s rRNA, PNA

S5-4

Integrative Community Analysis and Water Quality Assessment in Streams across Different Levels of Pollution

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Benthic macroinvertebrates serve as a linker between producers and decomposers in a food chain and have been widely used for assessing water quality with variousadvantages of taxonomic diversity, sedentary in behaviors and long life cycles [1]. In addition, microorganisms play an important role in aquatic systems regarding decomposition of organic matters in polluted conditions [2], and energy recovery in the high-level food chain through microbial loop [3]. Microorganisms sensitively respond to *in situ* availability of organic matters in polluted conditions and are considered as an efficient group to indicate water quality [4, 5].

If benthic macroinvertebrates and microorganisms are analyzed in an integrative manner, ecological status of aquatic ecosystemswould be more comprehensively revealed. There have been numerous accounts of research on community-habitat relationships and water quality assessment separately for macroinvertebrates (e.g., [5]) and for microorganisms (e.g., [6]). However, benthic macroinvertebrates and microorganism have been seldom investigated at the same sample sites except for a few cases [7, 8, 9, 10].

Materials and Methods

We selected the sample sites across different levels of pollution and collected benthic macroinvertebrates and microorganisms along with chemical factors. Macroinvertebrates were collected with the Surber net, while sampling of microorganisms was carried out by using a disposable syringe (100 *ml*) with its injection portion cut off. Denature Gradient Gel Electrophoresis (DGGE) was utilized to identify DNA sequences [6].

Data for communities for both macroinvertebrates and microorganisms were trained with the Self-Organizing Map (SOM) [14]. The Ward's linkage method was additionally applied to the weights of the nodes in SOM for further clustering [15]. We used the functions provided in the SOM toolbox [16]in Matlab. The nonparametric multiple comparisons were carried out according to the Kruskall-Wallis test [17].

Results, Discussion and Conclusions

When the combined data for abundance of microorganism and macroinvertebrate communities were trained with SOM, the patterned sample sites were vertically divided: clusters I-II for the samples from the clean sites in the upper area of the map, and clusters III-IV for the samples from polluted sites in the bottom area (Fig. 1a). In the clean area, the sample sites from DUK in the upstream area in cluster I was subdivided from the samples from the recovering site, DAG, in cluster II. In clusters III-IV representing polluted sites, the samples from DKS in cluster III, being located below the second source point of organic pollution, was separately grouped from the sample sites located in between the first and second sources of organic pollution, DKH and DDK, in cluster IV (Fig. 1a, b).

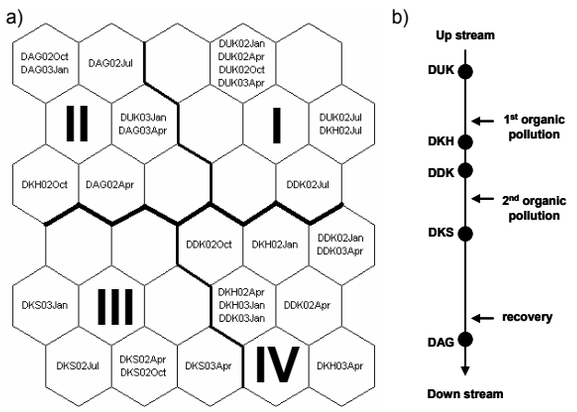

Fig. 1. Clustering of the sample sites on SOM based on the combined community data (relative abundance) of microorganisms and macroinvertebrates collected in the Daechon stream across different levels of pollution. Clusters (a), and location of the sample sites in the stream (b).

In both macroinvertebrates and microorganisms, there were taxa broadly covering the map. In macroinvertebrates, for instance, Gomphidae was widely present, being concentrated more at the upper area of the map around clusters I and II. Chironomidae and Oligochaeta were abundant at the lower area with higher densities, being concentrated in cluster IV. The widely-distributed species in microorganisms, however, were not as abundant as shown in macroinvertebrates. Species in macroinvertebrates appeared more selectively in the clean area (e.g. Lepidostomatidae (cluster II) and Leuctridae and Perlidae (cluster I)), while presence of the selective species in microorganisms were more limited to the polluted sample sites (e.g. *Acidovorax* and *Nostocoida* in cluster III).

Phosphate in water, nitrate in water, and ammonium in sediment were higher in the recovering site DAG than in the clean site DUK. At the pollutedsite DKS (cluster III), DOC was lower while nitrate in sediment was higher compared with DKH and DDK (cluster IV). The overall scopes of macroinvertebrate and microorganism communities and their associations with environmental factors were revealed through the inter-taxa community analysis with SOM. Feasibility of DGGE in identifying microorganism species from field collections suggested a possibility of using microorganisms as a means of field bioindicators [18].

In summary, the results from this study demonstrated that the inter-taxa community analysis would be useful for characterizing overall scopes of communities under various environmental impacts. In dealing with complex inter-taxa community data, SOM was efficient in clustering and visualization of communities, and showed the relationships with environmental variables. The inter-taxa presentation of communities would be an efficient means for revealing the full scope of polluted state in aquatic ecosystems and would be useful for integrative assessment of aquatic ecosystems.

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Keywords: Integrative assessment, Monitoring, Community dynamics, Biological indicators, Artificial neural network

S5-5

Prediction Model for Cyanobacterial Bloom in Daechung Reservoir

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Cyanobacterial blooms are general worldwide phenomena in eutrophic lakes and rivers in summer. Furthermore, some strains of cyanobacteria produce toxins, threatening public human health. Microcystin, a representative cyanobacterial hepatotoxin, was frequently detected in a large number of Korean lakes. To efficiently cope with such environmental problems, the Prediction System for Algal Blooms has been in operation since 1997 by the Korean Ministry of Environment. Three levels of alert are declared in this system, according to chlorophyll-*a* concentration and cyanobacterial cell density. However, the current system has no predictive ability actually. It only determines the present status of algal growth and thus cannot provide any preventive strategies in advance, at a proper time. In this study, we tried to develop a prediction model for cyanobacterial bloom in Daechung Reservoir. Linear regression and artificial neural network are two major techniques for model development. Weekly data measured from spring to autumn in 1999, 2001, 2003, 2004, and 2005 were used for model construction. The predicted values by simulation were compared with the measured values. Predictive power was fairly excellent in forecasting algal growth in three weeks earlier. Although model development requires long-term accumulation of field data, it deserves to be done, because it can provide a deeper understanding for complex ecosystems as well as practical usefulness.

Keywords: bloom, cyanobacteria, Daechung Reservoir, model, prediction

S6-1

Characteristic of Poly-γ-Glutamic Acid Producing Strain *Bacillus subtilis* (*Chungkookjang*) and Mass Production of Poly-γ-Glutamic Acid

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Poly-γ-glutamic acid (γ-PGA) is an unusual anionic polypeptide in which D- and/or L-glutamate is polymerized via gamma-amide linkages and, therefore, is an optically active polymer having a chiral center in every glutamate unit. γ-PGA is a biodegradable, biocompatible and water soluble material, thus can be applied for a thickener, humectants and matrices of drug delivery on the basis of its unique properties. At present, we succeeded in producing γ-PGA with high molecular weight industrially and developing new functional applications in foods and cosmetics. Some strains of *Bacillus subtilis*, including the starters of Natto, a traditional Japanese fermented food made from soybeans, and of Chungkookjang, a traditional Korean fermented seasoning made from soybeans, γ-PGA produce as a main component of the extracellular mucilage. γ-PGA from *B. subtilis* (*natto*) typically has a variable molecular weight (10 to 1,000 kDa), whereas high-molecular weight of γ-PGA (>2,000 kDa) can be obtained from the culture filtrate of *B. subtilis (chungkookjang)*. In particular, the potentials of the ester derivatives of γ-PGA, whose carboxyl groups were modified with various alkyl compounds, as biodegradable substitutes for currently used nonbiodegradable materials, including thermoplastics, fibers, films, and membranes, have been the focus of study. However, for the acceptance of this most promising biopolymer for practical industrial uses, two major problems remain to be solved: how to produce it more abundantly and at a moderate price and how to control its structural diversity. To address these problems, many attempts have been made to isolate and construct industrially useful producers of γ-PGA. [This work supported by the Cooperative Research and Development Program of Industry, Academy, and Research Institute from Seoul City.]

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Keywords: Characteristic, Mass Production, *Bacillus subtilis*, Poly-γ-Glutamic Acid

S6-2

A Platform Technology for the Production of Massive Recombinant Proteins in the Post-Genome Era

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Recombinant production of the proteins limited in naturehas been recognized as an essential field not only for human biopharmaceuticals but also for the functional genomics in the post-genome era. As a host for the recombinant protein production, yeast *Saccharomyces cerevisiae* has several advantages over other host such as *E. coli* and mammalian cells. It is a GRAS organism for human and has an excellent protein secretion pathway and post-translational modification function necessary for higher eukaryotic proteins in high quality and low cost. Although it has been chosen for the production of numerous human proteins, the yield was generally low and unpredictable. For the improvement of secretion level of such proteins, we have developed a target protein-specific translational fusion partner (TFP) technology. Around 4×10^3 TFP library was constructed from yeast genomic or cDNA library using a secretion reporter invertase. Simple insertion of target gene into TFP library vectors and a positive selection on sucrose media could find an optimal fusion partner in a high throughput manner from the constructed TFP library. Using this system, over 40different TFPs from the ORFs encoding yeast secretory proteins were already obtained and they were found to be useful for enhancing the secretion level of numerous 'difficult-to-express' proteins containing novel protein drug candidates. Thus, the TFP technology could be a platform technology for the production of massive recombinant proteins of bio-industry and basic research in the post-genome era.

Keywords: Yeast, recombinant protein, secretion, fusion partner

S6-3

T7 Helicase Recovers dsDNA Unwinding Ability by DNA Synthesis

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Helicases are molecular motors that use the energy of nucleoside 5'-triphosphate (NTP) hydrolysis to translocate along a nucleic acid strand and catalyse reactions such as DNA unwinding. The ring-shaped helicase of bacteriophage T7 translocates along single-stranded (ss)DNA at a speed of 130 bases per second; however, T7 helicase slows down nearly tenfold when unwinding the strands of duplex DNA. Here, we report that T7 DNA polymerase, which is unable to catalyse strand displacement DNA synthesis by itself, can increase the unwinding rate to 114 base pairs per second, bringing the helicase up to similar speeds compared to its translocation along ssDNA. The helicase rate of stimulation depends upon the DNA synthesis rate and does not rely on specific interactions between T7 DNA polymerase and the carboxy-terminal residues of T7 helicase. Efficient duplex DNA synthesis is achieved only by the combined action of the helicase and polymerase. The strand displacement DNA synthesis by the DNA polymerase depends on the unwinding activity of the helicase, which provides ssDNA template. The rapid trapping of the ssDNA bases by the DNA synthesis activity of the polymerase in turn drives the helicase to move forward through duplex DNA at speeds similar to those observed along ssDNA.

Keywords: helicase, polymerase, DNA unwinding, DNA synthesis

S6-4

Organization and Regulation of *arg* and *his* Operons in *Corynebacterium glutamicum*

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The Gram-positive bacterium *Corynebacterium glutamicum* is used for the large-scale production of amino acids. Complete genome of *C. glutamicum* has been sequenced and efforts are being made to understand many aspects in biotechnology of this organism. We have been studying organization and regulation of arginine and histidine biosynthetic genes in *C. glutamicum*.

The *his* genes encoding histidine biosynthetic enzymes from *C. glutamicum* were screened by complementation of several *Escherichia coli* mutants by transforming a *C. glutamicum* genomic library cloned in the vector pMT1. In this way, three plasmids that complemented of the *E. coli* histidine auxotrophs were obtained: pCH1 complemented *hisD, C,* and *B* in *E. coli* mutants; pCH2 complemented *hisH*, *A, F*, and *I* in *E. coli* mutants; pCH3 complemented *hisE,* and *G* in *E. coli* mutants. The histidine biosynthetic pathway, which requires 10 enzymatic reactions from ATP and phosphoribosyl-pyrophosphate (PRPP), has been extensively analyzed in *E. coli* and *Salmonella typhimurium*. The enzymes are encoded by only eight genes in these bacteria, since the products of the *hisD, hisB,* and *hisIE* genes are bifunctional. In *C. glutamicum*, the seven genes (*hisDCBHAFI*) are organized in an operon, which is regulated by attenuation.

The complete nucleotide sequence of a 9,672-bp region was determined. Analysis of this sequence revealed the presence of 9 open reading frames (ORFs). Nine ORFs are homologous to *E. coli hisDCBHAFI*, with the insertion of *orf1* and *impA* gene. The *his* genes were mapped in two unlinked loci. One loci containing *hisEG* genes, the other locus contains the main *his* gene cluster with eight ORFs (*hisDCBHAFI*). RT-PCR reactions resulted that *hisG* and *hisD* genes were separated in the different region of chromosome.

The primer extension showed that the first nucleotide of the mRNA was a C residue corresponding to position 246 in the DNA sequence. The 196-bp region upstream of the start codon of *hisD* contains the promoter P*his* and a 196-bp noncoding sequence that was named the *his* leader region. The *his* operon of *C. glutamicum* is effectively controlled by a tRNA-dependent mechanisms of attenuation. Two types of transcriptional fusions were constructed, one each in a promoter probe and a terminator probe vector, named pProm and pTer, respectively. The pProm series contains the *cat* genes under the control of the P*his* promoter followed by a modified *his* leader region. The pTer series contains derivatives of the *his* leader region without the promoter cloned in pET-*tac* as the parental vector. It was used to study the regulation of the histidine operon by termination and antitermination. Mutational analysis show that the interactions of the anticodon and the acceptor stem of the tRNA with the leader are necessary.

We have also cloned the entire arginine biosynthesis gene cluster in *C. glutamicum* by the ways we applied in the *his* genes. Northern hybridization and RT-PCR analysis showed that the arginine biosynthesis gene cluster has two transcripts corresponding to *argCJBDFR* and *argGH*, respectively. The respective transcriptional initiation sites in front of *argC* and *argG* were identified by primer extension experiments and promoter regions of these transcripts were investigated based on DNA sequences analysis. Thus, we have characterized the arginine biosynthesis gene cluster of *C. glutamicum* and the two transcripts of these genes along with their promoter regions of these genes in *C. glutamicum.*

Keywords: Corynebacterium glutamicum, genetic engineering, Amino Acid, *his*, *arg* operon

S6-5

Development of Bio-Related Functional Materials Based on Poly(γ-Glutamic Acid)

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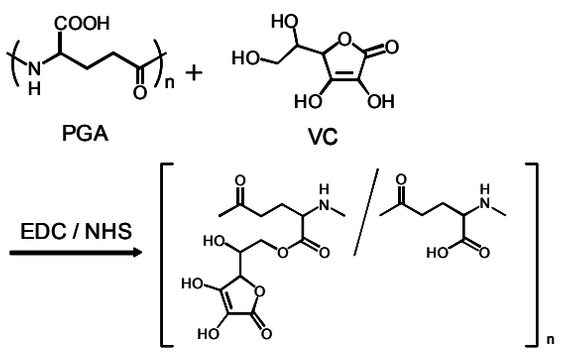
Poly(γ-glutamic acid) (PGA) is a bacterially synthesized biopolymer with γ-amide linkages.[1] Applications of PGA have been extensively studied from an industrial standpoint, since PGA is substantially biodegradable, nontoxic to humans, and edible. From these multifunctionalities, PGA has been applied in food, cosmetics, environmental and biomedical industrial fields.

We have established precise modification of PGA to produce bio-related functional materials based on PGA. This presentation deals with development of PGA-based conjugate polymers and PGA hydrogels. We developed two types of PGA-based conjugates, PGA-vitamin C conjugate and PGA-cholesterol conjugate.

Vitamin C is a natural compound having antioxidant, anti-cancer, anti-viral, and anti-aging properties. We synthesized PGA-vitamin C conjugate by the reaction of PGA with vitamin C in the presence of 1-ethyl-3-(3-dimethylaminopropyl)- carbodiimide (EDC) and N-hydroxy- succinimide (NHS) (Scheme 1).



Scheme 1

Antioxidant properties of the conjugate were evaluated by measuring the scavenging activity of superoxide anion. PGA did not scavenge superoxide anion, whereas the conjugate showed high scavenging activity for superoxide anion in the low concentration (Figure 1), indicating the good antioxidant property of the PGA-vitamin C conjugate. The PGA-cholesterol conjugate was synthesized by using amine-containing cholesterol derivative with 1,1'-carbonyl bis-1H-imidazole as dehydrating agent. Nanoparticles were formed by dispersion of the amphiphilic conjugate in water.

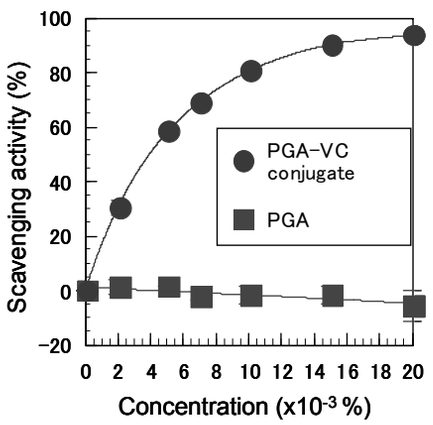


Figure 1. Superoxide anion scavenging activity of PGA-vitamin C conjugate.

We have investigated enzymatic synthesis of biopolymer hydrogels. The principle of the gel production is shown in Figure 2. The advantages of the enzymatic crosslinking is (i) high chemoselectivity, (ii) mild curing conditions, and (iii) rapid (instant) gelation. The peroxidase-catalyzed oxidative curing of the phenol-containing PGA derivative was performed using hydrogen peroxide as oxidizing agent in a phosphate buffer (PBS, pH 7). The gelation quickly took place after the addition of hydrogen peroxide to produce a transparent hydrogel.

For evaluation of intact structure of gels formed by the enzymatic curing, oscillatory measurement was carried out. Elastic modulus (G') of the hydrogel was higher than viscous modulus (G'') over all the

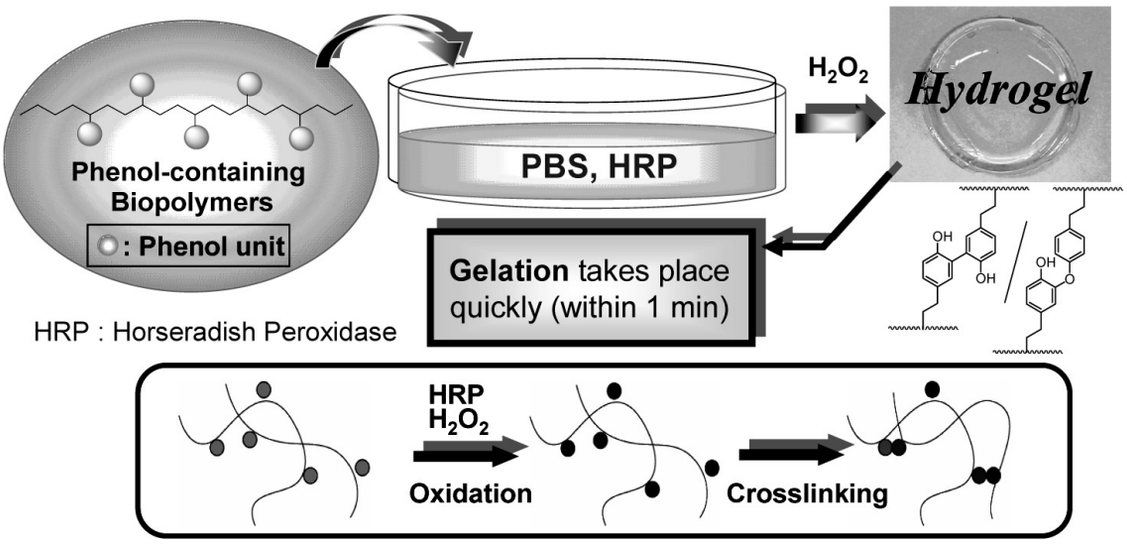


Figure 2. Enzymatic synthesis of biopolymer hydrogels.

frequency range. Moreover, only slight frequency dependence was observed in *G*' and *G*''. These data clearly indicate the homogeneous structure of the hydrogel. The effect of the enzyme amount on the elastic modulus of the hydrogel has been examined. Even a small amount of the enzyme gave the hydrogel quantitatively. The enzyme amount greatly affected the gel elasticity; the G' value increased as a function of the enzyme amount. These data strongly indicate that the elastic modulus could be controlled by the reaction parameters and the present enzymatic method affords the PGA hydrogels with a wide range of the elastic properties, which is significantly important for biomedical applications.

Furthermore, we have synthesized chemically PGA hydrogels by the reaction of PGA with diamins or polyamines as crosslinking agent. The gel properties were greatly dependent upon the amount of the crosslinking agent and curing conditions.

References

1) M.-H. Sung *et al.*, *Chem. Record*, **5**, 352 (2005).

Keywords: Poly(γ-Glutamic Acid), Conjugate, Hydrogel, Vitamin C, Cholesterol

S7-1

Omics Approach in Biology: Interactomics

Jong BHAK

KOBIC, KRIBB

The background and scope of protein interactomics are discussed. Interactomics is the study of molecular interactions in a holistic paradigm complementing the trend of reductionism in biology. It focuses on the action and interaction (and misinteraction) among information objects in biological cells. In other words, biological interaction networks are composed of the interaction pairs of information objects, having sequence order information. The fundamental unit of evolution is an interacting pair of objects. Interfaceomics is a study of all the molecular interfaces in living cells. It maps interfaces and their geometrical and chemical properties. Recent advances in the research is presented. Omics is a new paradigm of doing biological research. We can map most of biological research fields into various -omics such as genomics, proteomics and interactomics. An theoretical example of using omics for plant genome is presented.

Keywords: omics, interactomics, interface, bioinformatics

S7-2

Genetic Insight into Microbial Degradation of Aromatic Compounds

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Recently microbiological studies cope with the difficulties in understanding the biosphere and catabolic process due to the recovery of less than 1% of microorganisms from nature with the pure culture method and diversity of metabolic pathways in microorganisms. New biotechnological study, therefore, has been adapted to investigate the genetic information from environments directly or isolated microorganism exploring versatile microbial metabolism and recruiting a gene of interest. In this study, metabolic pathways of aromatic compounds were analyzed from anaerobic consortium and *Comamonas* sp. strain P19 by metagenomic and transposomic approach. An anaerobic consortium enable to degrade 4-chlorobenzoate or 4-bromobenzoate under denitrifying conditions was developed but isolation of a pure degrading strain from the consortium was not successful. A metagenomic cosmid library, thus, was constructed using pWEB::TNC™ cosmid. PCR primer set designed with 4-chlorobenzoyl-CoA dehalogenase genes from the known 4-chlorobenzoate degrading bacteria successfully amplified the product from the metagenome. Using the PCR approach two out of 1,440 library were found as recombinant cosmids harboring overlapped DNA fragments. One of the cosmid clones showing dehalogenation activity in whole cell assay was completely sequenced to identify the corresponding genes. A gene cluster encoding a CoA ligase, a hydrolytic dehalogenase, and a transport protein was identified. Interestingly, unlike similar gene clusters reported in aerobes, a gene encoding 4-hydroxybenzoyl-CoA thioesterase was not present. This makes metabolic sense since 4-hydroxybenzoate is degraded via a CoA intermediate without thioester hydrolysis in many denitrifying organisms. On the other hand, strain P19 was isolated as a biphenyl-degrader which was very close to *Comamonas testosteroni* on the basis of 16S analysis. We investigated the metabolic pathways of benzoate, anthranilate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, phthalate, and terephthalate in this strain by plasposon mutagenesis which was useful for high-throughput screening approach. *m*-Hydroxybenzoate, *p*-hydroxybenzoate, phthalate, and terephthalate were metabolized by corresponding oxygenases via protocatechuate which was known as a key metabolite in aerobic degradation of several aromatic compounds and further degraded by protocatechuate dioxygenase system in P19 strain. Whereas the degradation of aromatic compounds mentioned above proceeded by well-known pathway, benzoate and anthranilate were interestingly catabolized through new aerobic pathways mediated by CoA ligation which were discovered recently in *Azoarcus evansii*. Especially, two mutant strains incapable of growing on 5mM anthranilate had mutations on genes encoding isocitrate lyase and malate synthase, respectively. This indicates that anthranilate is completely metabolized through glyoxylate shunt pathway instead of TCA cycle in P19 strain since the proteins are essential in the bypass. Consequently, metagenomic and transposomic study suggested the metabolic insight into degradative pathway of aromatic compounds in the denitrifying enrichment and *Comamonas* sp. strain P19.

Keywords: Aromatic compound, Comamonas sp. strain P19, degradation, metagenome, transposon mutagenesis

S7-3

Genome Based Systematic Approaches for Development of Succinic Acid Producing *Zymomonas mobilis* ZM4

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In order to secure the forthcoming era of bio-ethanol and green chemicals, the top-value added chemicals have been focused on the microbial overproduction using renewable resources. Ethanologenic bacterium, *Zymomonas mobilis* ZM4 genome sequence and its metabolic pathways have been elucidated to attempt to develop the rational strain development. From the global transcriptome analysis, the central metabolic circuits were constructed systematically, and also uncoupled metabolism between catabolism and energy production were analyzed during ethanol production. Based on this distinct feature, succinic acid overproducing *Z. mobilis* strains have been developed by disruption of two genes for pyruvate decarboxylase (pdc) and lactate dehydrogenase (ldh). It shows high yields of succinic acid production at close to meet the industrial production (over 2.5 g/l/h productivity) under Na-bicarbonate and hydrogen gas. These characteristic higher yields and production rates of recombinant *Z. mobilis* strains make the mass production of succinic acid from renewable biomass possible which is much economic comparing chemical synthesis of succinic acid.

Keywords: *Zymomonas mobilis*, microarray, metabolic circuit, succinic acid, pyruvate decarboxylase, lactate dehydrogenase

S7-4

Protein Expression Profiles of Curdlan-Producing *Agrobacterium* sp. under pH-Controlled Batch Fermentation

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During the last decades, several exoplysaccharides-producing bacteria and yeasts have gained increasing interest, both for fundamental research and biotechnological application. Curdlan is a neutral, essentially linear, a water-insoluble biopolysaccharide, composed exclusively of β-(1→3)-linked glucose, and is synthesized by *Agrobacterium* species under nitrogen-limiting conditions. Unique rheological and thermal gelling properties of curdlan have been extensively exploited and could be advantageously applied in industries as diverse as admixtures of concrete, edible fibers, immobilizing supports, and new calorie-reduced products. In recent years, curdlan has also been attractively applied as a drug delivery polymer to sustain drug and control drug diffusion and curdlan sulfate was developed as antiviral agent for the inhibition of human immunodeficiency virus (HIV)-1 infection in pharmaceutical purposes. To increase the economic attractiveness of producing curdlan, it is important to increase productivity and minimize its production costs to allow curdlan to compete with other polysaccharides. Many research efforts were mostly focused on the optimization of several key factors (i.e. Temperature, pH, nutrients, agitation, and aeration) involved in the curdlan fermentation process for increasing the yield/productivity of curdlan biosynthesis. Furthermore, change of intracellular nucleotide levels and their stimulatory effects on metabolic flux of curdlan synthesis in *Agrobacterium* species were investigated under nitrogen-limited and sufficient culture conditions. Though previous studies have been focused extensively on cloning of the genes related to curdlan synthesis metabolism in *Agrobaterium* sp strain ATCC 31749 and expressed in heterologous hosts, those molecular studies have uncovered entirely new characteristics important for the development of industrial strains. With the completion of *Agrobacterium tumefaciens* genome sequencing, a new challenge has been launched by many projects whose goal is to enhance understanding of cellular physiology at the global level and thus to accelerate strain and metabolic pathway improvement in *Agrobacterium* strains-based bioprocesses. In the present study, the overall protein expression pattern of a curdlan-producing *Agrobacterium* sp. was investigated during pH-controlled batch fermentation, and demonstrated in detail on the synthesis level and its time-course change pattern of many intracellular proteins through comparative analysis of proteomic responses to growth condition tuning by pH shift. Also, comparative proteome analysis showed that the curdlan overproduction resulted from the significant change in the synthesis of important metabolic enzymes involved in curdlan biosynthesis. As for some selected proteins, peptide masses analyzed by automated MALDI peptide mass spectrometry were matched with the theoretical peptide masses of *Agrobacterium tumefaciens* database, and then functional characteristics in the cellular metabolisms have been elucidated.

Keywords: Proteomic responses, *Agrobacterium* sp., Curdlan production, Comparative analysis

S7-5

Complete Sequence and Comparative Genome Analysis of *Leuconostoc citreum*, a Key Player in Kimchi Fermentation

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There is a growing interest in lactic acid bacteria (LAB) that are commonly found in fermentation environments. As well as their importance in the food industry, they are recognized as probiotic bacteria that are beneficial to human health. Based on the population study using the PCR-denaturaing gradient gel electrophoresis technique (*Int J Food Microbiol* 102:143, 2005), *Leuconostoc citreum* is a lactic acid bacterium predominant in kimchi during the fermentation process. We sequenced the genome of *L. citreum* KM20, which is capable of inhibiting the growth of several food-borne pathogens and other harmful bacteria. The complete genome is composed of one circular chromosome (1,796,284 bp, 39.0% G+C) and four plasmids of 100,330 bp. Among the 1,826 putative protein-coding genes, functions of 1,621 (88.8%) could be assigned. No complete prophages were found, but several phage-related genes were identified including one site-specific recombinase. The complete genome also contained five complete copies of IS*3*-family insertion sequences and five derivatives of IS*30*-family elements. Average read depth of the contig sequences suggests that three plasmids are high-copy; presence of genes encoding the Mob protein coincides with this observation. Genome analysis revealed complete gene

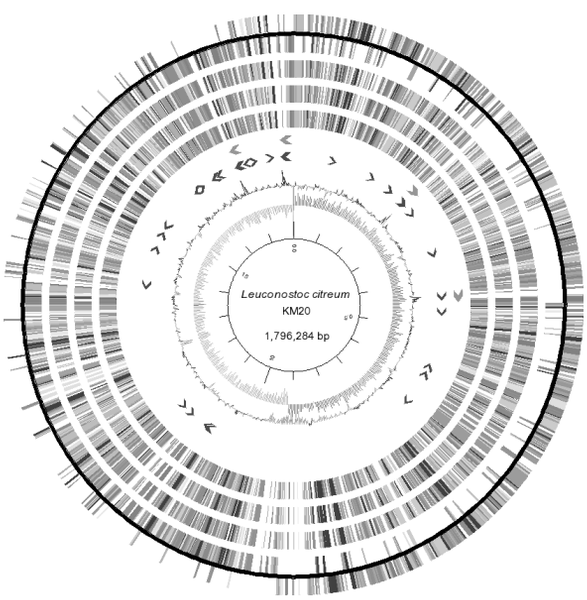

Figure 1. Circular representation of the chromosome of *Leuconostoc citreum* KM20. From outermost, circles 1 and 2, protein-coding genes transcribed clockwise and counterclockwise, respectively. Genes are color-coded according to the COG functional classification. Circles 3 to 5, the locations of homologs in *Leuconostoc mesenteroides* subsp. *mesenteroides* ATCC 8239 (draft), *Lactobacillus lactis* subsp. *lactis* I11403, and *Lactobacillus sakei* 23K. Circles 6 to 9, rRNA genes, tRNA genes, %G+C plot, and cumulative GC skew plot

set for heterolactic fermentation via phosphoketolase pathway with an incomplete tricarboxylic acid cycle, and their limited biosynthetic capacity for various amino acids and cofactors. Analysis of codon adaptation index based on ribosomal protein references indicates genes for phosphoketolase pathway and, in addition to well-known housekeeping genes such as transcription/translation processing factor genes or chaperone/degradation-related genes, some phosphotransferase systems are potential highly expressed genes. A plasmid-encoded, putative cell wall-anchored protein with five mucin-binding domains suggests that *L. citreum* may colonize on the surfaces of the gastrointestinal tract that are reported to be the sites of major probiotic effects in many *Lactobacillus* species via immune modulation. Various aspects of comparative genome analysis between *L. citreum* and eight completely sequenced lactic acid bacteria including draft sequence of *L. mesenteroides* will be discussed. This work would provide scientific insights into the probiotic effects and new biotechnological applications of traditional fermented foods. [This work was supported by the 21C Frontier Microbial Genomics and Applications Center Program, Ministry of Science and Technology, Republic of Korea.]

Keywords: lactic acid bacteria, probiotic, genome sequencing, kimchi

S8-1

The Avian Influenza Virus Gene Pool from Domestic and Aquatic Birds in Korea and Assessment of Their Pathogenic Potentials

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Background: Pandemic influenza is a zoonotic disease caused by the transfer of influenza A viruses or virus gene segments from aquatic bird reservoirs to humans and domestic animals. In wild aquatic birds – the natural hosts of influenza viruses – these viruses are in evolutionary stasis. After being transferred to other species influenza viruses evolve rapidly. The recent cases of human infection with H5N1, H9N2, and H7N7 avian influenza viruses demonstrate the continuing threat posed by influenza viruses that emerge from their natural reservoir in birds. On December 2003, South Korea reported an outbreak of the disease among chickens and ducks which was the first official report of disease for this outbreak of H5N1 and the first HPAI outbreak in South Korea's history. Although there were no more HPAI outbreaks in South Korea, there was an outbreak in North Korea on March, 2005. The role of migrating birds in the spread of H5N1 and exchange of viruses between domestic and wild birds in Asia is of great concern. Therefore, we investigated the avian gene pool of domestic poultry and migrating birds in South Korea. We conducted routine avian influenza virus surveillance of poultry farms and migrating birds in Korea. Most of the viruses used in this study were isolated by Korean National Veterinary Research & Quarantine Service (NVRQS) and Chungbuk National University by inoculating the specimens in embryonated eggs during the years 2002 - 2005.

Virus isolation and selection: Influenza viruses were isolated from 20 (2.1%) of 958 specimens tested in poultry farms. Sixty three avian influenza viruses were isolated in embryonated eggs from 5,876 fecal specimens of migrating birds. Viruses representative of the HA subtypes 3, 4, 5, 6, 7, and 9 were isolated. H6 HA type was the most predominant virus of migrating birds in Korea (41.3%, 26 isolates). Although H3, H4, H6 were common but they did not cause severe disease in migrating birds and poultry, H5 and H7 viruses were of great concern because of the role of migrating birds in the spread of H5 and exchange of viruses between domestic and wild birds. Therefore, we selected eight H5 and two H7 viruses isolated from migrating birds during this study and conducted genetic analysis.

Genetic and phylogenetic analysis: We found at least 2 subtypes of avian influenza viruses (H9N2 and H9N8) in chicken farms and reassortements events between H9N2 chicken viruses and influenza viruses of migrating birds. Diverse subtypes and gene pool were detected from migrating birds, but HPAI H5N1 was not detected from 2004 to 2005. To determine what proportion of the H5 and H7 viruses isolated in Korea had this trait, we compared their HA amino acid sequences (deduced from the HA gene's nucleotide sequences) with those of representative strains. None of the H5 viruses had dibasic cleavage site peptides in HA genes in contrast to A/duck/Korea/ESD1/03 H5N1 (data not shown). Phylogenetic analysis of HA genes revealed that the H5 viruses of migrating birds in Korea clustered separately with highly pathogenic HA genes of H5N1 viruses. Although we did not conduct animal experiments, genetic and phylogenetic results suggested that the H5 viruses isolated from migrating birds in Korea were not highly pathogenic viruses. Two H7 viruses were identified as H7N3 and H7N7 subtypes and had no dibasic cleavage site

peptides in the HA gene (P-G-R-G). Phylogenetic analysis of HA genes of H7 viruses showed that the H7 viruses of migrating birds in Korea clustered with Eurasian H7 virus lineage (Chicken/Pakistan/447/95 H7N3) viruses and separated with the American lineage (Chicken/Chile/4322/02 H7N3).

This report describes the gene pool of avian influenza viruses circulating in South Korea and demonstrates their continual evolution and their reassortment. These gene pool data and isolates can be used for the preparation of vaccine strain library that has all subtype combinations of aquatic bird derived hemagglutinin and neuraminidase in laboratory.

Keywords: Avian influenza virus, migrating birds, pandemic, Korea

S8-2

Current Understanding of Emerging Flaviviruses: from Basic Research to Vaccine Development

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The genus *Flavivirus* belongs to the *Flaviviridae* family, which contains two other genera, *Pestivirus* and *Hepacivirus*. As principal vectors for transmission, viruses in the genus *Flavivirus* are phylogenetically divided into three groups, specifically, mosquito-borne, tick-borne, and those with no known arthropod vector. Mosquito-borne flaviviruses include Japanese encephalitis virus (JEV), dengue virus (DV), yellow fever virus (YFV), West Nile virus (WNV), Kunjin virus (KUN), and Murray Valley encephalitis virus. All flaviviuses are spherical and enveloped with a diameter of 40-60 nm, and contains an electron dense core. The genome is composed of single-stranded positive-sense RNA ≈11,000 nucleotides in length, which is capped at the 5' end and unpolyadenylated at the 3' end. The genome contains a single long open reading frame (ORF) flanked by *cis*-acting nontranslated regions (NTRs) for viral replication/transcription/translation at both the 5' and 3' ends, which form highly conserved secondary and tertiary structures. The polyprotein is co- or post-translationally processed into mature proteins by cellular and viral proteases. The infectious virion is assembled by encapsidating genomic RNA into the core shell of capsid (C) proteins, which is, in turn, enveloped by two viral glycoproteins, premembrane (prM that is further processed into pr and M proteins) and envelope (E), embedded in the host-derived lipid membrane. The RNA genome replicates in the cytoplasm. This process is mediated by a complex of viral replicases, including NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5.

Mosquito-borne flaviviruses provide some of the most important examples of emerging and re-emerging diseases of global significance. Considerable information has been learned about flavivirus biology from comparison among several closely related flaviviruses. Recently, there has been substantial progress in understanding of transmission, epidemiology, and geographic distribution of these viruses and manifestations of disease produced by the infection. Recent development of an efficient reverse genetics system for most flaviviruses is enabling advancement of direct molecular genetic studies, previously hampered by the difficulty of manipulating the viral genome. Nevertheless, much more needs to be elucidated and demonstrated experimentally for better understanding of flavivirus biology. This talk aim to provide an update on knowledge of flavivirus biology that can be used to highlight the advances in the field during the past few years and help to define the questions that academic, industrial, and public-health communities must address in development of measures to control flavivirus-related diseases.

Keywords: Flaviviruses, Emerging and Re-emerging viruses, RNA viruses, RNA replication, Vaccine development

S8-3

Molecular Epidemiology and Immune Response of Noroviruses

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Norovirus is known as the leading cause of etiological agent causing more than 90% of nonbacterial gastroenteritis worldwide. The objectives of this study were to investigate the role of norovirus in causing traveler's diarrhea and to understand the immune response related to norovirus infection. Stool specimens from 124 U.S. students with acute diarrhea acquired during a short-term stay in Mexico during the summer of 2004 were studied for the presence of enteropathogens including noroviruses (NoVs) by nucleic acid extraction and subsequent RT-PCR assay. NoVs were the second most commonly identified enteric pathogen in diarrheal stool samples, found in 21/124 (17%) exceeded only by enterotoxigenic *Escherichia coli* (ETEC), identified in 50 of 106 (47%). NoV genogroup I (GI), identified in 18 of 21 (86%) of NoV positive samples, was more prevalent than NoV genogroup II (GII) which was detected in 4 of 21(19%). NoV infection was identified with a second co-infecting organism in 15 of 21 (71%) of the NoV positive patients with the common co-infecting organisms being ETEC or enteroaggregative *E. coli* (EAEC). Detection of NoV in the stool specimens was associated with clinical symptoms of vomiting ($P < 0.01$) and nausea ($P < 0.01$) supporting an etiologic role for these viruses. Both fecal cytokines and fecal lactoferrin were measured for NoV-associated diarrhea, mixed infection of NoV and enterotoxigenic *E. coli* (ETEC)-associated diarrhea and in pathogen-negative diarrhea cases. Both IL-2 and IFN-γ were significantly increased in NoV-associated diarrhea specimens, suggesting a predominant Th1 immune response to NoV infection in the gut. When a mixed infection of NoV and ETEC occurred, a combined Th1/Th2 response was observed suggesting a dual immune response secondary to infection by both pathogens. Intestinal inflammation associated with increased fecal lactoferrin, important in bacterial enteric infection, was not found in NoV-associated gastroenteritis. This study indicates that NoV is an underappreciated cause of travelers' diarrhea helping to explain failures of antibacterial drug therapy or chemoprophylaxis in this setting.

Keywords: Norovirus, traveler's diarrhea, Th1 immune response, gastroenteritis. foodborne disease

S8-4

Limited Genetic Variation of HIV-1 Korean Clade B Transmitted in Korea

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The rapid evolution of HIV is the result of an explosive combination of factors-a high rate of mutation, a remarkable replication dynamics and a frequent recombination and natural selection. Previous studies have described on Korean HIV-1 subtype B strain characterized a distinct clade.

To understand the evolution of distinctive Korean HIV-1 clade transmitted in Korea, we investigated the characteristics of the genetic variation of HIV-1 subtype B *env* gene in a group of MSM(men who have sex with men) for a ten years and transmission pairs.

We selected 197 HIV-1 subtype B infected MSM who were infected by Korean for a 10 year period (1996-2005) and 6 pairs of patients infected HIV-1 by sexual contact. The nucleotide sequences of HIV-1 *env* gene from target population were sequenced. The evolution of HIV-1 *env* gene was analyzed through its diversity and divergence from ancestor sequences to be likely by year of virus isolation.

The genetic expansion of diversity and divergence of HIV-1 infected MSM for a 10-year period were 2.1% and 2.5% respectively. We could not find statistically significant increase in the diversity and divergence of HIV-1 *env* gene. These results were much lower than those of other countries. The prevalent HIV in this group was very closely related and the evolution was not well progressed. In the *env* gene of pairs of MSM and hetersexual contacts, the mean numbers of amino acids and glycosylation sites did not show any significant change between sources and recipients. The CD4 binding sites and the region most associated with antigenecity were all consistently preserved between source and recipients. Our study on six infected pairs of Korean HIV subtype B has shown relatively low genetic variation of HIV-1 *env* gene during the transmission. This result would be helpful in understanding the evolution of currently distinct HIV-1 Korean clade B but further study on the immunological factors involved in transmission is needed.

Keywords: HIV-1, *env* gene, genetic variation, evolution, MSM, transmission pairs

S9-1

Lichen-Derived Culture and Its Application

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Methods of lichen-derived culture were spore- and thallus-derived cultures. Spore-derived culture was modified and thallus-derived culture was established for our two decades. We maintained about 500 lichen-derived cultures by using both methods. In recent several years, we have investigated to screen various pharmacological activities in lichen-derived cultures for the first time and found some activities in our cultures.

Introduction

Lichens are super organisms composed of fungal (mycobiont) and algal (photobiont) partners and have been used as medicines, dyes, perfumes, foods and drink stuffs since ancient times all over the world. However, mass harvesting of lichens as an industrial resource may lead to extinction of species. Therefore, if lichens are to be used in industrial applications, they must be cultured *in vitro*. In our laboratory, we succeeded in isolating, culturing and maintaining mycobionts as well as photobionts of about 500 cultures of lichens. We believe that lichens have an original potentiality for novel biological activities. Therefore, we have been screening cultures derived from lichens for several kinds of biological activities.

Materials & Method

Spore-derived culture established by Ahmadjian was modified by us and its procedure was described in the previous paper (Yamamoto *et a*l. 1998). Thallus-derived culture method for using thallus fragments, the lichen tissue culture method ("Yamamoto method") was described in previous paper (Yamamoto *et a*l. 2001). Extracts of randomly selected lichen-derived cultures were prepared for tests of several pharmacological activities. For screening tests of growth inhibition (bacteria and fungi), enzyme inhibition (tyrosinase and monoamine oxidase, MAO), virus activation inhibition and anti-oxidant were carried out (Yamamoto et al. 1993).

Results and Discussion

Lichen-derived cultures. The effects of various new conditions on discharge and germination of ascospores were investigated. The spore culture method has several disadvantages. When we started to develop this method, we were concerned that our cultured thallus fragments would be contaminated. According to Ahmadjian's comment in the book "The Lichens", thallus-derived cultures are always contaminated by microorganisms present in the thallus. We found that we could reduce this problem by using carefully selected, very small thallus fragments.

Pharmacological Activities in Lichen-derived Cultures. The extract of a *Haematomma puniceum* mycobiont showed the highest activity of growth inhibition of many bacteria among 36 tested mycobionts. *Thamnolia vermicularis* mycobiont inhibited the plant-diseased fungal growth. Eight mycobionts, *Acarospora fuscata*, *Arthonia cinnabarina*, *Cladia aggregata*, *Dibaeis absoluta*, *Haematomma puniceum*, *Ramalina exilis*,

Stereocaulon sorediiferum and *Xanthoria elegans*, inhibited the growth of wood decaying fungi. We screened inhibitory activity of tyrosinase in tissue cultures of lichens. The extract of *Hypogymnia physodes* tissue culture showed a highest inhibition activity (50 %) among them. Screening test on MAO inhibition was performed for the extracts of 26 species of cultured mycobionts. The extract of a cultured mycobiont of *Graphis scripta* showed the strong inhibition. The *Nephromopsis ornata* culture showed the highest activity of EVB activation inhibition. The extract of *Bryoria furcellata* culture showed high scavenging activity for superoxide anion. The *Graphis awaensis* and *Cladonia bellidiflora* cultures showed higher scavenging activity for DPPH radical than those of catechin and tocopherol.

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Keywords: lichen-derived cultures, screening of biological activities

S9-2

The Lichen Flora of Oases of Continental Antarctic, and the Ecological Adaptations of Antarctic Lichens

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Author have studies lichen flora of the most important ice-free areas of Continental Antarctic: Bunger Hills, and the vicinity of Prudz Bay (Larsemann Hills, and Radok Lake in Prince Charles Mountains).

Totally 44 lichen species from 22 genera were reported for Bunger Hills and 50 lichen species from 22 genera and 10 families: *Acarosporaceae, Lecanoraceae, Lecideaceae, Parmeliaceae, Pertusariaceae, Physciaceae, Rhizocarpaceae, Stereocaulaceae, Theloschistaceae*, and *Umbilicariaceae* were reported for the Prudz Bay Region. 20 lichen species were found in the region for the first time.

Phytogeographic analysis indicated a relatively high proportion of species with bipolar distribution – about 50% of recorded lichen species. About 30% of lichens normally don't extend into maritime zone occurring in continental Antarctic only. The most common lichen families in the region are *Buelliaceae, Lecanoraceae* and *Teloschistaceae*.

The water supply and not a temperature is the critical factor for lichens in the Continental Antarctic. Moisture appears to be supplied for lichens not only from snow-melt water but mainly from air. In Maritime Antarctic, due to high air humidity macrolichens form communities everywhere (*Himantormia, Usnea* and *Umbilicaria)*. In oases of Continental Antarctic extensive sites are lacking in lichen cover, even if the ground is normally snow free. Lichens occur at humid sites with moisture which were brought by winds over the ice cap and poorly developed or absent in dry areas.

Of particular significance for lichens are substrate characteristics, animals influence and salinity brought by wind in coastal areas. Most rich lichen vegetation developed in oases around nests of snow petrels, where the melt water is enriched by nutrients. In contrast, the most pure vegetation is on mobile sand and gravel and in salted coastal habitats.

Short introduction

Lichens are the major component of Antarctic and Subantarctic terrestrial flora (380 species) among vascular plants (66 sp.) and mosses (104 sp.). They are found luxuriantly growing on rocks, boulders, moraine and decaying cushions of moss tufts in ice free area. Because of their high degree of adaptation to the harsh climatic condition they are most interesting group of organisms, both for taxonomic and ecological studies. Antarctic lichens are being studied since 1830. Previously much of the work has been carried out in Antarctic Peninsula, than the East Antarctic region. Last decades the author have studied the lichen flora and vegetation of some very important areas in Continental Antarctic: Bunger Hills in eastern part of the Queen Mary Land, and in the region of Prudz Bay and Prince Charles Mountains (Larsemann Hills and the vicinity of Radok Lake).

Material and methods

The collection of lichens and the determination were carried out during Antarctic summers from 1988-1989 to 2004-2005. Research has concerned the main part of the oases. Specimens were collected with the purpose to reflect the whole diversity of conditions in habitats and the whole specific diversity of lichens. More than 2000 specimens of lichens were collected in different localities at different attitude, coast, mountains, slopes and at different exploitation, chosen in the most typical habitats and areas with the richest vegetation. During brief visits some other small rocks, areas and oases were skin-deep studied. Collected specimens are kept now in Lichen Herbarium of the Komarov Botanical Institute (LE) of the Russian Academy of Sciences in St. Petersburg (Russia).

Results and discussion

Cryptogams – lichens, algae, mosses, and fungi are the major components of the flora and vegetation of Polar regions. They are growing on rocks, boulders, moraine and on cushions of moss tufts in ice free area. Because of their high degree of adaptation to the harsh climatic condition they are most interesting group of organisms, both for taxonomic and for ecological studies.

Lichens are very tolerant to the environmental condition. They occur almost everywhere, from the northernmost part of Greenland to the latitude 86º in Antarctic.

The lichen flora of Antarctic, according to first fundamental work of C. Dodge consisted of 424 species, nearly 50% of which were described by C. Dodge himself as endemic species. The modern revisions of separate taxonomic groups of Antarctic lichens reduce the number of species in groups sometimes at once on 80 %. H. Hertel (Hertel, 1984) estimated the number of Antarctic lichens not more than 160 species, and about half of them has bipolar or wide distribution. Now the most important publication of D. Øvstedal and R. Levis-Smith (2001) includes 380 species. The whole lichen flora of Antarctic including Subantarctic islands could be estimated as not more then 400-500 lichen species.

The largest specific diversity is known in Subantarctic regions and in Maritime Antarctic. Number of species, known in Continental Antarctic is insignificant. It amounts about 25 % of the whole Antarctic lichen flora (about 100-120 species). The composition of species is almost identical for different and distant regions.

The most sensitive tool to estimate and to compare the biodiversity of regions is the local flora – rather good investigated flora of an area near 100 square km, which includes all possible habitats of the region. The comparison of local flora can display the peculiarities and tendencies of the whole regional flora. The local lichen flora of the rather big and most investigated areas of the Continental Antarctic (Bunger Hills, Radok Lake, Larsemann Hills and Syova Station) counts near 50 species each. The flora of single nunataks and small coast oases and islands counts even less – about 20 species.

Totally 44 lichen species from 22 genera were reported from Bunger Hills. The analysis indicated a relatively high (50%) proportion of species with bipolar distribution. 32% of lichens normally don't extend into maritime zone occurring in Continental Antarctic only. The most common lichen families in Bunger Hills are *Buelliaceae, Lecanoraceae* and *Teloschistaceae*.

The lichen flora of the vicinity of Radok Lak in the region of Prince Charles Mountains was first studied in 2004. 27 lichen species were discovered for the first time. Hilly, but in general rolling plain territory located 270 km away from the coastline of Prudz Bay near Amery Ice Shelf (70°48'S., alt. 0–350 m) has no permanent ice and snow cover.

The lichen flora of the Radok Lake Area is one of the richest in the Prudz Bay Region. 85% of the found lichens are crustose, 90% – saxicolous. About 90% of taxa have almost equally bipolar or Antarctic distribution. *Acarospora macrocyclos, Amandinea petermannii, Carbonea aggregantula, Lecanora mons-nivis, Lecidella wulfenii, Ochrolechia frigida* and *Stereocaulon antarcticum* previously were known from the area of the Antarctic Peninsula only.

Some specimens of *Pleopsidium chlorophanum, Rhizoplaca melanophthalma* and *Carbonea vorticosa* were obtained from three nunataks located on Lambert glacier even more southern: Shaw Massif (72°01'34"S., alt. 900-1333 m), Ely Ntk. (72°07'43"S., alt. 800 m), Mt. Izabelle (72°11'44"S., alt. 800-1164 m). They are the most southern collections in this sector of Antarctic.

As a whole the lichen flora of the Prudz Bay region numbers now 50 lichen species from 22 genera and 10 families: *Acarosporaceae, Lecanoraceae, Lecideaceae, Parmeliaceae, Pertusariaceae, Physciaceae, Rhizocarpaceae, Stereocaulaceae, Theloschistaceae*, and *Umbilicariaceae*. 20 lichen species were found in the region for the first time. The list of species is not considered complete, as far as some taxa need a further study.

Obviously humidity, slope exposition and degree of protection of habitats from prevailing winds in connection with the opportunity of the snow accumulation are major factors in conditions of the dry continental climate, influencing distribution of lichens on the territory of continental oases.

Strong southern winds blow off small amount of the winter snow and the summer precipitation are almost absent. Snow accumulates only in wind shadow, where the wind abrasive processes absent, evaporation of a moisture decreases and humidity of ground raises. Besides northern slopes are warmer. Salinity, especially in seaside part of the oases, character of destruction of rocks or ground stability, and abundance or lack of nitrogen are critical for the vegetation.

The colonization of primary substrates by lichens depends on their stability, and is possible only with sufficient amount of moisture. Mobile substrates - sands and gravel are normally uninhabited or covered by young specimens, because of low growth rate of lichens. The character of vegetation there remind the sites, located near glacier, where the number of species is insignificant and the vegetative cover is undeveloped because of recent deglaciation.

Elevated territories have richer flora and vegetation because of moisture, enriched with nitrogen and phosphor from snow petrel nests and also by accumulation of snow first of all on elevated sites near a glacier (Seppelt, 1986). Besides foots of hills can be much more cold than tops because of temperature inversions.

In general the water supply and not a temperature is the critical factor for lichens in the Continental Antarctic. Moisture appears to be supplied for lichens not only from snow-melt water but mainly from air. In Maritime Antarctic, due to high air humidity macrolichens form communities everywhere (*Himantormia, Usnea* and *Umbilicaria)*. In oases of Continental Antarctic extensive sites are lacking in lichen cover, even if the ground is normally snow free. Lichens occur at humid sites with moisture which were brought by winds over the ice cap and poorly developed or absent in dry areas. Of particular significance for lichens are substrate characteristics, animals influence and salinity brought by wind in coastal areas. Most rich lichen vegetation developed in oases around nests of snow petrels, where the melt water is enriched by nutrients. In contrast, the most pure vegetation is on mobile sand and gravel and in salted coastal habitats.

The most frequent and common species and main dominants of the vegetative cover in Continental Antarctic are: *Rhizoplaca melanophthalma, Candelariella flava, Lecidea cancriformis, Acarospora gwynii, Buellia frigida, Rinodina olivaceobrunnea, Pseudephebe minuscula, Umbilicaria aprina, U. decussata, Buellia pallida, Physcia caesia, Pleopsidium chlorophanum, Rhizocarpon flavum, Usnea antarctica*. All other lichen species are rather rare and do not play an essential role in formation of the vegetative cover.

The list of studied species is not considered complete, as far as some taxa need a further study.

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Keywords: Bipolar distribution, Coastal areas. Moisture, Prudz Bay, Phytogeography

S9-3

Gβγ-Mediated Signaling Pathway for Growth, Developmental Control and Toxin Biosynthesis in *Aspergillus nidulans*

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In the filamentous fungi, heterotrimeric G proteins play crucial roles in cell growth, asexual and sexual development, and pathogenicity and secondary metabolism. The basic unit of heterotrimeric G protein signaling is comprised of a seven-transmembrane domain G protein-coupled receptor, a heterotrimeric G protein consisting of α, β, and γ subunits, and downstream effector. In the model fungus *Aspergillus nidulans*, vegetative growth signaling is primarily mediated by FadA and SfaD, the α and β subunits, and a presumed Gγ subunit. To further understand heterotrimeric G protein signaling mechanisms in *A. nidulans*, we have identified and characterized the Gγ subunit GpgA and phosducin-like protein (PhLP) PhnA. Phosducin or PhLP is a positive regulator in Gβ function. Genome analyses in *A. nidulans* resulted in identifying a single Gγ subunit and three PhLPs, PhnA, PhnB and PhnC. Similar to Δ*sfaD*, deletion of each *gpgA* and *phnA* caused the restricted vegetative growth, defective sexual fruiting bodies (cleistothecia) in self-fertilization and severe impairment of outcrosses. Deletion of *phnA* resulted in asexual sporulation in liquid submerged culture, suggesting that PhnA is required for Gβ SfaD-mediated asexual development control. SfaD::GpgA (Gβγ) may function as a heterodimer in the growth and sexual development signaling pathways, but each component of heterodimer has somewhat different role in asexual development. Developmental defects caused by deletion of *flbA* encoding RGS (regulator of G protein signaling) protein negatively regulating FadA-mediated growth signaling were suppressed by deletion of *gpgA* and *phnA* respectively indicating that GpgA and PhnA function in FadA-SfaD mediated vegetative growth signaling. However, while FadA represses mycotoxin sterigmatocystin (ST) production, SfaD, GpgA, and PhnA are required for ST production. The Gβ SfaD is necessary for the expression of *aflR* encoding the transcriptional activator for the genes of ST biosynthesis. Over-expression of *aflR* is sufficient to restore ST production based on deletion of *sfaD* implying that SfaD-mediated signaling in ST biosynthesis may include transcriptional activation of *aflR*. Gβγ SfaD::GpgA and a positive regulator PhnA are required for normal vegetative growth, appropriate regulation of asexual sporulation, and the formation of sexual fruiting bodies. The identification of other G protein components and/or downstream effectors transducing SfaD::GpgA signals is critical for further understanding differential roles of G protein components associated with secondary metabolism and other physiological characteristics.

Keywords: *Aspergillus nidulans*, G protein subunits, signal transduction, growth, development, toxin biosynthesis

S9-4

Analysis of Genomic Structure of an Aflatoxin Biosynthesis Homologous Gene Cluster in *Aspergillus oryzae* RIB Strains

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Koji mould, *Aspergillus oryzae* has been widely used in food fermentations, such as sake, miso and soy sauce. Additionally *A. oryzae* has no history of producing aflatoxins or any other carcinogenic metabolites.

To investigate non-aflatoxin-production of *A. oryzae* at the molecular level, an afaltoxin biosynthesis gene homolog cluster of RIB 40 was analyzed. Although most genes in the corresponding cluster exhibited from 97 to 99 % similarity to those of *Aspergillus flavus,* three genes shared 93 % similarity or less. In addition, although slight expression of *aflR*, positive transcriptional regulator gene, was detected in some *A. oryzae* strains having seven aflatoxin bioxynthesis homologous genes, other genes related to aflatoxin production were not detected. RIB strains were mainly divided into group 1, having seven aflatoxin biosynthesis homologous genes (*aflT, nor-1, aflR, norA, avnA*, *verB*, and *vbs*), and group 2, having three homologous (*avnA*, *verB*, and *vbs*). Partial aflatoxin homologous gene cluster of RIB62 from group 2 was sequenced and compared with that of RIB40 from group 1. RIB62 showed a large deletion upstream of *ver-1* with more than half of the aflatoxin homologous gene cluster missing including *aflR*, a positive transcriptional regulatory gene. Adjacent to the deletion of the aflatoxin homologous gene cluster, RIB62 has a unique sequence of about 8kb and a telomere. Southern analysis of *A. oryzae* RIB strains with four kinds of probe derived from the unique sequence of RIB62 showed that all group 2 strains have identical hybridizing signals. Polymerase chain reaction with specific primer set designed to amplify the junction between *ver-1* and the unique sequence of RIB62 resulted in the same size of DNA fragment only from group 2 strains. Based on these results, we developed a useful genetic tool that distinguishes *A. oryzae* group 2 strains from the other groups' strains and propose that it might have differentiated from the ancestral strains due to chromosomal breakage.

Although it is extremely difficult to determine the reason for the non-aflatoxigenity of *A. oryzae* from the analysis of the genomic structure, this study may provide basic molecular information for the profound approaches. In succession, further research on aflR protein activity or other related signal transduction pathway and the deleted aflatoxin biosynthesis gene homolog cluster of group 2 strains together with group 3 strains may help in clarifying mechanism of the cluster deletion and differentiation.

Keywords: Aflatoxin biosynthesis gene cluster, *Aspergillus oryzae*

S10-1

Molecular Characterization of *Brucella abortus* of Human Isolates in Korea

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Brucellosis became a major zoonosis with a dramatic increase in case incidence since it was classified and controlled as type 3 notifiable disease in Communicable Disease Prevention Act in August 2000.

Brucellosis is a severe acute febrile disease caused by bacteria of the genus Brucella. There are eight species of Brucella that are currently recognized based on antigenic variations and host specificity. Among these species *Brucella melitensis,* and *B. suis* are the most pathogenic strains for humans and are classified as category B bioterrorism threat agents.

B. abortus is less virulent, but it nevertheless causes serious social- economic losses in cattle have occurred in Korea. Transmission of brucellosis occurs from ingesting, directly contacting, or inhaling the organism. Exposures most commonly occur by directly contacting, eating contaminated animal products from disease endemic areas.

Isolation of *Brucella spp*. from blood is irrefutable evidence of Brucellosis. Accordingly, the aim of this study investigated biotypes and antimicrobial susceptibilities of *Brucella* isolates from blood specimens suspected as human brucellosis in Korea. Over a period of 3 years (2003 to 2005), 326 cases were diagnosed by the standard tube agglutination (STA) and the enzyme-linked assay (ELISA) as brucellosis, Those blood specimens were cultured for isolation of the pathogen by the automatic blood culture system BACTEC 9050, biochemical tests and antimicrobial susceptibility tests were performed by the disc-diffusion method, according to the recommendations of the guidelines of the National Committee for Clinical laboratory. Genetic characterizations were performed by PCR for the common genes (BCSP 31kDa, OMP 36kDa, 16S rRNA) of *Brucella* spp. and compared with the sequences of *Brucella* spp. We have adopted Multiplex AMOS- PCR method for the species-specific typing of isolates and the vaccine strains specific PCR assay was based on the insertion of the genetic element IS711. The results of the antimicrobial disc-diffusion susceptibility tests showed that all of the 17 isolates were highly susceptible to the used antibiotics for human brucellosis. But RB51 was resistant to rifamphin, TMP-SMX, streptomycin and chephalothin. In genus specific PCR all of the 44 isolates amplified for the 3 common genes. The nucleotide sequence of BCPS 31kDa gene (223bp) of the 44 isolates showed 95-99% of sequence homology with *B. abortus*. In the profile of Multiplex AMOS-PCR, *B. abortus* (biovar 1, 3, 5, 6), *B. melitensis*, *B. canis*, and *B. suis* (biovar 3) showed characteristic patterns into respectively species and four different amplification patterns were observed. In the Multiplex AMOS-PCR, pattern 1(200, 600 and 900bp) was *B. melitensis* (biovar 1, 2), *B. abortus* (biovar 3, 6), pattern 2(200, 600 and 720 bp) was *B. abortus* (biovar 1), pattern 3(600 and 900 bp) was *B. canis*, *B. suis*(biovar 3) and pattern 4(200, 720 and 900 bp) was *B. abortus* ATCC 7705. The 44 isolates were identical patterns as *B. abortus* biovar 1 (patterns 2; 200, 600 and 720 bp). The brucella vaccine strain specific PCR assay was evaluated to determine its accuracy in differentiating *B. abortus* into three categories: *B. abortus* strains (species-specific, 498bp), vaccine strain S19 (S19: *eri* gene, 178bp), and vaccine

strain RB51/parent strain 2308(genetic element IS711, 364bp). RB 51 and 2308 strains were amplified of 498bp, 364bp and 178bp. But all isolates were amplified 498bp and 178bp. Our study demonstrates that all isolates from blood specimens were classified into *B. abortus* biovar 1, and showed different genetic characteristics among the isolates, compare with RB51 vaccine strain. And the 17 isolates were susceptible to doxycycline and rifampin. Further study will be provided an additional molecular tool for use in conformation and characterization of this important human and veterinary pathogen.

Keywords: Human brucellosis, genetic element IS711, Multiplex AMOS-PCR

S10-2

Characterization of Immune Responses to SARS-CoV Spike Induced by DNA Vaccine and/or Recombinant Adenovirus

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The spike protein (S) of severe acute respiratory syndrome (SARS) coronavirus (CoV) is a ~180-kDa glycoprotein and has been shown to be an important component of candidate vaccines. Recently, it has been shown that the neutralization antibody (nAb) to S is more important for the protection of mice against SARS-CoV infection than T-cell immunity.

Although the initial isolates of the SARS-CoV are sensitive to neutralization by antibodies to S, variants of S have been identified that are resistant to such inhibition. Clinical observations in SARS patients imply both humoral and cell-mediated immune responses may be needed to prevent SARS-CoV infection. Apparent depletion of T cells occurred in the early infection and a gradual increase to normal level was observed as the patients recovered. Therefore, to be an effective vaccine for SARS-CoV, both nAb and T-cell immunities are might be important.

Here, two different SARS vaccine strategies will be evaluated for their ability to induce cellular and humoral immune responses to S. For this studies, DNA vaccine and recombinant adenovirus vaccine which encoding S protein were constructed. Self-replicating DNA vaccine vector, pSINCP, which has been known to induce high level of immune responses in in vivo model, is in comparison with conventional DNA vaccine vector. Recombinant adenovirus combined with DNA vaccine is in evaluation in mice model to identify most efficient route of immunization (nasal, sublingual or intramuscular) for induction of optimal immune responses to SARS S protein.

Keywords: SARS Vaccine, DNA vaccine, adenovirus

S10-3

Mucosal Immunization with Surface-Displayed SARS Coronavirus Spike Protein on *Lactobacillus casei* Induces Neutralization Activity in Mice

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Severe acute respiratory syndrome (SARS) recently emerged as a zoonotic infectious disease with high morbidity and mortality. The causative agent, SARS-associated coronavirus (SARS CoV), belongs to Coronaviridae, a family of enveloped viruses containing a single strand RNA genome ranging from 27 to 32 kb in length. SARS CoV seemed to be transmitted through the mucosal areas of the upper respiratory or fecal-oral tracts, leading to pneumonia and enteritis in humans, with a limited occurrence of systemic diseases. Recently, several vaccine strategies have been examined for prevention of SARS infection, including utilization of inactivated virus, adenovirus, DNA, vaccinia virus and recombinant parainfluenza virus. Recent SARS vaccine trials have identified the SARS CoV spike (S) protein as a major target for vaccine trials because S protein mediates virus attachment to cell receptor and induces neutralizing antibodies.

Studies on the development of animal coronavirus vaccines have demonstrated that the systemic humoral or cell-mediated immune responses induced by parenteral administration may be not enough to prevent infection in mucosal areas. The prominent role of the mucosa in SARS transmission and infection suggests that direct mucosal immunization could be an effective strategy for prophylaxis by induction of systemic and mucosal immune responses. For mucosal vaccination purposes, lactic acid bacteria (LAB) are more attractive delivery vehicles because they are considered safe, have adjuvant effects, and show low immunogenicity by themselves, unlike many of the other commonly used live vaccine carriers (e.g., *Salmonella*, *Mycobacterium* and *Vibrio*). Additionally, the extracellulary accessible antigens expressed on the surface of bacteria are better recognized by the immune system than antigens remaining inside.

For the surface expression of the SARS coV S protein on LAB, we have developed a novel surface expression vector using the *pgsA* gene as the display motif. The PgsA is a transmembrane anchor protein derived from γ-PGA synthetase complex (PgsBCA system) of *Bacillus subtilis* chungkookjang. Two segments of the SARS CoV S protein gene SA (residue 2-114) and SB (residue 264-596) were inserted into this vector and displayed on the surface of *L. casei* and detected by immunoblot analysis

The surface localization was further verified by immunofluorescence microscopy and flow cytometry. Oral and nasal inoculations resulted in high levels of serum IgG and mucosal IgA antibodies that recognized S protein in ELISA and neutralized SARS pseudovirus *in vitro*. Orally immunized mice mounted a greater neutralizing antibody response than nasally immunized mice. We further identified three distinct neutralizing epitopes on the S protein (residues 291-308, 512-537, and 564-581) using a peptide neutralization interference assay. Collectively, these results indicate that mucosal vaccination with recombinant *L. casei*-expressing SARS CoV S protein on its surface may provide an effective mean for inducing mucosal and systemic immune response against SARS.

Keywords: SARS, surface-display, Lactic acid bacilli, immunogenicity

S10-4

Evolution of H9N2 Avian Influenza Viruses and Ongoing Active Surveillance Activities in Korea

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Background: Wild birds are considered as a natural reservoir of all of the known subtypes of influenza A viruses. Most subtypes of influenza virus are rarely endemic in poultry and only H1 to H3 subtype of influenza virus have usually infected human and swine. Recently, however, the human cases have been increasing by H5N1, H9N2 and H7N7 subtype of avian influenza virus (AIV), and H9N2 avian influenza (AI) is endemic in poultry especially in Asia since 1990s. In 1996, the first low pathogenic (LP) AI outbreak on Korean chicken farms was caused by H9N2 virus, and then it has been endemic. Therefore, ecology and pathogenicity of H9N2 AIV should be studied for human health as well as animal health. Meanwhile, South Korea had experienced an epidemic of highly pathogenic (HP) AI caused by H5N1 AIV between December 2003 and March 2004. Recently there is potential risk of re-entry of H5N1 HPAI to South Korea because outbreaks of H5N1 still occur in Asia, Middle East, Europe and Africa. Therefore, we introduced ongoing active surveillance conducted by national plan for prevention of H5N1 re-entry.

Evolution of H9N2 AIV: The first outbreak by H9N2 LPAI was reported at turkey farms in Wisconsin , USA in 1966. Since 1990s, many countries of Asia, Middle East, Europe and Africa were suffered by H9N2 AIV in poultry, especially in turkey and chicken. Although H9N2 subtype of AIV was LPAI by OIE criteria, but it could cause severe damage to poultry industry. H9N2 viruses of Asia differ from those of North America, and then Asian H9N2 viruses could be divided into three sublineages. The H9N2 viruses of Korea have close genetic relation to the A/duck/Hong Kong/Y439/97 (sublineage III). Most of the affected cases by Korean H9N2 virus showed typical clinical signs of influenza, such as drop in egg production and less than 30% rate of mortality. To understand the evolutional change of H9N2 viruses of Korea, we analyzed their mutation in Korean chicken farms during the period 1996 through 2004. The H9 viruses of Korea formed two antigenically distinct groups: those isolated from 1996 to mid-2003 and those isolated in late 2003 and 2004. Phylogenetic analysis of all eight genes of Korean viruses revealed three genotypes of H9N2 viruses and showed that reassortment had occurred. For example, one of the isolates, A/CK/Korea/164/04, belonged to the H9N8 subtype. Its HA and PB1 genes were similar to those of the other isolates, H9N2 viruses, but the remainder of its genes were closely related to those of an H3N8 virus from a wild aquatic bird. These results show that H9N2 viruses have been altered through antigenic drift and reassortment.

Ongoing active surveillance: For monitoring of H5N1 HPAI, we investigated wild bird feces extensively in wintering migratory habitat since December 2003. Entire 137 LPAIV have been isolated from 15,745 feces of wild bird (isolation rate: 0.87%). The subtypes of isolate from wild bird were H1 to H12 and N1 to N9 subtype. Most predominant subtype was H6 among viruses that their HA serotype defined (33 isolates in 134 isolates, 24.6%). Although 14 of H5 and 7 of H7 viruses were isolated, they were all LPAIV. We also investigated wild birds in Tadohae national park, in which usually 60% of summer visitor migratory bird pass through. None of AIV was isolated from summer visitor. On the other hand, one H5N2 subtype of LPAIV,

A/DK/Korea/GJ54/04 (H5N2), isolated from commercial duck during active surveillance activities for duck breeder farms. The HA gene of this virus was not related with those of current epizootic H5N1 nor those of H5N2 viruses in Japan, Taiwan and America. It was assumed to be from migratory wild bird.

Keywords: Avian influenza virus, H9N2, H5N1, surveillance, migrating birds

S11-1

Genome-Wide Analysis of Transcriptional Responses to Secretion Stress in the Methylotrophic Yeast *Hansenula polymorpha*

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As a microbial eukaryote, the traditional yeast *Saccharomyces cerevisiae* has been a favorable model to study the genetic control mechanism of protein secretion and modification. The similarity of protein secretion system between yeast and mammalian cells has also made this organism as a useful host for the production of human secretory proteins with pharmaceutical potentials. Noticeably, non-traditional yeasts, such as a methylotrophic yeast *Hansenula polymorpha*, have gained increasing attention as more suitable models for the study of protein secretion and modification processes and as more practical production systems, in that they possess secretion machineries closer to those of mammalian cells, compared to *S. cerevisiae* (1,2). Solid knowledge of the secretory systems at the global level should facilitate successful exploitation of *H. polymorpha* as an intelligent cell factory for the secretory production of correctly folded and processed recombinant proteins.

Secretion stress is caused by compromised folding, modification or transport of proteins in the secretory pathway. Accumulation of misfolded protein in the endoplasmic reticulum (ER) induces a signal transduction pathway called the unfolded protein response (UPR), which brings about multiple cellular protective events to ensure proper protein folding and secretion. A genome-wide analysis of gene expression profiles of UPR is expected to provide comprehensive information on gene function and regulatory networks of the protein secretion and modification. In the present study, we have investigated the global changes of gene expression induced by UPR in *H. polymorpha*, and compared with those found in *S. cerevisiae*. The *H. polymorpha* homolog (*HpHAC1*) of the *S. cerevisiae HAC1* gene, encoding the key UPR transcription factor, was identified and its characteristic non-conventional splicing upon UPR was used to determine the appropriate UPR induction conditions in *H. polymorpha*. After the treatment of DTT and tunicamycin to induce secretion stress by disrupting protein folding and modification, transcriptome analysis was carried out using the *H. polymorpha* whole-genome cDNA microarrays. The *H. polymorpha* genes up-regulated in response to secretion stress included a large number of protein secretion related genes, which can be categorized into secretion (translocation, vesicle transport), processing (folding, disulfide bond exchange, glycosylation), and quality control (ER-associated degradation pathway), as reported in *S. cerevisiae*. Interestingly, change of gene sets unique for each treatment was also observed, implying the differences of UPR induction mechanism between tunicamycin and DTT treatments. Comparison of the gene expression profiles was also carried out between the wild-type strain and the *Hphac1* null mutant strain, in which the *HpHAC1* gene had been deleted. Genes of which the expression levels were significantly changed in the *Hphac1* null mutant strain were categorized as targets of the *HAC1*-mediated signaling pathway in *H. polymorpha*. Our transcriptome analysis data revealed common features and several interesting differences in transcriptional responses to secretion stress between two different yeast species *H. polymorpha* and *S. cerevisiae*.

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Keywords: Secretion Stress, Unfolded Protein Response, Transcriptome, Yeast, *HAC1*

S11-2

Anti-Oxidative Enzymes and Cell Proliferation in Fission Yeast *Schizosaccharomyces pombe*

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In the fission yeast *Schizosaccharomyces pombe*, several anti-oxidative proteins play roles to protect cells against reactive oxygen species from both internal and external sources. These include CuZnSOD in the cytosol, MnSOD in mitochondria, thioredoxins and glutaredoxins in various cell compartments, glutathione peroxidase, and glutathione reductase. Depletion of these could result in auxotrophy, sensitivity toward oxidants, and in some cases no proliferation, depending on the enzymes depleted.

The *pgr1*$^+$ gene encoding glutathione reductase (GR) is essentially required for cell survival unlike in other systems such as budding yeast and many bacteria. Depletion of GR caused proliferation arrest at the G1 phase of the cell cycle in *S. pombe* under aerobic but not under anaerobic conditions. Multi-copy suppressors that restore growth were screened and one effective suppressor was found to be the *trx2*$^+$ gene encoding a mitochondrial thioredoxin. This suggests that GR is critically required for some mitochondrial function(s).

We found that GR resides in both cytosolic and organellar fractions of the cell. Depletion of GR lowered the respiration rate and the activity of oxidation-labile Fe-S enzymes such as mitochondrial aconitase and cytosolic sulfite reductase. Trx2 did not reverse the high GSSG/GSH ratio or the low respiration rate observed in GR-depleted cells. However, it brought the activity of oxidation-labile Fe-S enzymes to a normal level, suggesting that the maintenance of Fe-S enzymes is a critical factor for the survival of *S. pombe*. The activity of succinate dehydrogenase, an oxidation-insensitive Fe-S enzyme, however, was not affected by GR depletion, suggesting that GR is not required for the biogenesis of Fe-S cluster. The total iron content was greatly increased by GR-depletion and was brought to nearly normal level by Trx2. These results indicate that the essentiality of GR in the aerobic growth of *S. pombe* is derived from its role in maintaining oxidation-labile Fe-S enzymes and iron homeostasis.

Keywords: Glutathione, mitochondria, thioredoxin, Fe-S cluster, respiration

S11-3

Analysis of the Transcriptome During Asexual and Sexual Development in *Aspergillus nidulans*

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Although about 75 eukaryotic genomes, including those of Homo sapiens, *Drosophila melanogaster*, *Saccharomyces cerevisiae* and *Neurospora crassa*, have been sequenced during the last decade, the genomes of *A. nidulans* (Galagan et al., 2005), *A. fumigatus* (Nierman et al., 2005) and *A. oryzae* (Machida et al., 2005) have been sequenced only recently. Comparative genomic analysis of the three species revealed that they not only differ considerably in genome size but also display quite lower amino acid identity than expected (Galagan et al., 2005). *A. nidulans* has the intermediate genome size (30 Mb), which is quite much smaller than the *A. oryzae* genome (37 Mb) and bigger than the *A. fumigatus* (28 Mb) genome. Total numbers of genes encoding proteins with a length greater than 100 amino acid residues were predicted to be 9,396 in *A. nidulans*, 12,074 in *A. oryzae* and 9,009 in *A. fumigatus*.

In spite of the extensive efforts and prominent progress in addressing the functions of the genes involved in the fungal differentiation described in the previous papers, most of the genes responsible for the developmental process in *A. nidulans* have not yet been identified. To estimate the relative gene expression levels and changes in gene expression during asexual and sexual development, we analyzed the microarrays throughout a time course from asexual and sexual development using a AnURR (*A. nidulans* Universal Reference RNA; Gadgil et al., 2005) in all hybridization. The array revealed stage-dependent expression of distinct genes set. The most significantly regulated genes ($P < 0.001$) were grouped in five clusters based on their expression profile, respectively. The clusters analyzed using the cluster of orthologous group (COG) classification. Our transcriptional profiling data correlate well with biochemical and physiological processes associated with asexual and sexual development, respectively.

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Keywords: Aspergillus nidulans, Asexual development, Sexual development, Microarray, Transcriptome

S11-4

Siderophore-Iron Uptake in *Fusarium graminearum*: Production of Siderophores and Regulation of Iron Utilization by FgSit1, a Putative Ferrichrome Transporter in *F. graminearum*

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We observed that *F. graminearum* synthesized siderophores, such as ferrichrome (FC) and triacetylfusarinine C (TAFC), and their derivatives. This report investigated *FgSit1* that encodes a putative ferrichrome transporter of *Fusarium graminearum*. The identity of the deduced amino acid sequence of FgSit1 with the amino acid sequence of ScArn1p, an FC-Fe^{3+} transporter of *Saccharomyces cerevisiae,* was 51%; both the growth defect related to the *Δfet3Δarn1-4* strain of *S. cerevisiae* in an iron-depleted condition and the FC-Fe^{3+} uptake activity were recovered upon the introduction of *FgSit1* into the *Δfet3Δarn1-4* strain. Although ScArn1p was found in the late endosomal compartment in *S. cerevisiae*, FgSit1 was found on the plasma membrane in *S. cerevisiae*; when FgSit1 was expressed exogenously in *S. cerevisiae*, it showed greater FC-Fe^{3+} uptake activity than did ScArn1p. FgSit1 did not depend on endocytosis for FC uptake in *S. cerevisiae* and was localized to the plasma membrane in *F. graminearum*. Additionally, in *F. graminearum* FC-Fe^{3+} uptake activity in the *Δfgsit2* strain was found to be one-fourth that of the wild type. However, Fe^{3+} uptake activity in the *Δfgsit2* strain was 5-fold higher than that of wild type; the gene expression of *FgFtr1*, a putative iron transporter, was induced by the deletion of *FgSit1,* but was not induced by the deletion of *FgSit2*. Taken together, these results strongly suggest that *FgSit1* encodes a putative FC-Fe^{3+} transporter that mediates FC-Fe^{3+} uptake using a different mechanism than ScArn1p and plays an important role in the regulation of cellular iron availability in *F. graminearum*.

Keywords: *Saccharomyces cerevisiae*, *Fusarium graminearum*, Iron, Siderophore, Transporter, Desferol, Ferrichrome, Fet3p, FgSit1

S12-1

Emerging and Re-Emerging Animal Viral Diseases

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The impact of emerging and re-emerging viral animal infectious diseases on the global health issues is well recognized. Approximately 75% of all emerging diseases are zoonotic by either an association with animal populations or evolvement of the disease from animal species. The presentation will focus on a general description of the most recent infectious animal diseases, their distribution, involved species, pathogenesis, and preventive measures. Emphasis will be on preventive measures and preparedness.

Animal health researchers and veterinarians in general play a major role in skirmishing the spread of highly contagious animal diseases including their zoonotic element. Until recently the value of the veterinary input in this effort was not recognized by the scientific community or by the public research institutions. Most recently there have been several efforts to recognize these needs in order to understand the ecological aspect and other factors associated with these diseases and to establish reasonable control measures. Although vaccination measures have contributed significantly in reducing the impact of similar diseases in the past, the vaccination option may not be the only valuable solution to some of these emerging diseases. Thus, research avenues for the control measures however require further experimental and observational studies to optimize the control and eradication measures.

Risk seems to be more complex, if not greater in absolute terms. Disruptive and asymmetrical events have lead to threats of several diseases during the last two to three decades. Rabies, Foot and Mouth Disease, West Nile Virus, Avian Influenza, and Hendra viral infection will be used to demonstrate factors associated with the emergence of these diseases.

Public perception and political power are playing major rules in setting priorities for research and regulations to control these diseases. Scientists however have been influenced by the media and public pressure to shift their focus to the popularity of a disease instead of the scientific merits. The focus on Avian Influenza on several continents is a good example of the abuse of scientific findings and the shifting of research areas.

Preventive measures and preparedness plans will be presented with the emphasis on the value of applied and translational research.

Keywords: epidemiology, infectious animal diseases

S12-2

The Epidemiology of Bovine Viral Diarrhea Virus

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Abstract:

Bovine viral diarrhea viruses (BVDV), a group of viruses within the pestivirus genus of the family Flaviviridae, infect cattle worldwide with important economic consequences. These viruses are maintained in cattle by two modes of transmission: 1) vertical transmission from dam to fetus, and 2) horizontal transmission from animal to animal. The vertical transmission of BVDV from the blood of susceptible cows through the placenta to the developing fetus is highly efficient. The consequences of BVDV fetal infections depend on the stage of gestation in which the infection occurs. In the first 90 to 125 days of gestation, BVDV infection can result in early embryonic death and resorption of the conceptus, fetal death, abortion, a variety of congenital defects, and the birth of immunotolerant, persistently infected (PI) calves. Fetuses infected after 125 days may also die *in utero* and subject to abortion, be born as weak, non-viable calves, may be viable but exhibit decreased growth compared to uninfected calves, or may appear normal. The PI calf is the major source of BVDV infection shedding large amounts of infectious virus in urine, feces, milk, oral and respiratory secretions. Exposure of pregnant cows to PI cattle is the key to BVDV's survival in nature.

The epidemiology of BVDV depends on the herd management system as it affects the exposure of susceptible, pregnant female cattle to PI animals. In beef cattle ranches, breeding is synchronous resulting in the exposure of many pregnant cows to a PI calf during the first 4 months of gestation. As a result, epidemics of BVDV-associated reproductive losses occur following the first introduction of a PI animal into a beef herd; whereas, the occurrence of congenital defects is uncommon. In addition, epidemics can generate large numbers of PI calves. In contrast, dairy cows are bred throughout the year. Introduction of a PI animal into a dairy herd results in the infection of cows in all stages of pregnancy. Epidemics of BVDV-associated reproductive losses are uncommon in this scenario, and a wider range of congenital defects is observed in calves. While fewer PI calves are generated at one time, clusters may be born up to 9 months after the last PI is removed.

There are two possible outcomes following the introduction of BVDV into a herd. Either a PI calf is born and survives, and the herd enters an endemic state of infection; or, no PI calf survives and the infection ceases within the herd. In endemically infected herds, a large proportion of females will have been previously infected with BVDV; therefore, they are immune and able to protect their fetuses from infection. In this scenario, the number of PI calves born is low, usually comprising <0.5% of cattle in a herd. However, the larger proportion of calves are born fully susceptible to BVDV infection. Ironically, a high proportion of immune cows in a herd will result in the propagation of susceptible female cohorts which manifests as the recurrence of reproductive disease at 2 to 3 year intervals.

Acute BVDV infections result in peracute death, hemorrhagic disease, classic bovine viral diarrhea, primary viral pneumonia or unapparent infection. The presentation and outcome are determined by the specific strain of BVDV, environmental and nutritional factors, and the age of the animal. The hallmark of BVDV infections is immunosuppression resulting in depletion of lymphoid tissues, and death due to secondary bacterial or viral

infections. The epidemiology of acute BVDV infections is largely influenced by the circumstances in which cattle are exposed to PI animals. Epidemics of BVDV associated respiratory diseases are a common and costly occurrence in feedlots, beef or dairy heifer development facilities, veal barns and dairy calf raising facilities where cattle density is high. The major source of infection of BVDV in these outbreaks is PI cattle and the main risk factor is the mixing of cattle from multiple sources. The role of other sources of BVDV infection including other domestic and wild ruminant species and contaminated biological products should also be investigated. While the epidemiology of BVDV is complex and its manifestations legion, control of BVDV can be achieved by the identification and removal of PI cattle from breeding herds. Control programs based on this principle have been successfully implemented in several European countries at a national level, and on a herd basis in North America.

Keywords: bovine viral diarrhea virus, epidemiology

S12-3

Epidemiology of Ovine Herpesvirus type 2 in Sheep, Cattle, and Bison

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Introduction

Malignant catarrhal fever (MCF) is a severe lymphoproliferative disease affecting several ruminant species including cattle, bison, deer, and elk. It has a worldwide distribution. The disease is caused by a group of gammaherpesviruses collectively referred to as malignant catarrhal fever viruses (MCFV). MCF is observed in both domestic and wild ruminants. There are two etiological forms of MCF that are determined by the host reservoir of the virus. Wildebeest Associated MCF (WA-MCF) is caused by alcelaphine herpesvirus-1 (AlHV-1). The principal reservoir of AlHV-1 is the blue or white-bearded wildebeest (*Connochaetes taurins*). WA-MCF is seen primarily in Africa or in zoological parks where other ruminants have contact with Wildebeest. Sheep associated MCF (SA-MCF) is caused by ovine herpesvirus type 2 (OvHV-2). Domestic and wild sheep and goats are asymptomatic reservoirs of the virus. SA-MCF is the primary form of MCF that is observed outside of Africa.

Cattle and bison are the primary clinical hosts for SA-MCF. Common clinical signs of MCF include persistent fever, lymphnode enlargement, mucosal ulceration, keratitis and corneal opacity, mucopurulent nasal discharge, ocular discharge, diarrhea, and hematuria. In all cases, the disease is characterized by widespread lymphocytic vasculitis. Cases are generally sporadic with a high mortality rate although chronic and recovered cases are observed. There is no known curative treatment or prophylaxis.

Diagnostic Tests

While virus culture techniques exist for AlHV-1, efforts to isolate and grow OvHV-2 in cell culture have been unsuccessful. However, the development of competitive inhibition ELISA (CI-ELISA)[1,2] and polymerase chain reaction (PCR)[3,4] assays have greatly aided the study of OvHV-2 in both asymptomatic and clinically affected hosts. The CI-ELISA test is based on identification of serum antibodies directed against a homologous glycoprotein epitope that is conserved on both AlHV-1 and OvHV-2. This test utilizes a monoclonal antibody, 15-A, that reacts with antigen prepared from a MCFV isolated from a cow in Minnesota. This virus isolate has since been determined to be a variant of AlHV-1. The CI-ELISA test readily detects seroconversion to AlHV-1 or OvHV-2 but can not distinguish between the two. MCF CI-ELISA can be useful for detecting persistently infected asymptomatic carriers in sheep, cattle, and bison. It can also be used to confirm clinical cases of MCF, however, seroconversion is not always present in acute cases of MCF.

PCR is an extremely sensitive and rapid test that will detect AlHV-1 or OvHV-2 infection in blood and tissues. Differentiation between AlHV-1 and OvHV-2 can be obtained by proper selection of primers. The PCR tests readily detect infection in clinical cases of MCF and have a very high sensitivity for confirming clinical infection. However, viral DNA in blood may be below detection limits when screening for asymptomatic infections in cattle and bison.

Epidemology Of Ovhv-2

The prevalence of OvHV-2 infection determined by PCR in healthy adult North American domestic sheep has been reported as high as 99%.[5] This value correlates very well with the seroprevalence in the same population (94%). Positive CI-ELISA tests have been observed in bison (2-23%), domestic goats (74%), elk (9%), mule deer (2%), white-tailed dear (3%), pronghorn antelope (25%), bighorn sheep (37%), muskox (40%), and mouflon sheep (62). Black-tailed deer, llama, and mountain goats have been tested and were not found to be seropositive for MCF.[6,7]

The transmission of OvHV-2 in lambs has been studied.[8] The prevalence of viral infection in pre-suckling lambs is less than 5%. The majority of lambs remain uninfected up to 2.5 months of age. After 3.5 months of age, the lambs develop infections through close contact with adult sheep approaching 100% prevalence by 5.5 months. Shedding and transmission of OvHV-2 is believed to be through nasal and ocular secretions. Seroconversion and detection of OvHV-2 DNA in nasal secretions lags the detection of viral DNA in blood by several months. Separation of lambs from adults at 2 months of age reliably produces OvHV-2-free sheep.[9] These findings indicate that newborn lambs are not a major source of virus infection for cattle. Peak nasal viral shedding occurs around 7-8 months of age and then declines to lower levels as adults. Adult sheep continue to shed virus at variable levels throughout their life. OvHV-2 shedding from adult sheep may be higher during the periparturient period or while on feed in feedlots, resulting in a higher risk to commingled cattle. OvHV-2 is readily transmitted between in contact sheep, however, transmission by inoculation of whole blood or PBMCs is inconsistent.[10] This suggests that infectious virus is primarily produced and shed by nasal or ocular secretions and that infection of blood cells may be largely non-productive.

Studies of four Colorado dairies show that endemic OvHV-2 infections can occur within individual herds.[11] Dairies with a history of clinical MCF also have known history of sheep exposure. One dairy studied is located within 70 meters of a sheep feedlot and observes sporadic cases of MCF every year with an annual incidence of 0.1 to 0.6%. In this herd MCF seroprevalence increased from 0.8% in heifers <15 months of age to nearly 10% by 21 months of age. Seroprevalence in adult cattle ranged from 20-30%. Seroprevalence was associated with proximity to the sheep feedlot. The prevalence of asymptomatic OvHV-2 infections in adult lactating cattle from this dairy is 21.3%. Thirty adult cows were monitored monthly by CI-ELISA and OvHV-2 PCR for a 20 month period.[12] At the initial sampling 8/30 animals were seropositive. By the end of the 20 month period 21 of the 30 animals had at least one positive CI-ELISA or PCR test result. None of the animals developed clinical signs of MCF.

MCF can be a particularly devastating disease in bison.[6,13-15] MCF was observed in 45 of 163 bison exposed to sheep at an auction yard for a period of only 1 day.[14] Clinical disease occurred between 50 and 220 days after exposure and peaked around 60-70 days. No cases of MCF were observed among exposed bison on the destination farms indicating that bison with MCF do not transmit MCF to other bison. MCF was also observed in 51.2% (N=825) of bison exposed to sheep in a feedlot for 19 days. During that same time, only 1 of several thousand cattle at the feedlot developed MCF. This outbreak demonstrates the difference in susceptibility of bison and cattle to MCF and the high threat of OvHV-2 transmission from juvenile lambs in a feedlot environment.

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Keywords: Malignant Catarrhal Fever (MCF), Ovine Herpesvirus type 2 (OvHV-2), Cattle, Sheep, Virology

S12-4

Approaches to Eradicate Classical Swine Fever in Pigs in Korea

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Classical swine fever (CSF, hog cholera) is a highly contagious viral disease of swine causing significant economic losses worldwide. The disease causes significant economic losses worldwide and is classified as a notifiable disease by the OIE (Office International des Epizooties), formerly known as a List A disease. Successful eradication has been achieved in many countries, including North America, Austrailia, Japan, and parts of Northern Europe.

Classical swine fever virus (CSFV), the causative agent of CSF, is a member of the genus *Pestivirus* in the family *Flaviviridae*. Bovine viral diarrhea virus (BVDV) and border disease virus (BDV), which can infect cattle, sheep, and swine, are the two other members of this genus. CSFV has is enveloped and has a single-stranded, positive-sense RNA genome of approximately 12.3 kb. The genome consists of a large oopen reading frame (ORF) flanked by 5' and 3' non-coding regions (NCRs), which are highly conserved among viral isolates.

Although there are many CSF vaccines available worldwide, at present only live-attenuated viral vaccines using the Chinese (C) strain vaccine are routinely used. The live Chinese (C) strain vaccine is the most widely used. In Korea, live vaccine has been extensively used extensively since 1974 to control CSF in the field. A bovine kidney tissue-culture attenuated live vaccine (LOM strain), provided by the National Veterinary Assay Laboratory in Japan in 1964, was further attenuated and field-applied by the Korean National Veterinary Research and Quarantine Service (NVRQS).

This article will focuses on the facts mainly concerning outbreaks of the disease, the diagnosis of CSF, government control policy, the genetic typing of new virus isolates, and progress in diagnostic kit and vaccine development in Korea.

1. Classical swine fever outbreaks

CSF was first reported in the Republic of Korea in 1947 and subsequent outbreaks have made it one of the most devastating diseases to affectaffecting the Korean pork industry. However, the implementation of a national CSF eradication program by the government in 1996 resulted in a gradual reduction in the number of CSF outbreaks. For example, there were no cases of CSF between August in 1999 and December 1, in 2001.

Therefore Korean government declared that the country is free from CSF. All CSF vaccination has been banned since December 1, 2001. Unfortunately after the ban of vaccination, Unfortunately, however, there were two outbreaks of CSF in Kangwon province in April 2002 and another 11 outbreaks in Incheon and Kyeonggi provinces between October and December 2002. Therefore Aall only the pigs located only within the counties that CSF occurred were vaccinated to contain the disease prevalence. Any additional outbreak was not reported until February 2003. But Ffrom March to May 2003 there were 65 CSF outbreaks throughout the country. The reason for the outbreaks was that CSF infected breeding pigs were sold to many other farms. Therefore the Government's stamping-out policy was changed to mandatory vaccination and

quarantine policy.

2. Classical swine fever diagnosis

Veterinarians in the field can diagnose CSF by typical signs of the pigs. CSF is a notifiable notifiable disease and a tentative diagnosis based on clinical signs must be notified to thea provincial Veterinary Service diagnosis lLab (PDLVSL). The veterinarians working with PDL VSL visit the CSF suspected farms. Theyand collect samples for laboratory diagnosis, including whole blood in EDTA and tissues from the suspected animals. Movement restriction is applied to the farm pigs. The movement restriction couldcan be lifted after if the laboratory diagnosis of CSF is negative. In the Veterinary Service Lab, ELISA (enzyme-linked immunosorbent assay) could beis carried out to detect antibody toagainst CSF virus. WBC count and histopathological examination shouldalso performed be done. RT-PCR (reverse-transcription polymerase chain reaction) and antigen-capture ELISA can be done to detect CSF antigen in blood or tissue samples. The indirect fluorescent antibody test (IFA) can be used to detect CSF antigen in cryostat sections of tissue samples. For virus isolation, detection of SN (serum neutralization) antibody and molecular epidemiological analysis of the CSF, blood and tissue samples should be sent to NVRQS.

3. Government policy for CSF control

Korea submitted the report of declaration of CSF free status to OIE on December 1, 2001. After declaration of freedom from CSF in Korea in 2001, Korea experienced nationwide CSF outbreak in 2003. Thereafter government policy for CSF control changed completely.

No vaccination and stamping-out policy has been suspended. Instead mandatory vaccination of all pigs through a nationwide CSF vaccination campaign has been initiated again. Also an extensive serological surveillance to enhancecheckup the immune status and to detect viral antigen has been implemented. If there is no CSF outbreak for the time being and most of the pigs are completely immunized, Korea will declare freedom of CSF again and. Thereafter the ban on vaccination and stamping-out policy will be implemented.

4. CSF epidemiology and surveillance

Surveillance activity in Korea consists of a passive epidemiological surveillance system that investigates reported diseases, and of an active epidemiological surveillance system that examines statistically selected and targeted samples within hog pig population. Any suspicious case of CSF is followed by quarantine, confirmatory diagnostic tests and other necessary control measure.

A clinical surveillance team visits farms to clinically examinefor clinical examination of all pigs on the premises at least 5 times per farm a year for each farm. Each surveillance team is mainly composed of staffs from Livestock Health Control Association (LHCA). Any suspected case is immediately reported to city/county mayor and the provincial diagnostic labVSL.

To monitor immune response of pigs to enforcedetermine mandatory vaccination, a total of 200,000 serum samples are collected from abattoirs and farms. Indirect sandwich ELISA developed in Korea is performed to check antibody against CSF virus by VSL Provincial Diagnosis Lab (PDL) and NVRQS. If owners were found not to have performed vaccinations, they would receive a financial penalty. The test results should be reported to NVRQS through Confronted Animal Infectious Disease System on the World Wide Web every month.

After tissue and blood are obtained from infected pigs on farms and abattoirs, RT-PCR or antigen capture ELISA is performed to detect CSF antigen. If necessary, virus isolation could beis attempted using cell culture at NVRQS. A total of 50,000 samples are collected to detect viral antigen. The farms located near the CSF outbreak or showing a very high antibody titer againstto CSF virus belong to this surveillance program.

5. Genotypes of CSFV Korean isolates

Technological advances have facilitate d genetic typing of CSFVs based on the determination and comparison of nucleotide sequences for fragments of viral genomes. CSFV is divided into three major genetic groups. Group 1 comprises most of the historical isolates including vaccine strain and Group 2 consists most of the currents ones. Group 3 occurs solely in Asia. Only the CSFV Korean isolates between 80's and 90's belonged to Group 3.

6. Developments of vaccine and diagnostic kits in Korea

Enzyme-linked immunosorbent assays (ELISAs) for the detection of CSFV or anti-CSFV antibodies may be used for diagnosis and epidemiological surveys. CSFV antigen capture or antibody detection ELISA kits were developed by NVRQS and commercialized. Recently, differential ELISA for the E2 marker vaccine was developed and couldcan be used in the near future for the discriminatory ELISA for detection of anti-E^{rns} antibody. Baculovirus-expressed E2 recombinant subunit vaccines were developed by NVRQS .and Iit will be commercially available in near futuresoon. Also, Ddevelopment of a new type of live marker vaccine, using chimeric pestivirus is ongoing.

Keywords: Classical swine fever, eradication, diagnosis, genotype, vaccine

S13-1

Korea's Present State and Perspective on Biosafety

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Biosafety is the application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or biohazards. Biosafety defines the containment conditions under which infectious agents can be safely manipulated. The objective of containment is to confine biohazards and to reduce the potential exposure of the laboratory worker, persons outside of the laboratory, and the environment to potentially infectious agents. Global events in the recent past have highlighted the need to protect the biological agents in biomedical laboratories, which can be called biosecurity measures from being intentionally compromised in the ways that harm people or environment. Effective biosafety practices are the very foundation of laboratory biosecurity activities. Biosafety and biosecurity is also of growing importance in Korea with the increasing need for researches in area of infectious disease and the potential treat of bioterrorism. The development on biosafety in Korea was expedited with the introduction of the "Guidelines for researches involving recombinant DNA molecules (Recombinant DNA Guideline)" in accordance with the provisions of Article 15 of the Biotechnology Promotion Law in 1997. Also The Ministry of Health and Welfare recently revised the "Prevention of Contagious Disease Act." In the revised act, the high dangerous pathogens were defined and if researchers acquire the pathogens through isolation or transfer from other institutes, he must report the possession and transportation to the government.

Although the Recombinant DNA Guideline was promulgated in1997, biosafety circumstance in Korea is evaluated as relatively poor. In fact, the Recombinant DNA Guideline legislated for the purpose of the life science encouragement and Prevention of Contagious Disease Act was mainly focused on the biosecurity, therefore, only thirty-two species or genus pathogens could be controlled by the act.

As a result, we think a certain general biosafety guideline is necessary which defines the appropriate facility, risk assessment and pathogens transportation. To improve the biosafety circumstance in laboratory, Ministry Health and welfare is going to revise the Recombinant DNA Guideline in order to renew the standard according to international demands and improve practically the biosafety. In near future, KCDC will prepare the Laboratory Biosafety Manual and expect it will contribute to the improvement the national laboratory biosafety practices in related laboratories.

Keywords: Laboratory, Biosafety, Legislation, Guideline

S13-2

Smallpox Vaccine: *Yesterday*, *Today* and *Tomorrow*

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Smallpox is a dangerous life-threatening disease caused by variola virus, a member of the genus *Orthopoxvirus* of the family Poxviridae. The virus used as vaccination of variola virus is vaccinia virus, an orthopoxvirus of uncertain origin that induces cross-protective immunity against variola virus. Smallpox has been eradicated as a natural disease in 1977 as a result of intensive worldwide vaccination by World Health Organization (WHO). However, from the 1990s, the concerns about biological terrorism using smallpox were arisen and need for development of vaccine have been increased.

First generation vaccines have been produced from pulp lesions (calf lymph) on the skin of infected cattle or sheep. The calf-lymph product that is presently licensed in the USA is Dryvax (last produced in 1982 by Wyeth Laboratories, Marietta, PA, USA). Development of the pock lesion and scar has historically been accepted as evidence of successful immunization against variola, lasting 3–5 years after vaccination. Though the conventional smallpox vaccine is very effective, the need for a new smallpox vaccine has arisen because of the limited stocks of the approved, calf-lymph derived vaccine and the safety of it. Previous manufacturing methods using calf lymph are no longer acceptable because of the absence of controls in the process and the potential risk of contamination with the infectious agents including bacteria and prion disease such as bovine spongiform encephalitis (BSE). Therefore, second generation vaccines using cell culture techniques are developing now to lessen the risk of conventional vaccines.

In the future, recombinant vaccines and highly attenuated vaccines such as Modified Vaccinia Ankara (MVA) will be used as next generation of smallpox vaccines because the safety is more important than the effectiveness.

In Korea Center for Disease Control and Prevention (KCDC), we developed smallpox vaccine candidates using cell culture adapted vaccinia virus. We used vero cell line to attenuate Korean conventional vaccine strain. Through the virus isolation assay using agarose-overlayed plaque purification and the safety tests in mice and rabbits, we developed 3 candidates for new smallpox vaccine. We performed immunogenicity tests to identify both humoral and cell-mediated immune response in mouse and rabbit animal model Using 3 vaccine candidates. We expect that these new vaccine candidates will play a role in protection of bioterrorism.

Keywords: Smallpox, Vaccinia virus, Bioterrorism, Conventional vaccine, Second generation vaccine

S13-3

Countermeasures against Botulinum Neurotoxins: Detection, Diagnostics, and Therapeutics

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Botulism is a potentially life-threatening clinical syndrome of descending cranial nerve and neuromuscular paralysis caused by intoxication of botulinum neurotoxin (BoNT) produced by *Clostridium botulinum*. The BoNT, which is one of the most potent biological and chemical substances known, pose a significant bioterrorism threat, and as such are listed as Category A agents of bioterrorism by the National Institutes for Allergy and Infectious Diseases (NIAID) and the Centers for Disease Control (CDC). *C. botulinum* is an obligate anaerobic, endospore-forming bacterium that is ubiquitous in the environment, and the strains divided into serotypes A through G according to antigenic properties of BoNT. Of them, types A, B, E, and rarely F cause botulism in human. Since the possible presence of *C. botulinum* spores in the environment is a potential source of human botulism or biological attack by BoNT, environmental surveillance of *C. botulinum* is critical for an assessment of botulism hazards.

Detection of BoNT is important for laboratory diagnosis of clinical botulism, to isolate *C. botulinum* from environmental samples, and to cope with bioterror. Because BoNT cause death in human with 1.0 micrograms per kilogram orally, development of rapid and highly sensitive detection method is required. Korean National Institute of Health (KNIH) has been trying to develop rapid, sensitive and specific tools for the detection of BoNT and *C. botulinum* including immunological and PCR-based techniques. We have also attempted to isolate *C. botulinum* from environments for environmental surveillance and to analyze information of the isolates.

Because any case of suspected botulism represents a potential public health emergency and the early cases of botulism are commonly misdiagnosed, clinical diagnosis of botulism is necessary to be confirmed by specialized laboratory testing that relies on isolation and identification of the neurotoxins from sera or other samples. The standard laboratory diagnostic test remains the mouse protection bioassay (MPB). Because MPB is a reliable and sensitive test but is time-consuming and expensive, several *in vitro* assays have been developed for monitoring BoNT. KNIH is responsible for laboratory diagnosis of botulism, and has continuously been trying on approaches for the development of *in vitro* diagnostic methods.

Current therapy for botulism consists of supportive care and passive immunization with equine antitoxin. Continuous occurrence of clinical botulism in Korea since 2003 has emphasized the needs of antitoxin for successful therapy. For that reason, a pilot-scale production and the process development for the production of therapeutic antitoxin against BoNT is in progress under the initiative of KNIH.

In conclusion, because botulism has been reported as presenting a very high threat of being used in biological warfare, additional research in diagnosis and treatment of botulism is required to minimize its threat as a weapon. Therefore, the purpose of the researches has to be focused on the development of the next generation of countermeasures against botulinum toxins.

Keywords: Botulinum neurotoxin, botulism, Clostridium botulinum, Korean National Institute of Health

S13-4

Genome-Wide Screening of *Bacillus anthracis*-Specific Chromosomal Markers

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Bacillus anthracis is a gram positive spore forming rod-shaped bacterium and is a causative agent of anthrax that is an acute and almost fatal zoonotic infectious disease. It is also well known as one of the major biological weapons used in bioterrorism. Among genus *Bacillus*, most *Bacillus* species are innocuous but potential pathogens as well as *B. anthracis* belong to the members of so-called *Bacillus cereus* group. The group is comprised of *B. anthracis*, *B. cereus* , *B. thuringiensis*, *B. mycoides*, *B. weihenstephanensis* and *B. pseudomycoides*. *B. cereus* causes a mild form of food-borne gastroenteritis and opportunistic infections including an ophthalmic infection as well. *B. thuringiensis* is an insect pathogen and is reported potentially enteropathogenic. *B. anthracis* is exclusively a deadly pathogen due to its acute and fatal disease potential when infected. Humans rarely get an anthrax natural infection in most of developed countries but instead are exposed to more chances of a potential infection by bioterrorism. Thus, the prompt detection, diagnosis, treatment, prevention and control policy of this disease or the agent have been increasingly issued.

The reliable species identification of *B. anthracis* is rather complicated. The unambiguous identification of the organisms of the *B. cereus* group has been difficult due to their genetic and phenotypic similarity. A high level of genetic relatedness has been demonstrated by many of genetic analyses. These methods failed to discriminate among some members of the *B. cereus* group. There have been many efforts to utilize rapid DNA-based detection methods, such as PCR, to replace time-consuming biochemical or culture-based diagnostic tests. PCR-based methods can readily differentiate vaccine or fully virulent *B. anthracis* plasmid genotype. However, plasmid-cured B. anthracis, or near-neighbor species containing *B. anthracis* closely-related plasmids, are very difficult to distinguish from *B. anthracis*. In addition, plasmids or their virulence genes have been readily transferred within these groups by means of conjugation or transformation.

PCR methods developed for detection of the *B. anthracis* chromosome have suffered from lack of assay specificity; Ba813, *vrrA* gene, gyrase B gene, SG-850, the beta subunit of RNA polymerase gene and the gyrase A gene have shown false positive results in detection of the *B. anthracis* chromosome. These results imply that it is not easy to discover highly specific nucleotide sequences in the *B. anthracis* chromosome. Recently, we developed highly specific PCR-based assays for the *B. anthracis* chromosome using a sequence motif found within a spore structural gene (*sspE*) (GenBank accession number AF359938). These assays so far demonstrated 100% specificity in *B. anthracis* detection with more than 200 strains of *Bacillus* cereus group species. We developed some diagnostic tools of *B. anthracis* based on this gene.

In this presentation, we introduce how we found more *B. anthracis* chromosome specific gene candidates other than *sspE*. We retrieved all potential open reading frames (ORFs, 5738 frames) from the whole genome sequence of *B. anthracis* Ames (GenBank accession number AE016879) and compared all the ORFs of *B. anthracis* with *B. cereus* ATCC 10987 by nucleotide BLAST search at the institute for genome research (http://tigrblast.tigr.org) and recorded the identity % and E-value of each ORF. We sorted the ORFs in the

order of E-value to the lowest. We retrieved E-values of *B. thuringiensis* ATCC 35646, *B. cereus* ATCC 14579, *B. cereus* E33L, and *B. thuringiensis* 97-27 by BLAST search at the NCBI and ERGO Light with the nucleotide sequences of the top 1,000 ORFs of *B. anthracis* in the above sorted ORFs. We recorded the lowest E-value of the ones of the ORFS of these strains and sorted the ORFS in the order of the E-value to the lowest. The top 400 ORFs were collected and primers within each ORF sequence were designed using OLIGO V6.5 and PCR was carried out with highly purified 25 ng of genomic DNAs of *B. anthracis* Sterne 34-F2 and the most closely related *B. cereus* group species. We collected the PCR primer sets which do not produce any false positive reaction in detection of *B. anthracis*. 28 sets of primers were proved to have 100% specificity in *B. anthracis* chromosome detection with the template DNAs from 164 strains of *B. cereus* group species including the most closely related *B. cereus* group species and 54 more primer sets were specific when tested so far with the most closely related 21 strains of *B. cereus* group species and are still being tested with more strains for the further specificity analysis. We expect this study outcome will provide the foundation of many further studies and application developments.

Keywords: Bacillus anthracis, The Bacillus cereus group, Detection, Specific chromosomal markers, Polymerase chain reaction

S13-5

The Study on *Bacillus anthracis* Protein Associated with Sporulation-Germination and Surviving in the Host

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Bacillus anthracis is gram positive rod, aerobes, endospore-forming bacteria, and causes anthrax. The mortality of pulmonary anthrax is more than 80% and recently it has been noticed as it could be used for biological weapons. The attenuated strain and protective antigen (PA) has been broadly studied and used as vaccine. Also DNA vaccines against PA gene have been recently developed. Besides PA, other candidates have been also investigated as a vaccine or antigen. Among them, there are a capsular repeating epitope of $_D$-glutamic amino acid which could directly activate B lymphocytes and spore exosporium collagen like glycoprotein (BclA) which was immunodominant.

In the early pathogenesis, the germination and surviving inside macrophage should be a key step in the developing of systematic disease. Therefore, it was suggested that the treatment against some germination and other virulence factors should be very important in the control of early infection. So we studied the sporulation-germination factors and other virulence factor associated with surviving in the host.

1. Sporulation-germination factors

There are a few enzymes known to be indispensable for sporulation and germination in *Bacillus* species. One of them is 2,3-phosphoglycerate (23PGA) independent phosphoglycerate mutases (iPGMs) which play a important role in glucose metabolism to synthesize ATP during sporulation and germination. Also there is other indispensable protease, termed germination protease (GPR) in *Bacillus* species and it could degrade sequence-specifically small acid-soluble spore proteins (SASPs). Through the mutagenesis study and purification of recombinant *B. anthracis* GPR P_{41}, SASPs and iPGMs, we successfully produced the recombinant wild and each mutant protein, and assayed their characteristics based on mutant. Their results could be expected by further analysis like crystallography and the new drug development for anthrax.

2. Genome origin vegetable cell antigen associated with surviving in the host

Using patients serum obtained from the anthrax outbreak in Korea, genome-origin antigen was detected and was called as ba 35kDa antigen. It was neither plasmid-origin nor spore protein. The gene coding ba 35kDa antigen was found out from the screening genomic library of attenuated *B. anthracis* Pasteur strain (pXO1-, pXO2-). In silico study, its sequence analysis revealed that it would belong to the periplasmic solute binding protein of ABC family and it has lipoprotein like and metal ion transporter characteristics. So we established effective strategy of recombinant protein production from the mutagenesis study and we massively produced recombinant ba 35kDa antigen in *E. coli*, because wild antigen was lipoprotein to be so difficult to be purified.

Keywords: *B. anthracis*, sporulation, germination, GPR, iPGM, SASPs, ba 35kDa antigen

S14-1

Calcium Dependent Repression of *Streptococcus pneumoniae* Survival

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Streptococcus pneumoniae (the pneumococcus), a gram-positive and naturally transformable organism, is one of the most important human pathogens, and pneumococcal disease is endemic all over the world. For more than a century, *S. pneumoniae* has been known as the most common cause of acute otitis media, sinusitis, and pneumonia and one of the most important causes of bacterial meningitis. In developing countries pneumonia is a serious disease in children and it is estimated that more than a million children below the age of 5 each year die from pneumococcal pneumonia. In the United States the pneumococcus each year probably accounts for 3000 cases of meningitis, 500,000 cases of pneumonia, and 7,000,000 cases of otitis media. Pneumococcus is carried in the nasopharynx of healthy individuals, and this is a major reservoir for pneumococcal infections. Pneumococci are subject to a number of environmental stresses *in vivo*. *S. pneumoniae* encounters heat stress as a regular feature of its pathogenic life cycle after penetration from the nasal mucosa (30 to 34°C; 28) into blood and/or meninges (37°C). The elevated temperatures they encounter within a mammalian host serves as a key trigger for a rapid, transient increase in synthesis of a highly conserved set of proteins referred to as heat-shock proteins (HSPs). HSPs protect bacteria against such adverse effects as elevated temperatures, exposure to ethanol, or heavy metals thus increasing their survival rate. Hsp70 and Hsp60 (DnaK and GroEL, respectively, in prokaryotes) play a pivotal role in the folding of native and denatured proteins, and thus promote cell protection and survival. In the gram-positive organism, the expression of the *dnaK* and *groE* operons is negatively controlled by the HrcA repressor, which binds the highly conserved CIRCE operator sequence.

In both prokaryotes and eukaryotes, Ca^{2+} is required for a wide variety of cellular processes, such as cell division, signal transduction and protein stability. In eukaryotes, Hsp70 and Hsp60 levels are dependent on Ca^{2+}. In contrast to the relatively well-characterized eukaryotic system, the effect of Ca^{2+} on HSP expression in prokaryotes is poorly understood. HrcA may respond to environmental stress and various other factors that modulate the expression of the *dnaK* and *groE* operons. Here we show that heat shock-induced expression of the CIRCE regulon in *Streptococcus pneumoniae* is repressed in the presence of Ca^{2+}. Furthermore, the thermo-resistance of *S. pneumoniae* is significantly repressed in the presence of the Ca^{2+}, strongly suggesting that HrcA inhibits expression of the CIRCE regulon in a Ca^{2+}-dependent manner. Although HrcA does not bind directly to Ca^{2+}, its hydrophobicity is increased in the presence of the metal ion. Our observations collectively suggest that Ca^{2+} induces conformational changes such as exposure of the hydrophobic surfaces of HrcA, which facilitate binding to GroEL. This in turn enhances access to CIRCE and leads to repression of the *dnaK* and *groE* operons.

Keywords: Streptococcus pneumoniae, calcium, HrcA, stress protein, dnaK, groEL

S14-2

Vibrio vulnificus RtxA, a Key Virulence Factor, Mediates Contact Dependent Cytotoxicity

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Vibrio vulnificus, a halophilic estuarine bacterium causing a rapidly progressing fatal septicemia, is highly cytotoxic to eukaryotic cells. The cytotoxicity has been regarded as the hallmark of pathogenicity of the microorganism. The two most representive cytotoxins, the cytolytic hemolysin (*vvhA*) and the elastolytic protease (*vvpE*), have been regarded to play major roles in the cytotoxicity. However, single or double mutants of *vvhA* and *vvpE* showed no significant difference with the isogenic wild type strain in the cytotoxicity and lethality to mice of *V. vulnificus*. From these results, we came to speculate that a yet unidentified key virulence factor should play a major role in the *in vitro* and *in vivo* cytotoxic activity of *V. vulnificus*. Close encounter of *V. vulnificus* with host cells is a prerequisite to cytotoxicity. To identify new virulence factors associated with cytotoxicity, we constructed a mini-Tn5-*lacZ*1 transposon (Tn) mutant library of the highly virulent clinical isolate MO6-24/O. The Tn mutant library was extensively screened for the mutants showing decreased cytotoxicity to HeLa cells. We selected several cytotoxicity defective mutants. We found that a *V. vulnificus* transposon (Tn) mutant showing decreased cytotoxicity to HeLa cells had a Tn insertion inside *rtxA* ORF encoding a gigantic toxin (4,701 aa, 501 kDa). Specific mutations in the *rtxA* gene resulted in a drastic decrease in virulence. The expression of RtxA protein increased upon contact with host cells. A domain showing homology with eukaryotic ezrin/radixin/moesin (ERM) homologue was found in the first half (aa 1626-1,842) of the RtxA toxin. Host proteins interacting with the ERM domain were searched by a yeast two hybrid assay. One of the host proteins appeared to interact with the ERM motif by the assay was the actin cross-linking protein filamin. RtxA-filamin interaction was confirmed by immunoprecipitation and their cellular colocalization by confocal microscopy. Interestingly, cellular filamin was degraded after host cells contacted with *V. vulnificus* RtxA. In *V. vulnificus* wild type-treated HEp2 cells, RtxA and filamin showed overlapping intracellular distributions, and RtxA caused filamin to form globular aggregates. Also, *V. vulnificus* RtxA caused rounding of HEp2 cell and a dramatic actin aggregation, and the tight globular actin aggregates were removed from the cytoplasm by a weird pinching-off mechanism. The cell rounding and actin depolymerization were completely disappeared by specific mutations in *rtxA*, and the defect was fully complemented *in trans* with the wild type allele encoded by a plasmid. Filamin is known to serve the scaffold for stress-activated MAPK. Vv-RtxA-mediated cytotoxicity was significantly abrogated by JNK and p38 kinase inhibitors. Western blot analysis revealed increased phosphorylation of JNK after Vv-RtxA challenge. However, the cell death did not accompany DNA laddering, procaspase-3 cleavage, and apoptotic morphologic changes. The RtxA-mediated cell death could not be observed when calcium-free medium was used for the experiment. The cytotoxicity was dependent on the extracellular calcium concentration and calcium influx. Calcium influx antagonist diltiazem inhibited Vv-RtxA-mediated cytotoxicity in a dose dependent manner. When cellular calcium concentration was monitored with Fura-4 and

confocal microscopy, cytosolic calcium concentration gradually increased, cytosolic blebs protruded from the cell surface, and the cytosolic calcium was sequestered in mitochondria before the cell death. Electron micrographs of the host cells showed mitochondrial swelling and plasma membrane perturbation, which implied necrotic cell death. In conclusion, Vv-RtxA specifically interacts with host filamin and activated JNK signaling pathway significantly. Activation of MAPK seems to be a prerequisite to the necrotic cell death by Vv-RtxA. Another contributor to the necrotic cell death mechanism is calcium influx, which consequently disrupts mitochondrial integrity.

Keywords: *Vibrio vulnificus,* virulence, RTX, cytotoxicity, host-parasite interaction

S14-3

Regulation of *Salmonella* Virulence Functions

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The Gram-negative bacterium *Salmonella enterica* is the etiologic agent of gastroenteritis and typhoid fever in humans, which results in millions of cases and hundreds of thousands of fatalities every year. *Salmonella* has the capacity to cause lethal infections in a wide variety of animal species including several of economic importance. *Salmonella* is normally acquired by the consumption of contaminated water or food. Thus, *Salmonella* must be able survive the extreme acid pH of the stomach before reaching the small intestine where it elicits its internalization by intestinal epithelial cells. Those *Salmonella* serovars that cause systemic disease migrate to the liver and spleen, replicating within the macrophages of these organs. This raises the question: how does *Salmonella* know it is present outside an animal, in close proximity of a host cell or within a phagocytic cells so that the appropriate sets of genes are turned on and off?

Genetic studies have demonstrated that *Salmonella*'s ability to cause disease requires not only a variety of structural proteins that enable bacterial survival within host tissues and resistance to host microbicidal products but also regulatory proteins that ensure that the virulence structural proteins are expressed in the correct tissues and for the appropriate extents of time. The critical role that regulatory proteins play in *Salmonella* pathogenesis is demonstrated by the strong attenuating effect that results not only from inactivating certain regulatory genes but also from their constitutive activation.

It can be argued that the PhoP/PhoQ two-component regulatory system is the major regulator of *Salmonella* virulence functions. It consists of the membrane sensor kinase PhoQ and the response regulator PhoP. This system plays a major role in virulence because it controls the expression of determinants participating in several steps during the infection process, including the invasion of the intestinal epithelium and resistance within the macrophages of liver and spleen. In addition, a functional PhoP/PhoQ system is necessary for bacterial resistance to host antimicrobial peptides and acid pH.

The PhoP/PhoQ system is a the top of several regulatory cascades controlling the expression and/or activity of other regulatory proteins such as SlyA, two-component systems such as RstA/RstB, SpiR/SsrB and PmrA/PmrB, and the alternative sigma factor RpoS. In addition to the indirect regulation, PhoP controls the expression of several genes directly, by binding to the promoter region and stimulating gene expression. Using a method developed in our laboratory termed GPS for gene promoter scan, we have been able to identify the critical promoter features that determine the expression kinetics of genes that are under direct transcriptional control of the PhoP protein.

PhoP controls the expression of several *Salmonella*-specific virulence genes and also of genes that are found in the related commensal organism *Escherichia coli*. Interestingly, some of the virulence determinants that are regulated by PhoP in *Salmonella* are not regulated in the same fashion in *E. coli*, suggesting that the differential regulation of conserved proteins may be a critical element that distinguishes pathogenic from non-pathogenic organisms.

Keywords: gene regulation, pathogenicity, PhoP/PhoQ, RpoS, Salmonella, two-component system, virulence

S14-4

Quorum Sensing and Group Activity: The Role of Orphan Quorum Sensing Regulator, QscR in *Pseudomonas aeruginosa*

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The opportunistic pathogen *P. aeruginosa* possesses two complete acyl-homoserine lactone (acyl-HSL) quorum sensing (QS) systems, LasR-I and RhlR-I. LasI catalyzes the synthesis of *N*-3-oxododecanoyl homoserine lactone (3OC12) and LasR is a transcription factor that requires 3OC12 as a ligand. RhlI catalyzes the synthesis of *N*-butanoyl homoserine lactone (C4) and RhlR is a transcription factor that responds to C4. LasR and RhlR control the transcription of hundreds of *P. aeruginosa* genes. There is also an orphan LasR-RhlR homolog, QscR for which there is no cognate acyl-HSL synthetic enzyme. A *qscR* mutant is hypervirulent and QscR transiently represses a few quorum sensing controlled genes. To better understand the role of QscR and the relationship among three "R" proteins in *P. aeruginosa* QS regulation, we used transcription profiling to identify a QscR-dependent regulon. Our analysis revealed that QscR activates some genes and repressed others. Some of the repressed genes are not regulated by the LasR-I or RhlR-I systems while others are. The observation that some regulations required a DNA binding activity of QscR implied there should be a direct regulation by QscR. To test the hypothesis that QscR controls specific promoters directly, we purified QscR and characterized QscR activity in vitro and in recombinant *E. coli*. QscR binds to promoters that have elements similar in sequence to those found in LasR- or RhlR-dependent promoters but QscR does not bind to the LasR- or RhlR-specific promoters we examined. QscR binding to DNA requires 3OC12 produced by LasI, but QscR exhibits a relaxed acyl-HSL specificity compared to the 3OC12-cognate signal receptor LasR. Our results support the hypothesis that there is a specific QscR-dependent regulon and QscR directly controls this regulon in the presence of 3OC12. Because of its relaxed signal specificity QscR may also respond to acyl-HSLs made by other bacteria in mixed bacterial communities, independently of LasR-I system. This feature may be molecular basis of inter-species signaling. Since QscR also has relaxed signal binding affinity and the signal bind to QscR reversibly, QscR may respond to sudden decrease of signal concentration or change of signal composition. Thus QscR appears to be an integral component of the *P. aeruginosa* quorum sensing circuitry to cope with external signals from environment as well as internal signals.

Keywords: Quorum sensing, QscR, LasR, RhlR, Pseudomonas aeruginosa, Acyl-homoserine lactone, signal molecule, inter-species communication

S14-5

Quorum Sensing of *Burkholderia glumae*: The Causative Agent of Bacterial Rice Grain Rot

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Burkholderia glumae is a motile bacterium with two to four polar flagella and infects rice flowers to cause rice grain rot and wilt in many field crops. The bacterium produces non-selective phytotoxin, called toxoflavin, which is a key pathogenicity factor. Molecular and genetic analyses showed that quorum sensing (QS) regulates toxoflavin biosynthetic genes and its transporter genes. Toxoflavin biosynthesis genes were composed of five genes, *toxA* to *toxE*. Genes *toxF* to *toxI*, which are responsible for toxoflavin transport, were polycistronic and similar to the genes for resistance-nodulation-division efflux systems. Using Tn*3-gusA* reporter fusions, we found that ToxR, a LysR-type regulator, regulates both the *toxABCDE* and *toxFGHI* operons in the presence of toxoflavin as a co-inducer. In addition, the expression of both operons required a transcriptional activator, ToxJ, whose expression is regulated by QS. TofI, a LuxI homolog, was responsible for the biosynthesis of both *N*-hexanoyl homoserine lactone and *N*-octanoyl homoserine lactone (C8-HSL). C8-HSL and its cognate receptor TofR activated *toxJ* expression. This was the first report that QS is involved in pathogenicity by the regulation of phytotoxin biosynthesis and its transport in plant pathogenic bacteria.

QS of the bacterium regulates other phenotypes including flagella formation. C8-HSL deficient mutant of *B. glumae* was non-motile and did not possess flagella at 37℃. Mutagenesis of the bacterium with mini-Tn*5rescue* identified an IclR-type transcriptional regulator, called QsmR, affecting flagella formation. TofR activated *qsmR* expression by direct binding to the promoter region of *qsmR*. Nucleotide sequence analysis of one of the flagella gene clusters identified *flhDC* homologs whose expression is directly activated by QsmR. As if FlhDC is the master regulator of lateral flagella gene expression, FlhDC activated expression of genes involved in flagella biosynthesis, motor functions, and chemotaxis in *B. glumae*. Non-motile mutants except the QS-deficient mutant produced toxoflavin but lost pathogenicity in rice. We are the first to show that FlhDC is present in a polarly flagellated bacterium and its expression is regulated by QS. The fact that QS and functional flagella play critical roles in pathogenicity of *B. glumae* suggests a potential target for the control of rice grain rot.

Keywords: *Burkholderia glumae*, quorum sensing, pathogenicity

S15-1

Unexpected Invasiveness of *Helicobacter pylori* into Lamina Propria of Human Gastric Mucosa

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There are usually few lymphocytes and no phagocytes on normal gastric mucosa not infected with *Helicobacter pylori*. However, *H. pylori* infection usually induce inflammatory cell infiltrations such as lymphocytes, plasma cells, eosinophils or neutrophils in the lamina propria, some of which also have been used in the determination of the degree of chronic and active gastritis of the infected person. Long lasting infections with *H. pylori* cause adverse effects of these inflammatory cells, which may lead to thinner mucous layer, and the structural and atrophic deformity of epithelial gland, as well as intestinalization and gastric cancer. *H. pylori* infected gastric mucosa also shows CD4 positive T-cell infiltrates in the lamina propria with dominant Th1 and minor Th2 phenotypes.

Despite a lot of strenuous efforts, there have been very little cases of *H. pylori* observed in lamina propria, so most of researchers worldwide come to the following consensus of noninvasive *H. pylori* causing serious inflammatory cell infiltrations in the gastric lamina propria via epithelial cytotoxic factors (including CagA and VacA) secreted from *H. pylori*, via ammonia produced by urease, via emission of considerable chemotactic factors which may attract neutrophils like NAP (neutrophil activating peptide), via direct contact with gastric epithelial cells, which in turn produce higher amount of secretory chemokines like IL-8, IL-2, IL-6, IL-12, granulocyte-macrophage colony stimulating factor(GM-CSF), monocyte-chemoattractant protein-1 (MCP-1), tumor necrosis factor alpha (TNF-α).

However, marked inflammatory cell infiltrations including neutrophil and Th1 cellular response in the lamina propria without direct *H. pylori* invasion go beyond our conventional knowledge about the fact that the bacteria inducing the infiltration of serious phagocytes into lamina propria of small or large intestine mucosa are all those infiltrating visceral epithelial cell or lamina propria. If so, upon any infection with *H. pylori* as known so far to be noninvasive into gastric mucosa, why do immunologic and inflammatory reactions work primarily in gastric lamina propria against invasive bacteria?

If *H. pylori* intrude in the lamina prorpia, either macrophages or dendritic cells will phagocyte it and *H. pylori* or fragmented part of the bacteria will encounter the specific immune response of the host. Accordingly, *H. pylori* will be captured in the phagocyte such as macrophage or dendritic cell, or be combined with antibodies like IgG, IgM, IgA and IgE, or complement like C3, C1q, etc.

This study was intended to discover the invasive potential of *H. pylori* by means of confocal laser scanning microscopy (CLSM) using double immunofluorescent antibody staining.

Biopsy tissues of duodenal bulb, gastric antrum, and gastric body were sampled from a total of 30 subjects including 19 male and 11 female freshmen of medical college of Gyeongsang National University. They were among the volunteers for gastric endoscopy which is a part of community medicine experience, and had active gastritis with neutrophil infiltration in the gastric lamina prorpria and the positive reaction in urease test

within 1 hour.

Their tissues were examined for bacterial invasion by means of double imunofluorescent antibody staining with anti-*H. pylori* and one of anti-IgG, IgM, IgA, IgE, C3, and C1q antibodies, respectively.

Bacterial invasion observed via confocal laser microscopy was divided into epithelial invasion and lamina propria. *H. pylori* found in the lamina propria were all bound with cells. In gastric antrum, epithelial and lamina propria invasions were observed in 12 and in 15 cases, respectively, mostly in 10 or more spots of one subject. In gastric body, epithelial and lamina propria invasions were observed in 9 and in 21 cases, respectively, mostly in 10 or more spots of one subject. A total 17 and 24 among 30 cases showed epithelial and lamina propria invasion, respectively, in either antrum or body or both. All showed either epithelial or lamina propria invasion or both. As to binding of the invaded bacteria with immune materials, a total of 735 epithelial or lamina propria invasions were observed in either antrum or body. Among them, IgA binding was observed in 10 instances, IgG binding in 4 instances, and IgM binding in 3 instances in lamina propria. Despite the very small number of the invaded bacteria were bound with immune materials, the fact that none of *H. pylori* lying in the mucous layer were not bound with immune materials increases the specificity of the *H. pylori* invasion in the lamina propria of this study.

As the results of this study, it could be said that *H. pylori* invaded into the lamina propria very frequently than previously suggested in patients with *H. pylori* chronic active gastritis.

Keywords: Confocal laser scanning microscopy, lamina propria, invasion

S15-2

Supramolecular Assembly and Acid Resistance of *Helicobacter pylori* Urease

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Helicobacter pylori, an etiologic agent in a variety of gastroduodenal diseases, produces a large amount of urease, which is believed to neutralize gastric acid by producing ammonia for the survival of the bacteria. Up to 30% of the enzyme associates with the surface of intact cells upon lysis of neighboring bacteria. The role of the enzyme at the extracellular location has been a subject of controversy because the purified enzyme is irreversibly inactivated below pH 5. We have determined the crystal structure of H. pylori urease, which has a 1.1 MDa spherical assembly of 12 catalytic units with an outer diameter of approximately 160 A. Under physiologically relevant conditions, the activity of the enzyme remains unaffected down to pH 3. Activity assays under different conditions indicated that the cluster of the 12 active sites on the supramolecular assembly may be critical for the survival of the enzyme at low pH. The structure provides a novel example of a molecular assembly adapted for acid resistance that, together with the low Km value of the enzyme, is likely to enable the organism to inhabit the hostile niche.

Keywords: Helicobacter pylori, Urease, three-dimensional structure, gastroduodenal diseases

S15-3

Fur- and Growth Fhase-Dependent Regulation of *H. pylori* Protein Expression

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Helicobacter pylori is a micro-aerophilic gram-negative spiral-shaped flagellated bacterium. It resides in the mucus layer of the stomach and is often associated with chronic gastritis, peptic and duodenal ulcer, and gastric cancer. Extensive research has focused on the ability of *H. pylori* to survive in the acidic environment and to cause gastric diseases. Several virulence factors have been identified but little is known about the global mechanisms of the gene expression regulation in *H. pylori* under changing environmental conditions. The ferric uptake regulator (Fur) protein is a Fe^{2+}-dependent transcriptional repressor that binds to the Fur-box of bacterial promoters and regulates various gene expressions in response to environmental iron. In several gram-negative pathogens, Fur plays a role as a global regulator and regulates the transcription of genes involved in iron transport but also in detoxification of oxygen radicals, acid tolerance, production of virulence factors and metabolic processes. In our previous study, we compared the protein expression profiles of the *H. pylori* strain 26695 and its isogenic *fur* mutant and identified proteins whose expressions are modulated by Fur and iron. We also demonstrated that the *H. pylori* Fur protein plays as a classical transcriptional repressor but also serves as an activator, providing evidence for the presence of Fur-mediated positive regulation in *H. pylori*. In this study to understand the global regulation of *H. pylori* protein expression by Fur in response to environmental changes, we analyzed the proteome profiles of *H. pylori* 26695 and its *fur* mutant harvested during a course of *in vitro* culture. Total 193 protein spots were found to be up- or down-regulated greater than 2-fold by either a *fur* mutation and/or in a growth-phase dependent manner. Cluster analysis of protein expression profiles revealed a growth-phase dependent regulation as well as Fur-positive and Fur-negative regulations. RT-PCR analysis of the genes whose proteins were altered during a course of *in vitro* culture showed that, in the *fur* mutant, inactivation of Fur resulted in the premature turnoff of the transcriptional machinery. It also indicated that the transcriptome and proteome of *H. pylori* are differentially regulated in respect to growth phases. Taken together, these data demonstrate the presence of posttranscriptional and translational controls of *H. pylori* proteins *via* Fur in response to environmental changes.

Keywords: *H. pylori,* proteomics, Fur, growth phase

S15-4

Quantitative Effect of *luxS* Gene Inactivation on the Fitness of *Helicobacter pylori*

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There has been great interest in the ability of some bacterial taxa to signal their presence and abundance by secreting specific metabolites; to use these metabolites to monitor the density of members of their own species and of other species; and to respond with changes in patterns of gene expression, cellular phenotypes, and interactions with other microbes or host tissues-a set of behaviors termed “quorum sensing”. Quorum sensing within individual species is often achieved using acyl-homoserine lactones (collectively called autoinducer 1 or AI-1), with much of the species specificity of AI-1 action stemming from differences in acyl chain length.

Chemically distinct furanone metabolites called AI-2 are also used by some species as signals for sensing cell density, both of unrelated taxa and of the same species. These furanones are by-products of a cyclic pathway that uses *S*-adenosyl methionine (SAM) as a methyl donor and then regenerates it.

The *luxS*-encoded enzyme, in particular, participates in this activated methyl cycle by generating both a pentanedione, which is transformed chemically into these AI-2 compounds, and homocysteine, a precursor of methionine and SAM.

Despite use of AI-2 for quorum sensing by some taxa, AI-2s synthesized in other species could be simple by-products of the activated methyl cycle and of no regulatory significance.

Helicobacter pylori, the genetically diverse pathogen implicated in peptic ulcer disease and gastric cancer, contains a *luxS* gene and exhibits *luxS*-dependent AI-2 synthesis but seems to lack close homologs of genes known to be involved in AI-2 uptake or AI-2-responsive transcriptional regulation. Furthermore, no effect of *luxS* gene inactivation on overall growth in culture, VacA toxin synthesis, protein profiles in two-dimensional gels, or motility in liquid cultures was detected for the *H. pylori* strains tested. Additional studies indicated that a functional *luxS* gene diminishes the capacity of *H. pylori* to form a biofilm at air-liquid-glass interfaces and that a functional *luxS* gene contributes to a cell density-dependent induction of expression of *flaA-lacZ* and *flaA-cat* reporter constructs. However, *flaA* expression was less stimulated by conditioned medium than was typical of responses to AI-2 in well-established models. These studies were carried out using pure cultures of *H. pylori*, however, whereas *H. pylori*’s natural habitat consists primarily of gastric epithelial cell surfaces and overlying mucin, a niche that also can contain numerous other microbial species. Thus, if AI-2 signaling were important for *H. pylori* at all, this might be most evident in vivo, where AI-2 might affect coexisting microbes in ways that, in turn, impact on receptivity of the gastric mucosal environment to *H. pylori*.

Here we report that deletion of *luxS* in *H. pylori* reference strain SS1 diminished its competitive ability in mice and motility in soft agar, whereas no such effect was seen with an equivalent _*luxS* derivative of the unrelated strain X47. These different outcomes are consistent with *H. pylori*’s considerable genetic diversity and are reminiscent of phenotypes seen after deletion of another nonessential metabolic gene, that

encoding polyphosphate kinase 1. We suggest that synthesis of AI-2 by *H. pylori* may be an inadvertent consequence of metabolite flux in its activated methyl cycle and that impairment of this cycle and/or pathways affected by it, rather than loss of quorum sensing, is deleterious for some *H. pylori* strains. Also tenable is a model in which AI-2 affects other microbes in *H. pylori*'s gastric ecosystem and thereby modulates the gastric environment in ways to which certain *H. pylori* strains are particularly sensitive.

Keywords: *Helicobacter pylori, luxS*, autoinducer, fitness

S16-1

Human Pathogenic Enteric Viruses in Aquatic Environments of Korea

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Water sources are occasionally contaminated by human enteric viruses through discharge of untreated domestic and industrial wastewaters. The enteric viruses in aquatic environments are emerging issues for the cases and outbreaks of the viral diseases, even at low concentrations. So, it is necessary to investigate the occurrence of human enteric viruses in aquatic environments of Korea.

Human enteric virus was detected in tap water, drinking water reservoirs, river water tributaries, and sea waters. The viability was confirmed by the cell culture-PCR techniques and sequences. The infectious viral particles were estimated in streaming water sources, including drinking water reservoirs (Paldang and Jamsil) and a river tributary (the Wangsuk River) during 1997-1998. The Most Probable Number of Infectious Unit (MPNIU/100 liters) ranged from 0.93 to 84.0. Forty-six of the 69 samples (66.7%) had a cytopathic effect on BGMK cells under a microscope. Fifty-three of the 69 samples (76.8%) gave one or more positive results from the ICC-PCR assays of the tested viruses. The sequence analysis of enteroviruses showed that most of the detected enteroviruses (87.9%) were similar to poliovirus, particularly with poliovirus type 1. Several types of adenoviruses were detected in streaming water sources. A total of 50 treated tap water samples were included from September 1997 to September 2001, collected from 27 sites in three urban areas: Seoul, Busan, and Incheon. It was surprising that infectious viral particles were found in tap water samples: 21 of 50 tap water samples (42.0%) showed positive results for enteric viruses confirmed by the cell culture techniques. The frequency of the virus contamination of tap water increased and reached 58.0% of the cell culture-PCR results. The quantities of viruses in tap water within a range of 1.2 to 20.3 MPNIU/1000 liters were significantly high relative to the virus level in drinking water claimed by US EPA. The sequence analysis showed that the most positive strains of enteroviruses confirmed by the cell culture-PCR method had high similarities with poliovirus type 1, coxsackievirus type B, and echovirus type 6. Several types of adenoviruses found in tap water samples and other water samples were closely related to enteric adenovirus types, 40 and 41.

Monitoring surveys of infectious adenoviruses and enteroviruses were conducted based on the cell culture-PCR techniques using a combination of A549 and BGMK cell lines during 2002-2003 in four river water tributaries (the Sanbon River, the Hwajeong River, the Ansan River, and the Siheung River) in Gyeonggi Province and during 2003-2004 in three seawater sites (Goheung, Seosan, and Tongyeong). Thirty of the 40 samples (75.0%) in the River tributaries and two of six samples (33.3%) in seawater gave the positive results for enteric viruses. In the River tributaries, the frequencies of positive virus strains able to grow on A549 and BGMK (65.0%) were higher than those grown on BGMK alone (50.0%). Among the samples which did not exhibit any cytopathic effects, three of the River samples and four of seawater samples showed the positive reactions subsequently conducted by the cell culture-PCR method. The experiments showed a discrepancy between the combined and separate cell line uses for the cultivation of enteric viruses: The similar results for enteroviruses were obtained by either method using the two cell lines separately, but the separate uses of those cell lines for the detection of adenoviruses gave different results from one another: the A549 cell line was effective for all samples to detect adenoviruses more sensitively than the BGMK cell line. The results suggest that the developed cell culture-PCR method using a combination of A549 and

BGMK cell lines is applicable for monitoring infectious adenoviruses and enteroviruses, and for confirming the viabilities in aquatic environments.

These studies showcase how much Korean water sources are contaminated by infectious enteric viruses. In most cases, it makes us conscious that the water contaminations by human pathogenic enteric viruses are chronical, and the frequency and rate of the viral contamination becomes higher and faster in our water sources such as drinking water reservoirs, the River tributaries very near or at the Seoul Metropolitan areas, and coastal areas located near shell-fish farming areas. They are considered to act as potential reservoirs of human pathogenic enteric viruses. This means, our water sources used in drinking water, agriculture, aquaculture, and other domestic and recreational purposes are not safe any more to avoid human contacts with these infectious viruses. It is difficult to ignore that the contamination of our water sources by human pathogenic enteric viruses is very associated with the cases of the viral infection and outbreaks that increase in our society nowadays.

Keywords: human pathogenic enteric viruses, cell culture, cell culture-PCR, water contamination

S16-2

Microbial Diversity: Progress and Potential

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Mining microbial metabolites of small molecules for drug discovery is still promising! Recently, platensimycin and plectasin were identified as new potent antibiotics from an actinomycete strain and a saprophytic fungus. The new antimicrobial, called platensimycin and made by *Streptomyces platensis*, was identified from a soil sample that was collected in South Africa and subjected to an innovative screening procedure. This promising candidate from a novel class of antimicrobials acts by targeting an enzyme needed for making bacterial cell membranes, so effectively inhibits drug-resistant pathogens including MRSA. Another new small cysteine-rich peptide, called plectasin and made by *Pseudoplectania nigrella*, was identified from northern European pine forests. Plectasin showed potent activity against pneumonia caused by *Streptococcus pneumoniae* as efficaciously as vancomycin and penicillin. These findings identify microbes as novel sources of antimicrobials as ever.

At the same time, it is generally argued that less than one percent of all microorganisms are known and culturable. But are any microorganisms truly unculturable or have our attempts simply failed to isolate those microorganisms? Traditional culturing approaches have largely relied on growth media that attempt to mimic the natural environment. However we now recognize the value and challenges of developing new culture techniques that incorporate an understanding of the localized physical structure and chemical environment of an organism's habitat, for example, speciation of dissolved organic matter, minor and trace element compositions and mineral surfaces. Furthermore, many environmentally important microbes may grow slowly and prefer nutrient-limited conditions. This is especially true of microbes from the ocean or the deep subsurface. In traditional media, the amount of energy available from targeted metabolic reactions is at least several orders of magnitude higher than in most natural ecosystems. This kind of slow-growing is often dependent upon its associations with other microbes. Recognition of these associations may be crucial in our ability to culture either individuals or simple consortia in the laboratory. In future, emphasizing purified consortia may be as important as the historical microbiological emphasis on pure cultures.

Recent advances in the culture of novel microbes shows us the efficacy of using very dilute media for culturing soil microbes. The challenges now lie in overcoming the limitations of traditional culturing techniques and improving culturing techniques to isolate novel organisms known only from 16S rRNA sequences. To further efforts to culture more microorganisms, leading to increased understanding of the genetic potential and biogeochemical function of microbial life should be considered as properties.

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Keywords: Diversity, Microbial isolation, Drug discovery

S16-3

Diversity of Pumping Rhodopsins in Maxwell Bay, Antarctica

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Proteorhodopsin (PR), a retinal-containing seven transmembrane protein that functions as a light-driven proton pump was discovered in the genome of marine bacteria. They divided into two major groups: GPR (Green light absorbing PR) and BPR (Blue light absorbing PR). Previously, PRs have been classified by absorption maximum at pH 7.0: GPR (λ_{max}=527 nm) and BPR (λ_{max}=490 nm). However, we try to divide BPR and GPR by photocycle rate and pumping activity because color of PRs has nothing to do with ion- transport function. We isolated 18 PRs (GPR and BPR) from Maxwell Bay, Antarctica. It showed that there are not just BPRs in Antarctic Ocean as previously reported by Dr. Beja group. Interestingly, we discovered identical MBP, eBAC31A08 in Antarctic Ocean known as GPR. Especially, a retinal binding site Tyr200 in MBP is replaced with Asn which shows slow photocycle. The pKa of most PRs from Maxwell Bay is usually high. The fact there are BPRs with low pumping activity and high pKa in Antarctica surface water implies that proteobacteria might not need much energy in cold environment. We are trying to measure the laser–induced absorption changes to confirm the positive relationship between pumping activity and photocycling rate.

Results and Discussion

1. **PR diversity analysis from genomic DNA in Maxwell Bay, Antarctica**
   Proteorhodopsin genes were amplified with polar region genomic DNA from Antarctic ocean by PCR using both non-degenerative primers and degenerative primers which are designed by conserved N-terminal and C-terminal region. PR genes are divided into two groups. First group (747 nucleotides) has large homology with MBP on amino acid level. Second group (750 nucleotides) has about 80% homology with eBAc31A08 and HOT_75m4 and about 90% homology with RED23. In amino acid sequence alignment, 16 PRs (1st group) are different from each other at 23 positions out of 247 amino acids. Among 23 positions, three positions are retinal binding sites.

2. **Absorption spectroscopy and pKa of the acid and alkaline form transition**
   At pH 7.0, half of the 18 PRs are blue-shifted than λmax of MBP and the other half of them are red-shifted. At pH 4.0, the absorption maximum of almost all PR is blue shifted than MBP. Interestingly, the retinal binding site Tyr200 in MBP is replaced with Asn in ten PRs and nine of the ten PRs are blue-shifted at pH 10.0. The pKa of most PRs from Maxwell Bay is especially high in comparison with the pH of Antarctic ocean (~ 8.0).

3. **Proton pumping measurement and flash photolysis**
   PRs with blue-shifted at pH 10.0 tend to have slow photocycling rate and low pumping activity. We are comparing half life of M, O decay of newly found PRs from Antarctica with GPR and BPR. We will measure temperature dependency on photocycle rate and how rhodopsin functions differently in cold environment.

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Keywords: Rhodopsin, proteobacteria, proton pumping, Antartic ocean

S16-4

Fungal Diversity and Discovery of Bioactive Metabolites

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The fungal kingdom offers enormous biodiversity, with around 75,000 known species, and an estimated 1.5 million species in total(Deacon, 2006). Filamentous fungi are of considerable economic and social significance. It is very likely that the future will see an expansion in the number of species which are commercially exploited. Most of these are filamentous fungi, which differ from the yeasts not only in their more complex morphology and development(e.g. asexual and sexual structures), but also in their greater metabolic complexity. Microbial diversity already has contributed significantly to biotechnology industry. In particular, they are well known for production of secreted enzymes and secondary metabolites, many of which have been exploited by man(Turner, 2004).

Firstly, this lecture deals with the diverse fungi isolated from diverse sources in Korea. For example, *Bipolaris zeicola* was isolated from a maize grain using a standard blotter method. *Curvularia* species was isolated from a brown leaf spot lesion of *Euphorbia* plant using the blotter method. *Embellisia chlamydospora*, *Micromucor ramannianus* var. *angulisporus* and a new *Hypocrea* species were isolated from different soil samples by dilution plating method. *Xylaria holophylla* sp. nov. as a endophytic fungus was isolated from inner bark of Korean Manchurian fir. In addition, two new acid-tolerant fungal species, *Phaeomoniella zymoides* and *Phaeomoniella pinifoliorum* spp. nov. were isolated from pine needles as epiphytic fungi(Lee *et al*. 2006). *Colletotrichum boninense*, *Cristulariella moricola* and *Pseudocercospora paederiae* as plant pathogens were isolated from various leaf lesions of their host plants: *Euonymus japonica*, *Vigna vexillata* var. *tsusimensis* and *Paederia scandens*, respectively. The fungal isolates were identified based on the taxonomic descriptions, cultural characters and rDNA ITS region sequence analysis. Secondly, this lecture deals with bioactive metabolites and their exploitation. For example, cochlioquinone(CoA) and cochlioquinone A1(CoA1) were isolated from the culture extracts of *Bipolaris zeicola* as potent anti-angiogenic agents(Jung *et al*. 2003). Terpestacin(a bicyclo 5-15-fused sesterterpene) was isolated from *Embellisia chlamydospora* as a diacylglycerol acyltransferase(DGAT) inhibitor(Lee *et al*. 2003). Enzyme Inhibitors of PTP1B(protein tyrosine phosphatase 1B) were isolated from a zygomycete fungus, *Micromucor ramannianus* var. *angulispous*. Curvularol compound isolated from a *Curvularia* species was shown to inhibit the growth of various cell lines, and verrucarol anti-fungal and anti-bacterial activities. 4-hydroxy 3-(3'-methyl-2'-butenyl)-benzoic acid(HMBA) was isolated from the *Curvularia* species as a cell cycle inhibitor(Kim *et al*. 2004). Griseofulvin antibiotic first isolated from a *Xylaria* species(recently identified as a new fungal species, *Xylaria holophylla* sp. nov. Lee *et al.*) was shown to inhibit the growth of several phytopathogenic fungi(Park *et al*. 2005). Harzianums A and B isolated from a new *Hypocrea* strain showed cytotoxicity to tumour cell lines(Lee *et al*. 2005). In addition, two new fungal metabolites, 2,3-didehydropalitantin and culpin-1-β-galactopyranoside were isolated from an epiphytic fungus, *Paraphaeosphaeria* species and their bioactivities are now being investigated. Thirdly, this lecture deals with first reports of unknown plant diseases caused by several phytopathogenic fungi in Korea. Fourthly, this lecture also deals with phylogeny of the fungal species based on morphology or/and 18S rDNA sequence data.

Biodiversity and biotechnology are strongly interrelated and interdependent. With respect to microbial

diversity, filamentous fungi will be more widely used in a variety of ways in the bio-industry, constituting a rich and novel source for new bioactive metabolites.

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Keywords: Biodiversity, filamentous fungi, metabolites, phylogeny

S16-5

Genomic Heterogeneity and Geographic Structure in Endemic Genotypes of Fluorescent *Pseudomonas*

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We explored the genomic diversity and geographic structure in endemic genotypes of *P. fluorescent*, by using candidate molecular methods to bridge the gap resolution between rDNA RFLP and rep-PCR method. Focal group was selected based on our previous ARDRA result, and subjected to the analyses of 16S rDNA and *gyrB* sequences, whole genomic DNA-DNA hybridization (WDH), and microarray hybridization pattern (MHP). The 16S rDNA sequences were almost identical and *gyrB* sequences showed slightly more divergence than 16S rDNA sequences. WDH and MHP results showed finer resolution than the two sequence-based methods. WDH and MHP results were congruent, and incongruence between 16S rDNA method and *gyrB* method was also as higher as incongruence between the hybridization-based methods and sequence-based methods. We found no geographic clades in 16S rDNA and *gyrB* phylogenetic trees. However, WDH and MHP dendrograms showed week endemicity, and the genetic distances measured by WDH and MHP significantly increased with geographic distance. Additionally, genomic heterogeneity in the endemic genotypes of *Pseudomonas fluorescens* was observed with microarray analysis. MHP identified that 15.40% of the *P. fluorescens* genomes were hyper-variable sequences. Even the MHP-identified conserved parts, which comprised of 3.39% of the genomes, were not as conserved as 16S rDNA, but as much conserved as *gyrB*. These results suggest that the genetic distance continues to diverge over the detection limit of rep-PCR genomic fingerprinting, which was unclear with the rep-PCR fingerprinting due to the upper limit of its resolution. Also, we suggest that the genotypic level is a margin to observe geographically unique groups such as endemic genotypes, and the endemicity can be observed above the genotypic level up to *gyrB* sequence level only with the tendency that genetic distance increases with geographic distance.

Keywords: Genomic heterogeneity, Geographic structure, Microarray

Colloquia

백지

C1-1

Fungal Diversity of the Islands on the Yellow Sea of Korea

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To study the fungal diversity of sea islands, Soyunpyungdo (SY), Oeyeondo (OY) and Gageodo (GG) that are at a geographic distance each other were surveyed and soil samples were collected in spring, summer and autumn three times in 2005. There was no regular correlation between pH change and seasonal change. The soil texture of SY was sandy clay loam but OY and GG were sandy loam. The total organic carbon and total nitrogen contents of OY and GG were lower in summer but the total phosphorus content was higher in summer.

Eco-microplate was used for the profiling of carbon substrate utilization which can be represented as a measure of functional diversity. The substrate usage pattern was reverse to the change pattern of total organic carbon. The habitat disturbance in a smaller island affected the functional diversity of fungi less than in a larger island. The result of F-ARISA showed that the species richness of OY was higher than those of other samples, and SY showed the poorest species richness. Approximately 450 SSU rDNA isolates were cloned and analyzed per island. When the dendrogram of ARDRA test was estimated for the species diversity (species richness) using parameter C formula, the result showed that GG and OY samples consisted of more diverse fungal species than SY sample.

Total 740 clones were sequenced and the ARDRA test developed different band patterns. *Paecilomyces* was the dominant genus in all samples but there were few species in Zygomycota. The phylogenetic analyses based on sequences were the most effective method in evaluating community structures. Even though ARISA and ARDRA were low in resolution, they gave enough information for the comparison of communities. Three islands on the Yellow Sea of Korea contained different structural and functional diversities of fungi which might have been resulted from the differences of plant population, geographical and environmental properties.

Keywords: Fungal diversity, sea islands, soil fungi, Yellow Sea

C1-2

Taxonomic Study on Lichen Genus *Hypogymnia* in China

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The comprehensive study of the lichen genus *Hypogymnia* in China are performed using the methods of morphology, anatomy, chemistry, geography, ecology and molecular systematic biology. Thirty-nine taxa belonging to 36 species of *Hypogymnia* in China (constituting 35% of worldwide *Hypogymnia* species) are reported, among which 7 species and 1 variety new to science and 1 taxa new to China are included.

Based on the comprehensive analysis of phenotype and genotype, there are three important results: first, *Hypogymnia* is a monophyletic clade within *Parmeliaceae*; second, *H. flavida* and *H. hypotrypa* are not two separate species, *H. flavida* is treated as the synonym of *H. hypotrypa*; third, *Hypogymnia taibaiensis* and *H. taibaiensis* var. *noduliformis* with obviously different morphology are conspecific.

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Keywords: *Hypogymnia*, new to science, new to China, phylogenetic analysis

C1-3

Phenotypic and Genotypic Diversity of Fluorescent Pseudomonads Isolated from Rice Rhizosphere Soil

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Fluorescent pseudomonads are predominant among rhizosphere-inhabiting bacteria. The major objective of this investigation was to determine the phenotypic and genotypic diversity and antagonistic potential of fluorescent pseudomonads living in rice rhizosphere soils. These results as well as cluster analyses indicate the relative abundance of *P. fluorescens* (biovars III) and functional diversity of other fluorescent pseudomonad species in rice rhizosphere soils of India. Twenty-five of the 135 strains showed broad-spectrum antifungal activity towards phytopathogenic fungi. Strains were also screened for functional characters such as production of hydrogen cyanide (HCN), protease, catalase, lipase, cellulase, pectinase, chitinase, indole-3-acetic acid (IAA), aminocyclopropane-1-carboxylate deaminase (ACC) deaminase and *N*-acylhomoserine lactone (AHL). These bacteria were tested for the presence of genes that specify functions such as production of antibiotics, phenazine-1-carboxylic acid (PCA), phenazine-1-carboxamide (PCN), pyoluteorin (PLT), pyrrolnitrin (PRN) and 2,4-diacetylphloroglucinol (DAPG) as well as for denitrification genes, nitrate reductase (*narG*) and nitrous oxide reductase (*nosZ*). Genotypic diversity of antagonistic fluorescent pseudomonad strains were assessed by molecular typing using repetitive sequences (Rep)-based PCR such as enterobacterial repetitive intergenic consensus (ERIC), repetitive extragenic palindromes (REP), BOX and 16S rDNA-amplified ribosomal DNA restriction analysis (ARDRA). These results revealed a high degree of diversity among the antagonistic fluorescent pseudomonads of rice rhizospheric soil.

Keywords: Fluorescent pseudomonads, antibiotic gene; DNA Fingerprinting; ARDRA

C1-4

Clinicopathological and Immunological Characteristics of Thoroughbred Horses with Streptococcal Infectious Upper Respiratory Diseases

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The incidence rate of infectious upper respiratory disease (IURD) and the bacterial pathogens of IURD in Thoroughbred racehorses at the Seoul Race Park, Korea were evaluated. IURD of Thoroughbred racehorses has been a frequent problem at the Seoul Race Park, with an estimated incidence of 29.6% (IURD/resident horses) for the past five years (2001-2005). *Streptococcus equi* subsp. *equi* and *S. equi* subsp. *zooepidemicus* were the cause of approximately 15% of IURD from March 2003 to January 2004. They were resistant to neomycin, streptomycin, amikacin as well as trimethoprim which were previously effective for strangles. A number of the epidemiological and immunological risk factors for occurrence of IURD were identified, the most important being the season, age, and the stabled period in association with the immune status of the horses. There was significant movement of horses in and out of the Park from spring to fall and the least in winter. This coincides with i) the peak of infection, which was consistently observed at 6 to 8 weeks after arrival of young horses (2-3 years old), ii) the high isolation rate of multi-drug resistant (MDR) *Streptococcus equi*, the most important bacterial etiologic agent of equine IURD, from nasal specimens, and iii) the significantly lower proportions of $CD4^+$ and $CD8^+$ T lymphocytes and B lymphocytes expressing MHC class II in the IURD patient group compared with the healthy control group in summer and fall. It suggests the immune system of IURD patients may be depressed and not be able to respond to *S. equi* with MDR characteristics present in the stables properly. It may be desirable in future to take measures against equine IURD with the assessment of the immune status of horses at the arrival stage in the Park.

Keywords: IURD, *Streptococcus equi* subp. *equi*, Multi-drug resistance, Clinicopathology, Immunology

C1-5

Regulation of Tumor Necrosis Factor-Alpha Gene Expression and Signal Transduction during *Orientia tsutsugamushi* Infection

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Orientia tsutsugamushi, an obligate intracellular bacterium, is the causative agent of scrub typhus. The disease is histopathologically characterized by inflammatory manifestations, indicating that orientiae induce mechanisms that amplify the inflammatory response. To understand the pathogenesis of scrub typhus, I examined the induction of tumor necrosis factor alpha (TNF-α) after infection with *O. tsutsugamushi* in mice, peritoneal macrophage, and macrophage cell line. Peak expression of TNF-α gene was observed between 4 and 8 days after infection in mice. The TNF-α mRNA were induced and showed a transitory peak for 6 to 24 h after infection in macrophage cell line. TLR4 defective mice were found to lack the ability to respond to *O. tsutsugamushi* as measured by secretion of TNF-α by macrophages. Western blot analysis of cell lysates indicates that extracellular signal-regulated kinase 1/2 (ERK1/2), Jun N-terminal kinase 1/2 (JNK1/2), and p38 mitogen activated protein kinases (MAPKs) become phosphorylated, and hence activated in *O. tsutsugamushi*-stimulated macrophages. Selective inhibitors of ERK1/2 (PD98059), JNK1/2 (SP600125), and p38 (SB203580) MAPK pathways could all completely prevent TNF-α secretion. However, these drugs did not prevent either bacterial internalization and invasion into the host cells or TNF-α processing and secretion. Host TNF-α production via p38 and JNK pathways by this bacterium was found to be regulated by post-transcriptional mechanism, mainly by translational control. In contrast, ERK pathway mainly control the transcription step of TNF-α gene expression. Orientia inactivation by heat did not abolish induction of TNF-α production. However, inhibition of cellular invasion by treatment of host cell with cytochalasin D led to a diminished TNF-α induction, suggesting requirement of invasion by bacteria for this host cell response. In conclusion, our data indicate that MAPKs pathways are required to induce maximal TNF-α production in host cells during *Orientia tsutsugamushi* infection.

Keywords: *Orientia tsutsugamushi*, TNF-α, ERK, JNK, p38, signal transduction, regulation of gene expression

C1-6

Dissecting the Mechanism of Lipid Rafts Involvement in Mouse Hepatitis Virus Replication

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Thorp and Gallagher first reported that depletion of cholesterol inhibited virus entry and cell-cell fusion of mouse hepatitis virus (MHV), suggesting the importance of lipid rafts in MHV replication (J. Virol. 2004, 78:2682-2692). However, the mechanism of lipid rafts involvement is not clear. We explored the mechanism and extent of lipid raft involvement in MHV replication. We showed that cholesterol depletion by methyl beta-cyclodextrin did not affect virus binding but reduced virus entry. Furthermore, MHV spike protein bound to nonraft membrane at 4 C but shifted to lipid rafts at 37 C, indicating a redistribution of membrane following virus binding. Thus, the lipid raft involvement in MHV entry occurs at a step following virus binding. We also found that the viral spike protein in the plasma membrane of the infected cells was associated with lipid rafts, whereas that in the Golgi membrane, where MHV matures, was not. Moreover, MHV does not incorporate lipid rafts into the virion. However, MHV spike protein has an inherent ability to associate with lipid rafts. Correspondingly, cell-cell fusion induced by MHV was retarded by cholesterol depletion, consistent with the association of the spike protein with lipid rafts in the plasma membrane. These findings suggest that MHV entry requires specific interactions between the spike protein and lipid rafts, probably during the virus internalization step.

Keywords: Mouse Hepatitis virus, lipid raft, virus entrys

C2-1

Distribution of Waterborne Enteric Viruses in Korea Surface Water

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To survey the distribution of waterborne enteric viruses in Korea, a total of 192 surface water samples were collected by filtration with 1-MDS filter from July 2003 to January 2006. The presence of waterborne enteric viruses were investigated by total culturable virus assay using buffalo green monkey kidney (BGMK) cells, and the results showed that sixty three samples (32.8%) were positive for the presence of enteric viruses and the average concentration was 3.1 ± 18 MPN/100 L. The relationship between the detection frequency of enteric viruses and the physicochemical environmental factors was investigated, and our results suggested that turbidity of water samples had a significant correlation with the occurrence of the enteric viruses. Also, it was found that the frequency of enteric viruses was higher in low temperature or winter season. It was also assessed that total coliforms (T.C.) and fecal coliforms (F.C.) were compatible as indicator microorganisms of waterborne enteric viruses. The results of statistical analysis showed that T.C. and F.C. might not be sufficient for the microbial indicators of waterborne enteric viruses in the samples analyzed in this study. In conclusion, the waterborne enteric viruses were detected in 63 samples (32.8%) by TCVA, the detection frequency of enteric viruses was affected by the physicochemical environmental factors, and T.C. and F.C. might not be compatible as the microbial indicators of waterborne enteric viruses.

Keywords: waterborne enteric viruses, total coliforms, fecal coliforms, physicochemical environmental factors

C2-2

Mass Spectrometric Studies of Virus Structure and Dynamics

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Viral capsids are dynamic macromolecular machines that self-assemble and undergo structural transformations in a controlled manner to reach an infectious form. The viral capsid is a simple model to study macromolecule dynamics because it consists of multiple copies of one or a small number of chemically identical capsid proteins assembled using simple symmetry into a megadalton structure.

Although X-ray crystallography and cryo-EM provide high resolution structural information, they cannot follow structural dynamics, nor can they monitor continuous conformational changes of macromolecules. Solvent shielding and intra- or inter-molecular hydrogen bonding are directly related to protein structure, therefore the amide hydrogen exchange rate is a sensitive probe of protein structure and dynamics. Mass spectrometry makes it possible to apply this technique to large protein complexes such as viruses.

We have used mass spectrometry based hydrogen/deuterium exchange and chemical cross-linking to investigate structural transformations from precursor form to mature capsids of bacteriophage P22 and HIV-1 capsids. These studies provide a detailed model for the structural and biochemical changes accompanying viral maturation.

Keywords: Mass spectrometry, Virus structure, Virus dynamics, Hydrogen/deuterium exchange, Chemical cross-linking

C2-3

Regulation of Hepatitis C Virus RNA Replication by Phosphorylation of the Viral RNA Polymerase

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Hepatitis C virus (HCV) is a major etiologic agent of chronic hepatitis, and chronic infection of HCV often results in hepatocellular carcinoma. It is a member of the *Flaviviridae* family and has a single-stranded, positive-sense RNA genome encoding a polyprotein, which is processed into the four structural proteins and six nonstructural proteins. Among the nonstructural proteins, the NS5B protein is an RNA-dependent RNA polymerase that is important for the replication of HCV RNA genome. We identified a peptide that most closely resembles a short region of the protein kinase C-related kinase 2 (PRK2) by screening of a phage-displayed random peptide library with NS5B. Competitive phage ELISA with a synthetic peptide could bind NS5B proteins with a high affinity. Coimmunoprecipitation and colocalization studies demonstrated *in vivo* interaction of NS5B with PRK2. *In vitro* kinase assays and 2-dimensional phosphoamino acid analyses (2D-PAA) demonstrated that PRK2 specifically phosphorylates the Ser residue(s) of the NS5B. We also detected the Ser-phosphorylated form of NS5B by 2D-PAA through metabolic cell labeling. Knock-down of the endogenous PRK2 expression using a PRK2-specific siRNA and peptide nucleic acids (PNA) inhibited HCV RNA replication. In contrast, PRK2 overexpression dramatically enhanced HCV RNA replication. Treatment of PRK2 inhibitors in HCV subgenomic replicon cells effectively inhibited HCV RNA replication through inhibition of NS5B phosphorylation. The anti-HCV effect of PRK2 inhibitors was further enhanced by combination with interferon-α. Our results demonstrate that PRK2 effectively regulates HCV RNA replication by the NS5B phosphorylation and can serve as a cellular target for therapeutic intervention of HCV replication.

Keywords: HCV, NS5B, phosphorylation, PRK2, RNA replication, siRNA, PNA, PRK2 inhibitor, interferon-α

C2-4

Comparison of Gene Expression Profile of Prion Protein-Deficient Neuronal Cell Lines and Mice with Wild-Types

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A key factor in the development of prion diseases (also known as transmissible spongiform encephalopathies, TSE) is the change of a normal protein (PrP^{C}) to an abnormal, pathogenic protein (PrP^{Sc}). An important question related to investigation of the pathogenesis of these diseases is the normal function of PrP^{C}; this issue remains unresolved. It was thought that a useful approach would be to compare cell lines with PrP^{C} to those without PrP^{C}. To that end we developed lines from Rikn $PrP^{-/-}$ mice and from Zürich I $^{-/-}$. The former line overexpresses Dopple (Dpl) protein, whereas the latter line does not. In this study, we performed gene microarray analyses using these newly established $PrP^{-/-}$ cell lines, brains of $PrP^{-/-}$ mice, and their respective wild types to study the effect of loss of PrP^{C} on cell functioning. Using a large-scale gene array-based approach, which covers 40,000 characterized genes, we identified 39 genes in $PrP^{-/-}$ cell lines and 52 genes in $PrP^{-/-}$ mice which showed different expression levels compared to controls of at least 8-fold and 2-fold, respectively. In addition, 11 genes were identified to have similar expression patterns in both $PrP^{-/-}$ cell lines and mice. According to microarray results in vitro and in vivo, PrP^{C} is postulated to be involved in signal pathway, particularly in transcription, protein trafficking, and immune responsiveness. In this study using cDNA microarray method we have screened a large quantity of genes and based on the results we present a macroscopic schema of PrP^{C} function inside cells.

Keywords: normal PrP (PrPC), PrP-deficient cell line, dopple (Dpl) protein, cDNA microarray

C2-5

Activation of Mitogen-Activated Protein Kinases in Hamster Brains Infected with 263K Scrapie Agent

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We investigated the expression, activation, and distribution of c-Jun N-terminal kinases (JNKs), p38 mitogen-activated protein kinases (p38 MAPKs) and extracellular signal-regulated kinases (ERKs) using Western blotting and immunohistochemistry in the brains of hamsters infected with 263K scrapie agent to clarify the role of these kinases in the pathogenesis of prion disease. The immunoblot analysis demonstrated that activation of JNK, p38 MAPK, and ERK in whole brain homogenates were increased in infected animals. Phosphorylation of CREB, a downstream transcription factor of active ERK, was significantly increased in scrapie-infected hamsters. The immunohistochemical study showed that active ERK was enhanced in infected hamsters compared with controls. Active ERK immunoreactivity was observed within neurons in dentate gyrus and in GFAP-positive reactive astrocytes of infected animals. The expression level of c-Jun mRNA as well as protein, a substrate of active JNK, was increased in infected animals. Significant increase of the JNK activity upon glutathione *S*-transferase (GST)-c-Jun was observed in infected compared with control animals. Phospho-c-Jun immunoreactivity was observed only in neurons of thalamus in infected group. These findings indicated that the JNK pathway was activated in scrapie-infected group. The chronological activation of MAPKs using immunoblot analysis indicates that these kinases are sequentially activated during the pathophysiology of prion disease. Taken together, these findings lend credence to the notion that MAPK pathways are dysregulated in prion disease and also indicate an active role for this pathway in disease pathogenesis.

Keywords: c-Jun N-Terminal Kinase (JNK), p38 Mitogen-Activated Protein Kinase (p38 MAPK), Extracellular Signal-Regulated Kinase (ERK), Prion Disease.

C3-1

Identification of Phenotypic and Genetic Differences between *Listeria monocytogenes* Strains

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Listeria monocytogenes is a food-borne bacterial pathogen that can cause severe disease and high mortality in humans. Subtyping with DNA microarrays provides an alternative means to resolve genetic differences among isolates, and unlike PFGE and ribotyping, it can identify specific genes associated with strains. Therefore, microarray was constructed using 20 *L. monocytogenes* strains (6 serovars), and it distinguished serotypes and subtypes in accordance with PFGE and ribotyping and identified several subtype-specific genes. In vivo model, which can determine the ability of strains to penetrate the intestinal mucosa and invade organs, was established with A/J mice using 6 epidemic and 6 environmental strains, and epidemic strains were significantly more invasive than environmental strains ($P< 0.05$). Among the strains tested, one epidemic strain had higher infectivity as compared to the environmental strain that had an identical PFGE subtype. Suppression subtractive hybridization was used to identify genetic differences that might account for the difference in phenotype, and 18 regions (similar to phage proteins) were found to be unique to the environmental strain. Whole genome microarray expression analysis indicated that transcription of two genes in the *agr* locus was significantly down regulated in the environmental strain. Subsequently a single nucleotide insertion was identified in the *agrC* ORF of the environmental strain, which resulted in a severely truncated protein and appeared to cause a significant decrease in listeriolysin O secretion.

Keywords: *Listeria monocytogenes*, invasiveness, phage, *agr* locus, listeriolysin O, microarray

C3-2

Toward the Interdisciplinary Research at the Biology

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The ultimate goal of researches in biology is to expand our understanding of living systems at all scales. On a point of view of bottom-up approach, elucidating biological phenomena at the molecular level is needed to describe living systems in terms of chemistry and physics. On a point of view of top-down approach, on the other hand, accumulated biological data, including genome sequencing and high-throughput experimentation, are offering an opportunity for understanding biology from a systems perspective. To effectively explore biological problems from molecular level to integrated systematic level, interdisciplinary research between scientists working in various fields has been considered vital. Experimenters in biology, chemists, physicists, computer scientists, mathematicians and engineers are creating new knowledge in their own disciplines and integrating it together to solve biological problems. Here, I introduce the general investigation trend of this multidisciplinary science, especially focusing on biology and computer science, and present several recent researches.

Keywords: Multidisciplinary science, computational structural biology, bioinformatics, systems biology

C3-3

Coactivation of *Vibrio vulnificus putAP* Operon by cAMP Receptor Protein and PutR through Cooperative Binding to Overlapping Sites

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The CRP (cAMP receptor protein) positively regulates the expression of *Vibrio vulnificus putAP* genes encoding a proline dehydrogenase and a proline permease. In the present study, an open reading frame encoding PutR was identified downstream of the *putAP* genes and a mutational analysis revealed that the PutR protein was also involved in regulating the *putAP* transcription by activating P_{put} promoter. Although CRP acts as a primary activator and the influence of PutR on P_{put} is mediated by CRP, the level of P_{put} activity observed when PutR and CRP functioned together was greater than the sum of P_{put} activities achieved by each activator alone. Western blot analyses demonstrated that the cellular levels of PutR and CRP were not significantly affected by each other, indicating that PutR and CRP coactivate P_{put} rather than function sequentially in a regulatory cascade. Two adjacent binding sites for PutR mapped by *in vitro* DNase I protection assays were found to overlap the CRP binding sites and were centered -91.5 (PCBI) and -133.5-bp (PCBII) upstream of the transcription start site of P_{put}, respectively. PutR and CRP bind to the sites cooperatively and a dissection of the role of the binding sites revealed that CRP at PCBI plays the most crucial role in the activation of P_{put}. Accordingly, the present results revealed that PutR and CRP coactivate the expression of P_{put} and exert their effect by cooperatively binding to the promoter.

Keywords: *Vibrio vulnificus*, *putAP,* CRP, PutR

C3-4

Escherichia coli Enzyme IIA^{Ntr} Interacts with the K^+ transporter TrkA to Regulate *ilvBN* Derepression

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The phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) phosphorylates sugars and regulates cellular metabolic processes. While the proteins of carbohydrate PTS have been shown to regulate numerous targets, little such information is availble for the nitrogen-metabolic PTS. To elucidate the physiological role of the nitrogen-metabolic PTS, phenotype microarray (PM) analysis of *E. coli* K-12 strain MG1655 deleted for the *ptsP* gene encoding the first enzyme of the nitrogen-metabolic PTS was carried out. Together with the PM data, further studies revealed that the dephospho-form of $EIIA^{Ntr}$ is required for derepression of the *ilvBN* operon encoding acetohydroxy acid synthase I catalyzing the first step common to the biosynthesis of the branched-chain amino acids. Ligand fishing experiments were performed by using the purified His-tagged $EIIA^{Ntr}$ as bait to find out factors involved in the derepression of the *ilvBN* operon. Interestingly, the $EIIA^{Ntr}$ interacted with the potassium transport TrkA in a phosphorylation state-dependent manner.

Keywords: nitrogen-metabolic PTS, phenotype microarray, leucine toxicity, *ilvBN* operon, TrkA

C3-5

Revolutionary Recombinant Hybrid Mussel Adhesive Protein fp-151

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Mussel adhesive proteins (MAPs) have attracted public attention as potential environmentally friendly adhesives in aqueous conditions and in medicine. However, attempts to produce functional recombinant MAPs (mainly foot protein type 1, fp-1) in several expression systems have failed. Previously, we reported that recombinant foot protein type 5 (fp-5) can be produced as a functional form with significant adhesive ability in *Escherichia coli* system. However, practical usage of recombinant fp-5 was limited by several problems such as low production yield, low purification yield, and high insolubility after purification. Here, we designed and constructed novel hybrid bioadhesive fp-151 that is fusion comprising 6 repeated fp-1 decapeptides at both terminus of fp-5 to solve these limitations. Recombinant hybrid fp-151 was successfully expressed with higher production yield (~2 g/L, fed-batch fermentation) in *E. coli*. Interestingly, this hybrid version could be easily purified by acetic acid extraction with high yield (~62%) and purity (~90%). Importantly, purified hybrid fp-151 showed superior solubility in aqueous solution. Therefore, we could formulate this bioadhesive with relatively high concentration (~200 g/L) in 5% acetic acid solution and this enabled bulk adhesion test. Comparative bulk adhesion study using plastic pillar showed about 6 times higher tensile strength of hybrid fp-151 than that of commercial fibrin glue. We also demonstrated that hybrid fp-151 could attach various types of mammalian cells on culture surface with good biocompatibility. Therefore, this novel hybrid fp-151 can be a revolutionary and practical bioadhesive with strong adhesive ability, simple purification, and proper manipulation properties.

Keywords: Mussel adhesive protein, bioadhesive, hybrid fp-151, *Escherichia coli*, cell adhesion, bulk adhesion

C3-6

Central Carbohydrate Metabolism in Extremely Thermoacidophilic Archaea: Comparative Studies of Archaeal and Bacterial *D*-gluconate Dehydratases, a Key Enzyme of the Modified Entner-Doudoroff Pathways

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Extremophiles are attractive biological resources for industrial biotechnology and for dramatically improving bioprocesses as environmentally-friendly technologies (1). Unlike conventional microorganisms, several extremophiles including hyperthermophilic microorganisms have a modified version pathway which can synthesize nonphosphorylated intermediates in the glycolysis reactions. The thermoacidophilic archaeon *Sulfolobus solfataricus* utilizes *D*-glucose as a sole carbon and energy source via the nonphosphorylated Entner-Doudoroff (ED) pathway (2,3). This microorganism metabolizes *D*-gluconate, the oxidized form of *D*-glucose, to pyruvate and *D*-glyceraldehyde by using two unique enzymes, *D*-gluconate dehydratase and 2-keto-3-deoxy-*D*-gluconate aldolase. Although D-gluconate dehydratase (EC 4.2.1.39) is a key enzyme involved in this pathway, little is known about the enzyme. In this study, we report the purification and characterization of *D*-gluconate dehydratase from *S. solfataricus*, which catalyzes the conversion of *D*-gluconate to 2-keto-3-deoxy-*D*-gluconate (3). We also demonstrated the identification and characterizations of two bacterial counterpart enzymes involved in the partially nonphosphorylated ED pathways from *Achromobacter xylosoxidans* and *Clostridium acetobutylicum*. These comparative studies will allow the evolutionary relationship between archaeal and bacterial *D*-gluconate dehydratases through phylogenetic analyses and emerging biochemical data (4). Finally, the results of this research could be used for the development of biocatalyst systems to produce other carbohydrates and amino acids derived from the ED pathway in hyperthermophiles.

Keywords: gluconate dehydratase, Extremophiles, modified ED pathway, archaea, glycolysis, sulfolobus solfataricus

•Poster Sessions

A. Systematics and Evolution

B. Environment and Ecology

C. Differentiation and Morphology

D. Physiology and Biochemistry

E. Fermentation and Metabolites

F. Genetics and Genome

G. Infection and Pathogenesis

H. Immunology and Signal Transduction

I. Biotechnology

J. Food Microbiology

K. Others

면지

A001

Notes on Three Species of the Genus *Euphoriomyces* (Laboulbeniales) from Korea

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Three species of the genus *Euphoriomyces*(Laboulbeniales) were found newly from Korea. *E. agathidii*(Maire) Tavales was collected from *Agathidium sp*.(Coleoptera, Leiodidae). The characteristic traits of this species are the distal portion of the primary axis composed of 5-11 superposed layers and bearing more or less the elongated appendages. *E. cybocephali*(Thaxter) Thaxter was collected from *Pseudocolenis hilleri* Reitter.(Coleoptera, Leiodidae). The primary axis of this species composed of 5-9 superposed layers is simple, not branched and a single antheridium occurs on the apex of the secondary axis. *E. sugiyamae* Majewski was collected from *Scaphisoma rufum* Achard.(Coleoptera, Scaphidiidae). This species is very unique in having the antheridia formed as coner cells with lateral necks.
Keywords: Laboulbeniales(Ascomycotina), Euphoriomyces, Korea

A002

Taxonomic Evaluation of the Genera *Ruegeria* and *Silicibacter:* A Proposal to Transfer the Genus *Silicibacter* Petursdottir & Kristjansson 1999 to the Genus Ruegeria Uchino *et al*. 1999

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The taxonomic positions of the genera *Ruegeria* and *Silicibacter* were evaluated by polyphasic investigation. It is evident from 16S rRNA gene sequence analysis that both genera are closely related as they formed a monophyletic clade with high sequence similarities (96.95 to 98.2 %). Several properties commonly found in these taxa strongly suggest that they should be classified in the same genus. Further comparative study based on DNA-DNA hybridization, phenotypic characterization and fatty acid analysis indicated that the members of this clade, namely *Ruegeria atlantica*, *Silicibacter lacuscaerulensis* and *Silicibacter pomeroyi*, can be readily differentiated from each other. On the basis of the polyphasic data obtained in this study, all species of the genus *Silicibacter* should be transferred to the genus *Ruegeria*, since the latter has nomenclatural priority. It is therefore proposed to transfer S*ilicibacter lacuscaerulensis* and *Silicibacter pomeroyi* to the genus *Ruegeria* as *Ruegeria lacuscaerulensis* comb. nov. and *Ruegeria pomeroyae* comb. nov.
Keywords: Ruegeria, Silicibacter, Roseobacter clade

A003

Taxonomic Studies on *Myelochroa* from Korea

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Myelochroa is typical foliose lichen characterized by having the yellow-colored medulla. There are 26 species in this genus. Eleven species of *Myelochroa* have been reported from Korea so far. However, there have been no revisions about them. Careful examination of the specimens collected from the main mountains of South Korea and deposited in the Korean Lichen Research Institute (KoLRI) has been attempted. Nine species have been revealed to occur and confirmed. As the other three species reported by the former Korean lichenologists are not supported by Korean voucher specimens, they were not traceable for this study. A key to the species is provided. The differences among the nine species and the other related species are also presented. This is the first report on taxonomic revision of *Myelochroa* in Korea.
Keywords: lichen, lichen-forming fungi, Korea, taxonomic revision, Myelochroa

A004

Molecular Analysis of Korean *Anzia opuntiella* (Lichenized Ascomycota) Based on ITS-rDNA Sequences

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Recent taxonomic study on *Anzia* in Korean peninsula confirms that only 2 species of *A. colpota* and *A. opuntiella* are distributed in South Korea. *A. opuntiella* is the most common species and can be separated into 2 groups according to morphological difference. Although there was no anatomical difference between 2 groups, one has larger and wider thalli than the other. There was no difference in chemical analysis between 2 groups as well. Molecular analysis was attempted to figure out the separation of the species. DNA was extracted from whole thalli and the ribosomal ITS region was amplified by PCR using fungal specific primers. Resulting products were sequenced to analyze the relationship of *A. opuntiella* species. A total of 564 bp of rDNA sequence including ITS1 and ITS2 were determined for all specimens. Sequence analysis of *A. opuntiella* lichens clearly separated the species into 2 groups same as the morphological groups. It was shown that 6% sequence divergence of the ITS region was found within *A. opuntiella* lichen species tested. The sequence divergence was contributed to the variation of ITS regions between 2 groups, whereas only 1 base was different in 5.8S rDNA region. This sequence divergence implies that *A. opuntiella* currently treated as same species based on morphological and chemical characteristics can be separated into 2 groups. Further study will be needed to confirm our findings.
Keywords: Anzia, lichen, lichen-forming fungi, Korean Peninsula, ITS sequence

A005

New Record of *Karoowia saxeti* (Stizenb.) Hale in South Korea

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Karoowia saxeti was recorded during the lichen field expedition in southern part of Korea in 2005. The lichen was found on the rock surface along coastal line. This species was easily recognized by chemistry (K^+ yellow) and the presence of isidia. Thallus saxicolous, subcrustose, more or less lobate at the center with clearly lobed margins, 2 - 6 cm broad, pale yellowish green; lobes irregular, variable, upto 1.0mm wide, not branched, flat to more or less convex, contiguous to subimbricate; upper surface continuous, emaculate, moderately isidiate, the isidia subglobose to cynlindrical, darkening at the tips, unbranched; low surface black with a spongy rhizoidal and lamellar layer. Apothecia initially aspicilioid but soon emergent and sessile, the disk brown, 0.5-0.8mm in diameter; spore 5-6×9-11 μm. HPLC analysis proved the presence of stictic acid (K^+ yellow), norstictic acid and usnic acid. ITS sequence analysis showed that Korean *K. saxeti* has a high homology (97 %) with Taiwan *K. saxeti* (AY581063.1) registered in NCBI. This is the first record of the species in South Korea.
Keywords: new record, Karoowia saxeti, lichen, lichen-forming fungi, Korea

A006

Isolation and Taxonomy of Hydrogen Producing Bacteria

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Hydrogen is a clean energy source, producing water as its only by-product when it burns. Besides, hydrogen can be produced from renewable raw materials such as organic wastes. Therefore, hydrogen is a potential clean energy substitute for fossil fuels. Biological hydrogen production has several advantages over hydrogen production by photoelectrochemical or thermochemical processes. Biological hydrogen production by microorganisms requires low energy and efforts. It is very important to purify and identify bacteria to produce high efficient hydrogen. Bacterial samples were collected from wastewater treatment plants and rice-fields. Hydrogen producing bacteria were cultured under anaerobic condition. Among total 225 isolates obtained, 55 bacteria represented high hydrogen production capability. Through analysis of their 16S rDNA partial sequence, these isolates belonged to 3 bacterial phyla (Actinobacteria, Proteobacteria, Firmicutes). Novel strains were also detected.
Keywords: Hydrogen producing bacteria, anaerobic condition, 16S rDNA sequence

A007

Diversity of Bacteria Estimated by Phylogenetic Analysis in Oak Forest Soil

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The cultivated and uncultivated bacterial communities of oak forest soil was investigated by use of 16S rDNA approach. Restriction analysis method of the amplified 16S rDNAs was performed with *Hae* III. One hundred thirty-nine different ARDRA types were detected from bacteria clone library (338 clones). Clone associated with the Proteobacteria phylum was most dominant as 55% and second was Bacteriodetes phylum (30%). The rest of clones were detected as Verrucomicrobia (6.4%), Acidobacteria (5.1%), Chloroflexi (2.6%) and Planctomycetes (1.3%), respectively. Eighty strains were isolated from oak forest soil. In the isolated strains, strains belonging to the β -Proteobacteria (18.0%), ɣ-Proteobacteria (12.8%), low G+C Gram-positive bacteria (33.3%), high G+C Gram-positive bacteria (28.8%) and Bacteroidetes (7.7%) were detected, respectively. Among them, Gram-positive bacteria was dominant as 62.1%. On the other hand, Acidobacteria and Verrucomicorbia were not appeared. On the basis of 16S rDNA similarity and phylogenetic data, cultivated 9 strains from oak forest soil propose novel strains.
Keywords: Phylogenetic analysis, soil bacteria, 16S rDNA sequence

A008

Dyadobacter koreensis sp. nov., Isolated from Freshwater

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A non-motile, rod shaped and light yellow pigmented bacterium, designated strain WPCB159^T, was isolated from freshwater of Woopo wetland, Republic of Korea. Cells were Gram-negative, aerobic, catalase- and oxidase-positive. The major fatty acids were $C_{16:1}$ ω 7*c*/iso-2OH (34.8%), $C_{15:0}$ iso (24.2%) and $C_{16:0}$ (9.4%). The DNA G+C content was 44 mol%. A phylogenetic tree based on 16S rRNA gene sequence showed that strain WPCB159^T forms an evolutionary lineage within the radiation enclosing the members of the family *Flexibacteraceae* and, in particular, a coherent cluster with *Dyadobacter hamtensis* JCM 12919^T. The level of 16S rRNA gene similarity between strain WPCB159^T and *D. hamtensis* JCM 12919^T is 97.8%. The phenotypic characteristics and DNA-DNA hybridization relatedness data indicate the WP159^T should be distinguished from *D. hamtensis* JCM 12919^T. On the basis of the evidences presented in this study, a novel species, *Dyadobacter koreensis* sp. nov. is proposed for strain WPCB159^T (KCTC 12534^T = NBRC 101116^T)
Keywords: Pedobacter koreensis, 16S rDNA sequencing, freshwater

A009

Sphingopyxis sp. nov., Isolated from Sea Water of Jeju

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Gram negative and aerobic bacterial strains, designated FR 1087^T and FR 1093^T, were isolated in a course of analyzing diversity of surface seawater off Jeju Island, in Republic of Korea. Phylogenetic analysis based on 16S rRNA gene sequence indicated that the two isolates shared 98.5% similarity, with the highest sequence similarity to *Sphingopyxis flavimaris* SW-151^T (97.9%), and belonged to the genus *Sphingopyxis*. The two isolates shared low DNA-DNA relatedness (20.7%) and were differentiated from *S. flavimris* (21.2% and 36.3%). Two strains grew optimally at 23.7-31.8 °C and in the presence of 3-5% seasalts. Two isolates have sphingoglycolipid as major polar lipid. It is evident from the data that the two organisms might be classified as new *Sphingopyxis* species.

Keywords: Sphingopyxis, sea water, 16S rRNA

A010

Re-identification of KACC *Penicillium* Strains Isolated in Korea

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Korean Agricultural Culture Collection (KACC) holds more than 3,000 fungal strains and distributes more than 600 strains to microbial researchers every year. The accurate identification of individual strains is an essential prerequisite for applications to taxonomy and applied researches. However, traditional methods have mainly depended upon morphological, physiological, and biochemical characteristics, so they have limitations for accurate identification of fungal strains. We have been re-identifying fungal strains using sequence analyses of internal transcribed spacer (ITS) and another genes useful for identification since 2005. We carried out re-identification of 367 KACC *Penicillium* strains using sequence analyses of ITS and beta-tubulin, and reported the results from 30 strains isolated in Korea and identified by scientists in domestic agricultural colleges or institutes.

Keywords: Penicillium, internal transcribed spacer, beta-tubulin

A011

Complete Sequence and Secondary Structure of rRNA Gene of the *Nosema* sp. C01

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We present here for the first time the complete DNA sequence data (3779 bp, GenBank Accession No. AY383655) of the ribosomal RNA (rRNA) gene of the Lepidoptera-infecting microsporidia species, *Nosema* sp. C01. The SSU rRNA consists of 1236 bp which is much shorter than a typical prokaryotic SSU rRNA. The predicted secondary structure of SSU rRNA consists of a core (formed by 1, 2, and 31 helices) and 4 branches (formed by 1-21, 22-30, 32-48, and 49-50 helices) from the 5' end clockwise to the 3' end. The helices 10, 11, 18, 37, 43, 45 and 46 were missing. The LSU rRNA is greatly reduced in length (2506 bp). In LSU rRNA secondary structure of LSU rRNA, eleven hypervariable areas are shown and nine helices (B6, B7, B8, B14, B21, D5, E9, E15, and G5) are missing. B7-B9 and D4 helices can be used for taxonomic studies. The ITS region (37 bp), positioned between the SSU and LSU rRNA genes. The establishment of microsporidial rRNA sequences and their secondary structure might contribute to their somewhat limited taxonomic classification based on morphology.

Keywords: Microsporidia, rRNA

A012

Isolation and Characterization of *Chryseobacterium* Associated with Roots of Sand-dune Plants

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Seven Gram-negative, yellow-pigmented bacteria designated RBA2-6, RHA2-9, RHA2-18, RHA3-1, RSB3-1, PSB1-1 and PSB1-20, isolated from three sand-dune plant species inhabiting coastal areas in Tae-an, Korea, were subjected to taxonomic investigation. The 16S rRNA gene sequence analysis indicated that these isolates should be placed in the genus *Chryseobacterium* of the family *Flavobacteriaceae*. The phenotypic properties of the strains were also consistent with their classification into this genus. The levels of 16S rRNA gene sequence similarity between seven isolated *Chryseobacterium* strains, and other *Chryseobacterium* species were less than 97%. The DNA-DNA relatedness data indicated that seven strains grouped into three clusters that were clearly different from the nearest species, the one cluster (RBA2-6, RHA3-1 and PSB1-1) showed 97% similar to *C. joosteii*. The another cluster (RHA2-18, RSB3-1 and PSB1-20) showed 97% similar to *C. gleum* and *C. formosense*. The other cluster (RHA2-9) showed 96% similar to *C. piscium*. The major fatty acids were 13-methyltetradecanoic acid (iso-C15:0), 3-hydroxy-15-methylhexadecanoic acid (iso-C17:0 3-OH) and summed feature 3 (iso-C15:0 2-OH and /or C16:1w7c). On the polyphasic taxonomic analysis results, it is evident that each of these strains represents a novel species of *Chryseobacterium*.

Keywords: *Chryseobacterium*, sand dune plant, endophytic bacterium

A013

Phylogenetic Study of the Lichen-forming Fungi from King George Island, Antarctica

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Lichens are predominant and important flora in the terrestrial ecosystem of Antarctica. However, relatively few phylogenetic researches on Antarctic lichen-forming fungi have been accomplished. Phylogenetic study using molecular techniques and advanced analysis algorithms leads us to identify and classify them comprehensively, and it will also enable us to understand the evolutionary relationships among species with various geographical distributions. During the floristic surveys from January to February 2006 in King George Island, Antarctica, 225 lichens were collected and morphologically identified according to macroscopic and microscopic characteristics. To provide information for phylogenetic relationships, nuclear large subunit (nLSU) rDNAs of 89 specimens were sequenced and compared with 201 sequences of lichen-forming fungi retrieved from the GenBank. Most of the collected Antarctic species were grouped together with species from other continents of the earth. However, Antarctic species, which include several members of *Catillaria, Cladonia,* and *Xanthoria,* formed unique phylogenetic lineages.

Keywords: Antarctic, Lichen, Phylogeny

A014

Insulimonas litoralis gen. nov., sp. nov., a Novel Gammaproteobacterium Isolated from a Coast of the Yellow Sea

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A bacterial strain named IMCC1877^T was obtained from the surface seawater of a coast of Deokjeok island, Yellow Sea, using a standard dilution plating method. The strain was Gram-negative, chemoheterotrophic, facultatively anaerobic, slightly halophilic, catalse- and oxidase-positive, and motile rod that had a single polar flagellum. Cell size was 1.2-2.0 μm in length and 0.5-0.8 μm in width. Colonies on marine agar were very small; average size was 0.1 mm in diameter. Strain IMCC1877^T metabolized a variety of sugars and carbohydrates for growth. The DNA base composition of the strain was 60.6 mol% of G+C. The strain was most closely related to members of the genus *Marinobacterium*, sharing 91.8-93.7% of 16S rRNA gene sequence similarity. The phylogenetic analyses based on 16S rRNA gene sequences showed that this marine isolate belonged to the class *Gammaproteobacteria* and formed a distinct lineage together with members of the genus *Marinobacterium*. The robustness of this clade, however, could not be confirmed by bootstrapping analyses. In addition to the phylogenetic position, biochemical and phenotypic differences, such as 5 mol% difference in DNA G+C content and relationship to oxygen, between the new strain and the genus *Marinobacterium* justify the proposal of a novel genus and species, named *Insulimonas litoralis* gen. nov., sp. nov. (type strain = IMCC1877^T).

Keywords: Insulimonas litoralis, Gammaproteobacteria, 16S rRNA gene, polyphasic taxonomy

A015

Oceanobacillus caenosus sp. nov., a Novel Bacterium Isolated from a Bio Best *Bacillus* System

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An *Oceanobacillus* strain S-11, was isolated from the return sludge of Bio Best *Bacillus* system which was a wastewater treatment system in Ulsan. Strain S-11 was studied phenotypically, genotypically, and phylogenetically. Cells are Gram-positive rod and motile. Cell size is 0.58 X 2.0~2.17 um. They bear ellipsoidal endospores that lie in midway positions in swollen sporangia. 16S rDNA sequence analysis revealed that strain S-11 was a member of genus *Oceanobacillus*. *Oceanobacillus picturae* showed the highest sequence similarity of 95%. The G+C content of DNA was 33.6 mol%. Major cellular fatty acid of strain S-11 was iso-C15:0, anteiso-C15:0, and iso-C16:0. Based on phenotypic, genotypic, and phylogenetic evidence, it is proposed that the unknown bacterial strain isolated from the sludge should be classified as *Oceanobacillus caenosus* sp. nov., and the type strain is S-11. [Supported by grants from Eco-STAR Project]

Keywords: *Oceanobacillus caenosus*, 16S rDNA sequencing, wastewater treatment system

A016

Maritimibacter alkaliphilus gen. nov., sp. nov., a Novel Bacterium of the *Roseobacter* Clade Isolated from the Western Sargasso Sea

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A bacterial strain designated HTCC2654^T was isolated from the Bermuda Atlantic Time-series Study site in the western Sargasso Sea, Atlantic Ocean, by a dilution-to-extinction culturing. The strain was Gram-negative, strictly aerobic, non-motile rod, requiring sodium ions for growth. The pH range for growth was 4-12 and optimum pH was 10.0, and thus the strain was considered to be alkalophilic bacterium. The temperature and NaCl concentrations for optimal growth were 25-30℃ and 3.5%, respectively. The strain oxidatively utilized several kinds of substrates, including oligosaccharides, sugar acids, and amino acids. The DNA G+C content of the strain was 61.7 mol%. The predominant fatty acid constituents were $C_{16:0}$2OH (27.3%), 11-methyl $C_{18:1}\omega7c$ (19.6%), and $C_{18:1}\omega7c$ (17.3%). The most closely related and validly published species to the strain, as determined by 16S rDNA sequence comparison, was *Roseovarius tolerans* (94% of sequence similarity). Phylogenetic analyses using several treeing algorithms strongly indicated that this strain formed a distinct clade together with uncultured and unidentified marine bacteria within the *Roseobacter* clade of the *Alphaproteobacteria*. Based on 16S rDNA sequence analyses and chemotaxonomic data obtained in this study, a novel genus and species, *Maritimibacter alkaliphilus* gen. nov., sp. nov., is proposed; HTCC2654^T is the type strain of *Maritimibacter alkaliphilus*.

Keywords: Maritimibacter alkaliphilus, Roseobacter clade, dilution to extinction, polyphasic taxonomy

A017

Sufflavibacter litoralis gen. nov., sp. nov., a New Marine Bacterium within the Family *Flavobacteriaceae*

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A yellow colored marine bacterium, designated IMCC1001^T, was isolated from the surface seawater in a coast of Gosung, the East Sea, and characterized by polyphasic approaches. Phenotypic data and phylogenetic analyses showed that the strain belongs to the family *Flavobacteriaceae*. Strain IMCC1001^T was Gram-negative, chemoheterotrophic, facultatively anaerobic, and gliding motile straight rod that divide by binary fission. Several kinds of macromolecules, including gelatin, starch, casein, elastin, aesculin were degraded. The predominant fatty acids were $iC_{15:0}$ 2OH plus $C_{16:1}$ ω7c (25.0%), iC17:0 3OH (18.4%), and $iC_{15:0}$ (16.3%). Based on comparative 16S rDNA sequence analyses, the most closely related species were *Salegentibacter mishustinae* (95% of sequence similarity) and *Mesonia motionis* (93%). Phylogenetic analysis using three treeing algorithms based on 16S rRNA gene sequences indicated that the strain formed a distinct lineage containing several uncultured marine bacteria in the family *Flavobacteriaceae* and the strain was only distantly related to previously defined genera. Therefore, it is proposed from the polyphasic studies that strain IMCC1001^T (=KCCM42359^T) represent a new genus and species named *Sufflavibacter litoralis* gen. nov., sp. nov.
Keywords: Sufflavibacter litoralis, Flavobacteriaceae, Sea water, Polyphasic Taxanomy

A018

Isolation and Characterization of Acidophilic Actinomycetes Isolated from Soil of Tae-An Area

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The taxonomic position of thirty-two acidophilic actinomycetes isolated from *pinus* soils at Sambong and Anmyeon, near coastal areas in Tae-An, Chungnam was taxonomically characterized using a polyphasic approach. For the isolation of acidophilic actinomycetes, starch casein agar with pH 4.5 was used. Complete 16S rRNA gene sequences determined for the strain were aligned with corresponding sequences of representatives of the genera *Steptacidiphilus* and *Streptomyces* and phylogenetic trees inferred using neighbor-joining tree-making algorithms. The organisms formed four distinct clusters is within the *Streptacidiphilus* and *Streptomyces* 16S rRNA gene tree.Cluster 1-4 are related several to soil actinomycete CN668, *Streptacidiphilus jiangxiensis, Streptomyces psammoticus* and *Streptomyces misawanensis*. Representative strains were also tested for optimal pH profiles. From the phylogenetic analysis, it was clear that the isolates formed a distinctive phylogenetic line within the genera *Streptomyces* and *Streptacidiphilus*. Further taxonomic works will reveal their relationships with existing species of *Streptomyces* and *Streptacidiphilus* isolates. it will propose that these organisms be assigned to a new *Streptomyces* species.
Keywords: Streptacidiphilus

A019

Intra-specific Diversity of *Hormonema dematioides* Isolates from Pine Needles Assessed by rDNA ITS Sequencing, RAPD, Microsatellite Primed PCR, and Physiological Tests

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Hormonema dematioides is an endophyte in the needles of pines and firs. The rDNA ITS sequencing, RAPD, and microsatellite primed PCR were used for the characterization of the genetic diversity of Korean *H. dematioides* isolates from pine needles. The rDNA ITS sequences were highly conserved (99-100% similarity for 599 bp alignment), but the results of RAPD and microsatellite primed PCR revealed that Korean isolates of *H. dematioides* were separated into two distinct genotypes. The physiological analyses showed that the divergence pattern of *H. dematioides* strains in nutrient availability was congruent with that of *H. dematioides* strains in genotypes. The physiological and molecular analyses were useful in characterizing phenotypic and genetic diversities of *H. dematioides* strains, together in finding correlations between genotypes and phenotypes. Based on present results, it is suggested that RAPD and microsatellite primed PCR can be a powerful tool for differentiating closely related yeast-like fungi with similar morphologies and ITS sequences. Supported by grants from KOSEF and BK21.
Keywords: Hormonema dematioides, intra-specific diversity, pine needles, rDNA ITS, RAPD, microsatellite primed PCR, physiological tests

A020

Identification and Characterization of Surfactin-producing *Bacillus* sp. YJ2 from Chonggugjang

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A Gram-positive, endospore-forming, rod-shaped bacterium, designated YJ2, was isolated from the Chunggugjang. The isolate produced a surfactin-like potent biosurfactant and amylase. The strain was strictly aerobic and motile by means of peritrichous flagella. The strain grew optically at 37 °C and pH 5.0-5.5. Chemotaxonomic data (G+C content: 52%, major fatty acids: anteiso-$C_{15:0}$, $C_{17:0}$, and iso-$C_{15:0}$) supported the affiliation of the isolate to the genus *Bacillus*. Comparative 16S-rDNA sequence analysis showed that the isolate formed a distinct phylogenetic tree within the genus *Bacillus* and was most closely related to *Bacillus* sp. Based on phenotypic, chemotaxonomic characteristics and phylogenetic inference, this strain *Bacillus* sp. YJ2 was assigned to the genus *Bacillus*.
Keywords: surfactin, Bacillus sp.

A021

Some New Species of *Boletus* and *Cantharellus* from Korea

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Many higher fungi were collected at Korea from 2002 to 2005. They were identified. As the result , three *Boletus* and one *Cantharellus* are new to world. Among them, *Boletus tabicinus* D.H. CHo is pileus light yellow, stipe yellowish rough furrow-net and none exchanged color when bruised. *B. albopores* D.H.Cho is pileus darkish, rugulose, tubepores and stipe white. *B. nigrriaeruginosa* D.H.Cho is pileus mixed green, negro and yellowish , tubepores and stipe yellowish.. *Cantharellus mionr* f.*pallid* color with white at edge.
Keywords: New Species, Boletus tabicinus, B. nigrriaeruginosa, Cantharellus minor f.pallid

A022

Taxonomic Study on Korean Aphyllophorales (5) - On Some Unrecorded Genera and Species

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Total 149 species and 209 strains of Korean Aphyllophorales maintained in Seoul National University Fungus Collection (SFC) were analyzed by taxonomic and phylogenetic methods. Among those examined fungal specimens, nine genera *Abundisporus, Antrodiella, Cyphellopsis, Dendrothele, Dichomitus, Laxitextum, Piloderma, Skeletocutis* and *Tublicrinis*, and 23 species, *Abundisporus fuscopurpureus, Antrodiella semisupina, Auriporia pileata, Cantharellus subalbidus, Clavulina cinerea, Cyphellopsis confusa, Dendrothele acerina, Dichomitus campestris, Haplotrichum aureum, Heterobasidion annosum, Hyphoderma argillaceum, Hyphodontia tropica, Inonotus dryophilus, Ischnoderma benzoinum, Laxitextum bicolor, Phanerochaete radicata, Phellinus lonicericola, Piloderma byssinum, Skeletocutis nivea, Tomentella terrestris, Trametes elegans, Trametes tenuis* and *Tubulicrinis accedens* were confirmed as new to Korea and registered here with descriptions. Supported by grants from KOSEF, RDA and BK21.
Keywords: Korean Aphyllophorales, taxonomic study, unrecorded genera, unrecorded species

A023

Paenibacillus ginsengihumi sp., a Novel Bacterium from Ginseng Field

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The taxonomic study of strain J10-54^T which was isolated from the ginseng field was examined. This strain contained manaquinone 7 as the main respiratory quinone and *meso*-diaminopimelic acid in the cell-wall peptidoglycan. The major cellular fatty acid of the isolate was anteiso-$C_{15:0}$. Levels of 16S rDNA similarity between strain J10-54^T and other *Paenibacillus* species were 90.1 to 96.4%. The results of 16S rDNA sequence comparisons revealed that the strain J10-54^T formed an evolutionary lineage distinct from other *Paenibacillus* species. On the basis of morphological, physiological and chemotaxonomic characteristics, together with 16S ribosomal DNA sequence comparison data, we propose the new species of the genus *Paenibacillus*, *Paenibacillus ginsengihumi* sp. nov., the type strain of which is J10-54^T(=KCTC 13063^T).
Keywords: Paenibacillus, taxonomy

A024

L*actococcus chungangense* sp. nov. Inhibit *Bacillus anthracis* and Methicillin Resistant *Staphylococcus aureus* (MRSA)

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An Gram-positive, anaerobic, bacterial strain, CAU 28 was isolated from activated sludge in Cheonan city wastewater treatment and subjected to a polyphasic taxonomic study. Strain CAU 28 grew optimally at pH 7.2 and 30℃ and showed antibacterial activity against Bacillus anthracis and Methicillin resistant Staphylococcus aureus (MRSA). Based on cellular morphology and the results of biochemical tests, CAU 28 was tentatively identified as a Lactococcus species. Comparative 16S rRNA gene sequencing studies confirmed that CAU 28 is a member of the genus Lactococcus, with Lactococcus raffinolactis as its closest phylogenetic relatives (96.9% similarity). DNA:DNA pairing studies showed that CAU 28 displayed less than 70% relatedness to the type strains of Lactococcus raffinolactis, Lactococcus piscium and Lactococcus plantarum. On the basis of phenotypic and molecular genetic evidence, it is proposed that CAU 28 was classified as a novel subspecies, Lactococcus chungangense sp. nov.
Keywords: 16s rDNA, activated sludge, antibacterial activity, Lactococcus

A025

Corynebacterium *Chungangensis* sp. nov. Isolated from Wastewater Treatment in Yeongdeok-Gun

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An Gram-positive, non-motile, facilitative anaerobic, catalase-positive, diphtheroid-shaped bacterial strain, CAU 212 was isolated from wastewater treatment from the Yeongdeok-Gun, Gyeongsangbuk-Do, Korea and subjected to a polyphasic taxonomic study. Strain CAU 212 grew optimally at pH 7.0 and 30℃. Based on morphological and biochemical characteristics and the presence of a murein based on meso-diaminopimelic acid, CAU 212 was tentatively assigned to the genus Corynebacterium. Phylogenetic analysis based on 16S rRNA gene sequences indicated that CAU 212 belonged to the genus Corynebacterium. Levels of 16S rRNA gene sequence similarity between strain CAU 212 and the type strains of Corynebacterium species were below 95.0%. On the basis of phenotypic and phylogenetic distinctiveness, CAU 212 was classified in the genus Corynebacterium as the type strain of a novel species, for which the name Corynebacterium chungangensis sp. nov. is proposed.

Keywords: 16s rRNA, wastewater treatment, Corynebacterium

A026

New Records of Helotiales and Pezizales from Korea (II)

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A total of 1274 specimens of discomycetes have been collected from various localities of Korea since 2002. In the previous report, seven species were recorded new to Korea. This is a continuation of 'New records of Helotiales and Pezizales from Korea'. In the present study, *Hyaloscypha albohyalina* var. *albohyalina* (Hyaloscyphaceae), *Mollisia discolor* var. *discolor*, *Mollisia discolor* var. *longispora*, *Pezicula carpinea* (Dermateaceae) in Helotiales and *Otidea bufonia* (Pyronemataceae), *Pachyella babingtonii* (Pezizaceae) in Pezizales are newly added to the mycobiota. *Mollisia discolor* var. *longispora* is distinguished from var. *discolor* by its longer ascospores. *Pezicula carpinea* collected at Jeju island has host-specificity on *Carpinus laxiflora*. All materials are housed at MCKU (Mushroom Collections of Korea University).

Keywords: discomycetes, Helotiales, Pezizales, taxonomy, Korea

A027

Taxonomic Concordance of Albugo Species on *Brassicaceae* and *Capparaceae* Based on Phylogenetic and Morphological Analyses

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The taxonomic positions of *Albugo candida*, *A. capparis,* and *A. chardoni* were molecularly and morphologically compared. The *Albugo* isolates infecting two genera *Capparis* and *Cleome* in the *Capparaceae* were originally identified as *A. capparis* and *A. chardoni*, respectively, while *A. candida* infects various host plants belonging to *Brassicaceae*. The phylogenetic tree was inferred from the Bayesian analysis of the mitochondrial COX2 sequences, and the former two species formed a well-supported monophyletic group with *A. candida* and shared a high level of sequence homology of 99.8-100%. Morphological observation also confirmed conspecificity of the two species and *A. candida*. From these results, we propose that both *A. capparis* and *A. chardoni* should be reduced to taxonomic synonyms under *A. candida*.

Keywords: white rust, *Capparis, Cleome,* COX2 mtDNA, taxonomic synonyms

A028

Reidentification of *Colletotrichum gloeosporioides* and *C. acutatum* Isolates Stored in Korean Agricultural Culture Collection (KACC)

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Korean Agricultural Culture Collection (KACC) have been re-identifying KACC fungal strains using molecular phylogenetic analyses since 2005. We carried out re-identification of KACC *Colletotrichum* strains, and found the interesting results from *C. gloeosporioides*. Therefore we studied 41 strains of *C. gloeosporioides* based on phylogenetic analyses of the nuclear ribosomal internal transcribed spacer (ITS) region and partial β-tubulin gene, and cultural characters on potato dextrose agar (PDA) and Benomyl-added PDA. From the result, 21 strains out of them were considered as *C. gloeosporioides* but the rest *C. acutatum* (15 strains), *C. boninense* (2 strains), *C. coccodes* (2 strains) and *C. truncatum* sensu lato (1 strain). We also discussed taxonomy and distribution of *C. gloeosporioides* and *C. acutatum* isolates from major crops in Korea.

Keywords: colletotrichum, internal transcribed spacer, beta-tubulin

A029

Jejuana flavecens gen. nov., sp. nov., a Novel Member of the Family *Propionibacteriaceae*

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A novel actinobacterium, designated strain SST-39^T, was isolated from a sandy sediment sample collected in Jeju, Republic of Korea. The cells were aerobic, Gram-positive, non-endospore-forming and non-motile rods; the colonies were circular, slightly convex, opaque and brilliant-yellow in colour. The cell wall peptidoglycan of the organism contained LL-diaminopimelic acid as the diagnostic diamino acid. Polar lipid includes phosphatidylglycerol and diphosphatidylglycerol. The predominant menaquinone is MK-9(H_4). The cellular fatty acid profile consisted of straight-chain saturated and branched fatty acids, with major component of ai-$C_{15:0}$. The G+C content of the DNA is 68·4 mol%. A phylogenetic tree based on 16S rRNA gene sequence exhibited that strain SST-39^T belonged to the family *Propionicbacteriaceae* and formed a unique cluster with a strain of *Tessaracoccus bendigoensis*. The highest 16S rRNA gene sequence similarities of SST-39^T were found with *Tessaracoccus bendigoensis* ACM 5119^T (97.0%), *Propionibacterium propionicum* DSM 43307^T (95.1%) and *Luteococcus peritonei* CCUG 38120^T (95.1%). On the basis of the phenotypic and genotypic properties, this novel strain represents a member of novel genus and species in the family *Propionibacteriaceae*, for which the name *Jejuana flavecens* gen. nov., sp. nov. is proposed. The type strain is strain SST-39^T.

Keywords: Jejuana flavecens gen. nov., sp. nov., 16S rRNA gene sequence, Propionicbacteriaceae

A030

Analysis of Genetic Diversity in *Agaricus* as Determined by RAPD Analysis

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In this study, as being differentiation of individual strains, grouping and identification of isolates, for systematics of taxonomic aggregates in Agaricus, it is to examine phylogenetic relationship of them, and to offer taxonomic data to classify species similar in shape. RAPD profiles may present several advantages for taxonomic identification and grouping of isolates in the species complex. RAPDs (Random Amplified Polymorphic DNAs) was adopted to discriminate between 7 species of Pleurotus spp, which were 36 strains. Genetic variation in Pleurotus, 36 strains (seven species), were investigated by random amplified polymorphic DNA(RAPD) marker using eight oligonucleotide primers including the OPA08. The result of UPGMA cluster analysis using the PCR data showed that seven species are clustered into various groups. It appeared variation among isolates within genus and species and in Agaricus, and was divided small groups and tended to be classfied by each species in most of species. Agaricus edulis was close relationship with A. bitorquis .A. bisporus group except of a strain was distinguished form other species clearly. It appeared to be able identification isolates as well as being group each species within the genus through analysis of polymorphism using of RAPD.

Keywords: RAPD, Agaricus, Genetic Diversity

A031

Use of *amiB* Gene for Species-specific PCR Detection of *Vibrio anguillarum*

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Vibrio anguillarum is the causative agent of the fish disease vibriosis and is the most intensely studied species of *Vibrio*. In the present study, we designed specific primers and a PCR assay to detect *V. anguillarum*. The primers were designed to amplify a 429-bp internal region of the *V. anguillarum amiB* gene, which encodes the peptidoglycan hydrolase N-acetylmuramoyl-L-alanine amidase. PCR specificity was demonstrated by successful amplification of DNA from *V. anguillarum* and by the absence of a PCR product from 25 other *Vibrio* strains and various enteric bacteria. The PCR produced a 429-bp amplified fragment and had a sensitivity level of 1 pg of *V. anguillarum* DNA. The limit of detection for this PCR technique was approximately 20 bacterial colonies in 25 mg of infected flounder tissue. These results suggest that this PCR method is a sensitive, species-specific, and is possible to use as a diagnostic tool to detect *V. anguillarum*.

Keywords: Vibrio anguillarum, amiB gene, PCR, flounder

A032

Molecular Phylogenetic Analysis of HIV-1 *vif* gene from Korean Isolates

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Phylogenetic studies on *nef*, *pol* and *env* gene sequences of HIV-1 isolated from Koreans suggested the presence of Korean clade where Korean sequences are clustered exclusively of foreign sequences. We attempted to identify and characterize the Korean clade using all (n = 233) *vif* gene sequences isolated from Koreans registered in NCBI GenBank database. Most (77%) of the Korean isolates belonged to the Korean clade as a big subcluster in subtype B, and named as Korean clade subtype B (KcB). KcB sequences were relatively homogenous compared with the other Korean subtype B sequences that did not belong to the KcB (NKcB: non-Korean clade subtype B). Comparison of the frequencies of amino acid between KcB and NKcB sequences revealed several positions where the amino acid frequencies were significantly different. These amino acid residues were critical in separating the KcB from NKcB or foreign sequences since substitution of these amino acids in KcB with the NKcB amino acids relocated the KcB sequences to NKcB, and vice versa. Further analyses of the KcB will help to understand the origin and evolutionary history of the KcB.

Keywords: HIV-1, phylogeny, vif, Korean clade

A033

Taxonomic Study of Strain po4, a Novel Species Candidate in Genus *Flavobacterium*

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An aerobic, gram-negative bacterium, strain po4 was isolated from diesel contaminated soil. The taxonomy of strain po4 was studied by using phenotypic and phylogenetic methods. Cells of strain po4 were yellow with clear margins, rod-shaped and contained menaquinone-6 (MK-6) as the major respiratory quinone. The nearly complete 16S rRNA gene of strain po4 was amplified and sequenced. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain po4 is related to the genus *Flavobacterium*, with the highest sequence similarity of 98% to *Flavobacterium psychrolimnae* (LMG22018^T). Strain po4 differed from some phylogenetically related Flavobacterium species in several phenotypic characteristics. On the basis of phenotypic and phylogenetic distinctiveness, po4 was concluded to be novel species in the genus *Flavobacterium*.

Keywords: Flavobacterium, species novel

A034

Polyphasic Estimation of Anaerobic Population from Freshwater Sediments by Culture-Dependent Methods

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Anaerobic population in freshwater sediments was estimated by culture-dependent methods in unamended condition. The freshwater sample with sediment was taken from a small lake and the total number of bacteria was counted by DAPI staining with MPN statistics. The freshwater was collected for use as a medium. According to the total count, the sample suspension was diluted to extinction and twofold dilution series were made in 96-well plates, which were incubated anaerobically in the light and dark condition for 6 months. The scale of the anaerobic population from the sediment was determined at several different levels; by MPN-Flx, MPN-LTB, plate count CFU for anaerobe, and MPN-CFB. For sensitive detection of wells that bacteria grow in but neither show any turbidity nor give a colony on a plate, the flx800 fluorescence reader (Bio-Tek, CA) was employed. It successfully substituted the classic method like an epifluorescence microscopy in that it saved both times and labors immensely. It was revealed that the plate-count-number of anaerobic culture was 1,117 times less than the total count, but the extinction arrays contained over one seventh of the total number of cells, suggesting this manner promises diversity of anaerobic population from a given sample considerably better than that classic way does when the multiplication of the microplates are expanded as much as possible. This work was supported by the 21C Frontier Microbial Genomics and Applications Center Program, MOST.
Keywords: Most Probable Number(MPN), Extinction Culture, Unamended Cultivation, 4',6'-diamidino-2-phenylindole(DAPI)

A035

A New Species CJ4^T of the Genus *Flavobacterium* isolated from the Pond of Chung-Ang University in Anseong, Korea

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A Gram-negative yellow-pigmented and aerobic bacterial strain, designated CJ4^T, was isolated from the pond of Chung-Ang University in Anseong, Korea. 16S rRNA gene sequence analysis revealed an affiliation with the genus *Flavobacterium.* The strain CJ4^T showed the highest sequence similarity of 97.08% with *Flavobacterium psychrolimnae.* According to physiological data and 16S rRNA gene sequence analysis, CJ4^T was assigned to the genus *Flavobacterium*, but could be discriminated from recognized species of the genus. Therefore, the strain CJ04T represents a novel species that belongs to *Flavobacterium*.

Keywords: Flavobacterium, Systematics

A036

Phylogenic Analysis of ITS Region Sequences of rDNA from *Flammulina velutipes* Collected from Asian Countries

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Flammulina velutipes, one of edible mushroom belongs to Tricholomataceae of Basidiomycota, has been cultivated for 25 years in Korea. Eighteen fruiting bodies of *F. velutipes* were collected from Korea, China and Japan. Genomic DNA were isolated from the 18 collected strains. The Internal Transcribed Spacer (ITS) regions of the ribosomal DNA gene from *F. velutipes* were amplified using Polymerase Chain Reaction(PCR). The base sequences in each isolate of *F. velutipes* were compared. The length of bases within ITS region I and II were 214~249 bp and 243~263 bp among 18 tested strains, respectively. The analysis of ITS region sequences indicated that *F. velutipes* in Korea, China and Japan were classified into three clusters.

Keywords: Flammulina velutipes, ITS region, rDNA, PCR

A037

A Polyphasic Taxonomic Study on Some New *Kaistia* Species Isolated from Daegu Industrial Complex

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4-Chlorobiphenyl (4-CB) has been used as a model substrate to investigate polychlorinated biphenyl degradation by aerobic bacteria. For evaluating the *in situ* degradative capabilities by bacteria, we tested the existence of gene encoding biphenyl dioxygenase which is responsible for initial degradation of 4-CB. Sewage samples from Geumho River and Dalseo Stream in Dague were initially stimulated with 100 ppm of 4-CB and then stimulated cultures were spread onto R2A. Selected colonies were directly used for PCR. Newly synthesized PCR primers, BphAf-m1 and BphAr-m1, were used. Colonies showing the band of expected size were subcultured onto new R2A, 4-CB degrading capabilities of which were checked. Totally 46 strains were isolated and three strains (B1-1^T, B6-8^T and B6-12^T) of them, considered to belong to novel species, were subjected to a polyphasic taxonomic investigation. Comparative 16S rRNA gene sequence studies showed a clear affiliation of these three strains into the *α-Proteobacteria*, which were most closely related to *Kaistia adipata* KCTC 12095^T (94.5, 95.5 and 97.0% similarity, respectively), sharing 96.1-97.1% similarity among them. In the phylogenetic tree, the strains formed three separate lineages among related taxa. Combined phenotypic data and DNA-DNA hybridization data confirmed that they represented three novel species in the genus *Kaistia*. [Supported by the Eco-Technopia-21 of Ministry of Environment, Republic of Korea]

Keywords: Kaistia, 4-CB

A038

New Strain Isolated from Jang-Baek Waterfall

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Sixteen isolates were obtained from Jang-Baek waterfall in North Korea. 16S rDNA full sequence data had suggested that these isolates might contain previously undescribed species. On the basis of 16S rRNA gene sequence similarity, the degree of homology percent between strains JB10 and the family *Moraxellaceae* related to *Acinetobacter haemolyticus* was 97%. The results of physiological and biochemical tests allowed genotypic and phenotypic differentiation of strains JB10 from the validly published *Acinetobacter* species. A Gram-negative, aerobic, short-rod-shaped bacterium desingnated JB10. Colonies on Nutrient agar are circular, convex, smooth and slightly opaque. Oxidase negative and Catalase positive. Strain JB10 growth occurs at 37℃ and 41℃, but not at 44℃. Growth at 41℃ usually occurs but may be reduced. Sheep blood is haemolysed and gelatin is not hydrolysed. Malonate, mannitol, sucrose and DL-aspartate are all utilized. L-arginin and L-phenylalanine are not utilized.

Keywords: Acinetobacter, 16S rDNA, phylogeny

A039

Polyphasic Study of *Hymenobacter* Strains Isolated from Air

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Twelve Gram-negative bacteria were isolated from the air sample in Suwon, Taean and Jeju. The strains were studied by phenotypic, genotypic and chemotaxonomical methods. 16S rRNA sequence comparisons demonstrated that these isolates were clustered phylogenetically with the type strains of the genus *Hymenobacter*, with sequence similarity levels of 87.2-99.1%. Major fatty acids are iso-$C_{15:0}$(22.2±11.9%), mixture of $C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH(18.8±9.62%), $C_{16:1}$ ω 5c(13.6±7.14%) and anteiso-$C_{17:1}$B/iI(12.3±7.37%). The G+C content of the genomic DNA ranged from 50 to 60 mol% G+C. Cells are strictly aerobic, rod-shaped, non-motile, catalase- and oxidase-positive. On the basis of polyphasic evidence, we describe several novel *Hymenobacter* species.

Keywords: Air-borne bacteria, Polyphasic taxonomy, Hymenobacter, Novel bacteria

A040

Characterization of Novel 4-Chlorophenol Degrading Bacteria Isolated from Flowed Sewage into the River Geumho near the Daegu Industrial Complex

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In order to evaluate the *in situ* degradative capabilities by bacteria, we examined the existence of genes encoding enzymes catalyzing the cleavage of the chlorinated aromatic ring during transformation of the objective pollutant, 4-chlorophenol (4-CP). Sewage samples from the River Geumho were initially stimulated with 0.8 mM of 4-CP and then stimulated cultures were spread onto R2A agar. Different kinds of colonies were selected, which were directly used for colony PCR. PCR primers, C12Of and C12Or, were used to amplify the gene encoding chlorocatechol 1,2-dioxygenase. Colonies showing the band of expected size (282 bp) were subcultured onto new R2A agar, 4-CP degrading capabilities of which were checked. As a result, 2 novel bacterial strains capable of degrading 4-CP were isolated and characterized.Phylogenetic analysis based on 16S rRNA gene sequencing showed the strains, 4C1-a^T and 4C1-b, belonged to the genus *Arthrobacter*, which were most similar to *Arthrobacter chlorophenolicus* DSM 12829^T (98.4 % similarity). Combined phenotypic data and DNA-DNA hybridization data supported that they represented a novel species in the genus *Arthrobacter*, for which the name *Arthrobacter defluvii* is proposed; the type strain is 4C1-a^T. [Supported by the Eco-Technopia-21, Ministry of Environment]

Keywords: 4-Chlorophenol, Arthrobacter

A041

Glaebimonas ginsengisoli gen. nov., sp. nov., the Pure-Culture Representative of Subdivision Ⅳ, *Glaebibacteria* classis nov. of the Phylum *Acidobacteria*

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The phylum *Acidobacteria* is increasingly recognized as an environmentally significant group of bacteria. Multiple culture-independent microbial community analyses indicate that the *Acidobacteria* are ubiquitous and abundant across a wide variety of soil type, suggesting that they play important roles in soil microbial communities. At least eight subdivision of the *Acidobacteria* are resolved by comparative analysis of 16S rRNA gene sequences. To date, only two of these subdivisions (Ⅰ and Ⅷ) have validated pure-culture representatives. Strain Gsoil 1619 was isolated from soil of the ginseng field of Pocheon and was characterized in the pure-culture representative of subdivision Ⅳ. Strain Gsoil 1619 is aerobic heterotrophic bacterium that is able to grow with a few of the carbon source components. Cells are Gram negative, white, non-motile and rod-shaped. It was characterized chemotaxonomically as having meso-DAP in cell-wall peptidoglycan and MK-8 as the predominant menaquinone. The G+C content of the genomic DNA is 63.4 mol%. We will propose a new genus and species, *Glaebimonas ginsengisoli* gen. nov., sp. nov., with isolate Gsoil 1619 as the type strain, and a new class for the subdivision to which it belongs, *Glaebibacteria* classis nov. [This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, MOST (Grant MG05-0101-4-0).]
Keywords: *Glaebimonas ginsengisoli, Glaebibacteria, Acidobacteria,* Polyphasic taxonomy

A042

Streptomyces panacagri sp. nov., Isolated from Soil of a Ginseng Field

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A Gram-positive, hyphae, non-motile, non-spore-forming, and aerobic bacterium (Gsoil 519^T) was isolated from soil of a ginseng field in Pocheon province. The novel isolate was characterized in order to determine its taxonomic position. On the basis of 16S rRNA gene sequence similarity, strain Gsoil 519^T was shown to belong to the family '*Streptomycetaceae*' and was related to *Streptomyces sodiiphilus* (97.6%), *Streptomyces almquistii* (97.2%), *Streptomyces rangoonensis* (97.2%), and *Streptomyces flocculus* (97.2%). The G+C content of the genomic DNA was 64.8%. Phenotypic and chemotaxonomic data (major menaquinone – MK-9(H_6), LL-DAP in cell wall peptidoglycan, major cell wall sugars – ribose, xylose, mannose, and glucose, major fatty acids – anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$, $C_{16:0}$, and anteiso- $C_{17:0}$) supported the affiliation of strain Gsoil 519^T to the genus *Streptomyces*. The results of physiological and biochemical tests enabled strain Gsoil 519^T to be differentiated genotypically and phenotypically from the *Streptomyces* species with a validly published name. The novel isolate, therefore, represents a novel species, for which the name *Streptomyces panacagri* sp. nov. is proposed, with the type strain Gsoil 519^T (= KCTC 19139^T = DSM 41871^T). [This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, MOST (Grant MG05-0101-4-0).]
Keywords: *Streptomyces panacagri,* soil of ginseng field, 16S rRNA gene sequence, Polyphasic taxonomy

A043

Paludimonas yongneupensis gen. nov., sp. nov., Isolated from a Wetland, Yongneup, in Korea

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One ivory-coloured bacterial strain 5YN8-15^T was isolated from a wetland, Yongneup, in Yanggu region in Republic of Korea. It is a facultatively anaerobic, Gram-negative, motile with one polar flagellum, curved rod-shaped bacterium. On the basis of 16S rRNA gene sequence analysis, the strain 5YN8-15^T is clearly affiliated within the β-*Proteobacteria*. The most closely related genus is *Chromobacterium violaceum* (94.1 % similarity). All other species with validly published names showed sequence similarities below 92%. The strain had ubiquinone 8 (61%) and ubiquinone (30%) as the major isoprenoid quinines. The major fatty acids were Summed Feature 3(iso-$C_{16:1}$ w7c and/or $C_{15:0}$ 2OH), $C_{16:0}$ and $C_{18:1}$ w7c. The DNA G+C content was 63.0 mol%. On the basis of polyphasic evidence, strain 5YN8-15^T (=KACC 11601^T) is proposed as the type strain of gen. nov., sp. nov.
Keywords: Novel bacterium, Polyphasic Taxonomy, Yongneup, Peat Bog

A044

Fimbriimonas ginsengisoli gen. nov., sp. nov, a Gram-negative Aerobic Bacterium, the First Pure-Culture Representative of OP10 clade, and the description of a novel bacterial phylum, OP10

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A phylogenetically novel aerobic bacterium was isolated from soil of a ginseng. The isolation strategy used targeted growing all bacteria on one fifth modified R2A agar medium in 150 mm plastic plate. The isolate, designated strain Gsoil 348^T, was a Gram negative, strictly aerobic, non-motile, and rod shaped, 0.5-0.7 mm in width and 2.5-5.0 mm in length for two week's culture on 1/2 R2A agar. Cells grow at 18-30 °C with the optimum temperature at 30 °C and at pH 6.0-8.5 with the optimum at pH 7.0. Comparative analyses of 16S rRNA gene sequences indicated that strain Gsoil 348^T belongs to candidate division OP10, a phylum-level lineage in the bacterial domain, to date comprised exclusively of environmental 16S rDNA clone sequences. The G+C content of genomic DNA of strain Gsoil 348 was 66.7 mol%. Menaquinones MK-11 (65%) and MK-10 (32%) were detected predominantly. The fatty acid profile of strain Gsoil 348 was mainly comprised of iso-$C_{15:0}$ (30.9%), 19.5% iso-$C_{17:0}$ (19.5%), $C_{16:0}$ (17.1%), $C_{16:1}$ ω11*c* (11.3%), and iso-$C_{13:0}$ 3-OH (5.8%). Here, we describe a first pure culture representative of candidate phylum OP10. [This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, MOST (Grant MG05-0101-4-0).]
Keywords: OP10, *Fimbriimonas ginsengisoli,* Polyphasic taxonomy, uncultured bacteria, *Fimbriimonadetes*

A045

Chitinophaga ginsengisegetis sp. nov., *Chitinophaga ginsengisoli* sp. nov., *Chitinophaga soli* sp. nov., and *Chitinophaga terrae* sp. nov., Isolated from Soil of a Ginseng Field

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Four novel strains of *Cytophaga-Flexibacter-Bacteroides* (CFB) group, designated Gsoil 040^T, Gsoil 052^T, Gsoil 219^T and Gsoil 238^T, were isolated from soil of a ginseng field of Pocheon province, and were characterized in order to determine their taxonomic positions using a polyphasic taxonomical approach. All the isolates were Gram-negative, aerobic, non-motile, non-spore-forming, and rod-shaped. Phylogenetic analysis based on 16S rRNA gene sequences indicated that all these isolates belong to the genus *Chitinophaga* but were clearly separated from established species of this genus, e.g. *C. arvensicola*, *C. japonensis*, *C. filiformis*, *C. pinensis*, *C. sancti* and *C. skermanii*. Phenotypic and chemotaxonomic data (major menaquinone – MK-7, major fatty acids – iso-$C_{15:0}$ and $C_{16:1}$ ω5c, major hydroxy fatty acid - iso-$C_{17:0}$ 3OH, major polyamine- homospermidine) supported the affiliation of the strains Gsoil 040^T, Gsoil 052^T, Gsoil 219^T and Gsoil 238^T to the genus *Chitinophaga*. The sequence similarities between these four isolates range from 93.1 to 96.8%, and DNA–DNA relatedness value between strain Gsoil 219^T and Gsoil 238^T was 23 %. Furthermore, the results of physiological and biochemical tests allowed genotypic and phenotypic differentiation of four strains from the other validated *Chitinophaga* species. [This work was supported by the 2004 Agricultural R&D Promotion Center Program, Ministry of Agriculture and Forestry.]
Keywords: *Chitinophaga,* Polyphasic taxonomy, a soil of ginseng field, Phylogenetic analysis

A046

Emmella ginsengisoli gen. nov., sp. nov., in the class *Spartobacteria* of the Phylum *Verrucomicrobia* Isolated from Soil of a Ginseng Field

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The phylum *Verrucomicrobia* is increasingly recognized as an environmentally significant group of bacteria, particularly in soil habitats. Two strains designated as Gsoil 133^T and Gsoil 144 which belong to the class *Spartobacteria* of the phylum *Verrucomicrobia* were isolated from soil of a ginseng field. The taxonomic positions of two environmental isolates were established using a combination of genotypic and phenotypic data. The organisms were Gram-negative, aerobic, non-motile, non-spore-forming, and short-rod shaped. Both strains have identical 16S rRNA gene sequence similarity and genotypic fingerprinting pattern, which shows they are clonal isolate. They have highest 16S rRNA gene sequence similarity with '*Candidatus Xiphinematobacter rivesi*' (89.0%) and *Chthoniobacter flavus* Ellin 428^T (88.5%). Chemotaxonomic data revealed that both strains possess MK-9 as the predominant quinone, iso-$C_{14:0}$ and $C_{16:0}$ as predominant fatty acids, and meso-DAP in cell wall peptidoglycan. Since strain Gsoil 133^T and Gsoil 144 are clearly distinct from closely related species with a validly published name, we propose the name *Emmella ginsengisoli* gen. nov., sp. nov. for these strains with the type strain Gsoil 133^T. [This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, MOST (Grant MG05-0101-4-0).]
Keywords: *Emmella ginsengisoli, Verrucomicrobia*, *Spartobacteria,* Polyphasic taxonomy, a soil of ginseng field

A047

Morphological Properties of a *Leptographium* Species Isolated from the Roots of *Pinus thunbergii Parlatore*

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Leptogrphium spp. are anamorph of *Ophiostoma, Ceratocystis, Grossmania,* and some of species the species are associated with serious diseases of trees and cause blue or black stain in coniferous trees. A *Leptogrphium* fungus was isolated from black stained area in the roots of unhealthy Japanese Black Pine in Janghang, Chungnam. In this work we examined morphological and genetic properties of the isolated fungus. The fungus grew optimally at 25℃ and showed a high level of tolerance to cycloheximide (200ug/ml). Its conidiophores are occurring singly or in group of four, arising directly from the mycelium, slightly bended at center of hyphae, occasionally erect,81-313㎛ mecronematous, mononematous with rhizoid like structure present their base. Stipes are dark brown, not constricted, 2-7septate, 42-250㎛ long and 2-8㎛ wide, apical cell not swollen, basal cell not swollen. Conidiogeneous apparatus is 30-80㎛ long, excluding the conidial mass, 3-5 series cylindrical branches. Overall this *Leptographium* isolate has similar morphology to that of *L. pini-densiflorae* described in the literature. However, this fungus has a distinct conidium apparatus, conidiogeneous cell, conidiophore shape, occasionally constricted branches, and conidia shape. Phylogenetic analysis using sequence data of the rDNA and β -tubulin gene showed that this fungus diverged from *L. pini-densiflorae* and other known *Leptographium,* suggesting it is an undescribed taxon.
Keywords: Leptogrphium, Japanese Black Pine, Morphology

A048

Molecular Epidemiology of Korean Rabies Virus Isolate in 2006 and Comparison with Isolates from throughout the North-East Asia

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Rabies is a zoonotic disease that caused severe destruction to the central nerve system and is usually fatal. After the recurrence of sylvatic rabies in 1993, the number of raccoon dog mediated rabies cases in Korea has increased annually until 2006. To better understand the current rabies epidemics in Korea, Korean rabies isolate (SKRBV0601GY) from Gyeonggi province in 2006 was compared with isolates previously in Korea and with isolates originating from the North-East Asia, such as Japan, China and Russia, based on complete nucleoprotein (N) gene sequences. Phylogenetic analyses of the isolates reveled that the Korean isolate in 2006 belonged to Korean group B. The topology of the phylogenetic tree in Korean isolates related not the species and year of isolation but the geological location of the virus isolates. All of the Korean isolates showed close relationship to the "Arctic-like" virus (Russian group B) more than the "Arctic" virus (Russian group A) and all of the Chinese isolates (Chinese group A, B and C). The "Arctic-like" virus group contained the Japanese isolate and Russian group B viruses, originating from the south of East Siberia and Far East in Russia. These molecular data demonstrated that the current rabis epizootic in Korea developed independently of China.
Keywords: Rabies, Molecular epidemiology, Korea, Arctic-like, North-East Asia, nucleoprotein

A049

A Novel Bacterial Species of the *Algibacter* Isolated from Yellow Sea Water in Korea

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An aerobic, motile and Gram negative bacterium, DDC6, was isolated from Yellow sea water in Korea. The strain grew at between 20℃ and 40℃ with optimal growth of 30-35℃. The strain grew at 2% (w/v) NaCl optimally, but neither in the presence of more than 3% NaCl, nor at 0% NaCl. The strain had ability to hydrolyze urea, and showed catalase-negative and oxidase -positive reactions. The strain was the most closely related to *Algibacter gromovii* strain KMM 6038 with 16S rDNA seqeunce similarity of 96%. Therefore, we propose the strain DDC6 represent a novel species within the genus *Aligibacter* on the basis of physiological and phylogenetic properties.

Keywords: *Algibacter* sp., new species, marine bacterium

B001

Identification and Degradation Activity of PBSA Degrading Microorganisms Isolated from Soil

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Microorganisms capable of degrading poly(butylene succinate-*co*-butylene adipate) (PBSA) were isolated from various soils such as cultivating soil and activated sludge soil of landfill sites locating in Korea by using the enrichment culture and the clear zone test techniques. Based on the 16S rDNA sequences and morphological characterization, the microorganisms were identified to be *Burkholderia cepacia*, *Pseudomonas aeruginosa*, *Streptomycese* spp., *Aspergillus* spp. The PBSA degradation activity of the isolated microorganisms was enhanced through the serial acclimation and the addition of yeast extract as a nitrogen source in PBSA plate medium. The clear zone diameter and the clear zone thickness were increased by 25~134% and 88~173% respectively through the acclimation. The clear zone diameter formed by *Streptomyces* sp. PBSA-3 was enlarged by 88% as the amount of the added yeast extract increased from 0.25g to 0.6g. The PBSA degrading microorganisms appeared to be highly active for the PBSA degradation, because 65~83% of PBSA was mineralized as a result of 40days of the modified Sturm test with 0.01% of PBSA film as a sole carbon source. *Streptomyces* sp. PBSA-3 showed the highest PBSA degrading activity in the modified Sturm test as was the case for the enhancement of the degradation activity through the acclimation.

Keywords: PBSA, Degradation

B002

Isolation and Analysis of the *Pseudomonas* sp. HK-6 Mutant Showing Hypersensitivities to TNT

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A TNT-degrading bacterium, *Pseudomonas* sp. HK-6 was mutagenized in order to isolate mutants which show higher sensitivities to TNT. After screening of about 12,000 colonies, 10 mutants that grew much more slowly on TNT-containing LB agar were identified and designated N1 - N10, respectively. When these mutants were cultured in LB liquid broth supplemented with 0.5 mM TNT, and concentrations of remaining TNT were measured using HPLC every 6 hrs, N5 were capable of degrading 0.5 mM TNT within 48 hrs. Meanwhile, the wild-type strain took only 18 hrs for complete degradation, that is about 2.7-fold faster degradation. As far as the rates of degradation were concerned, N1 - N4 and N6 - N10 mutants were almost equivalent to the wild-type strain. This indicates that other mechanisms rather than slower degradation may render N1 - N4 and N6 - N10 mutants TNT-sensitivities. Comparison of N5 and the wild-type strain has revealed several proteins spots differently induced. They include proteins involved in stress-shock response and metabolic pathways. [This work was supported by grant No. R01-2005-000-106080 from the Basic Research Program of the Korea Science & Engineering Foundation.]

Keywords: TNT degradation, Pseudomonas sp. HK-6, TNT-sensitive mutant

B003

Construction of an Improved *Escherichia-Pseudomonas* Shuttle Vector Containing an Aminoglycoside Phosphotransferase Gene and a *lacZ'* Gene for α-Complementation

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A new 4.87-kb *Escherichia-Pseudomonas* shuttle vector has been constructed by inserting an 1.27-kb DNA fragment with a replication origin of a *Pseudomonas* plasmid pRO1614 into an 3.6-kb *Esherichia coli* plasmid pBGS18. This vector designated pJH1, contains a aminoglycoside phosphotransferase gene (aph) from Tn903 that confers kanamycin-resistancy, a *lacZ' gene for α-complementation and a versatile multiple cloning site as well as a CoE1 origin and a Pseudomonas* origin of pRO1614. [This work was supported by grant No. R01-2005-000-106080 from the Basic Research Program of the Korea Science & Engineering Foundation.]

Keywords: Pseudomonas, shuttle vector, kanamycin-resistant gene, cloning

B004

Characterization of Biofilms on Fouled RO Membranes

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Biofouling is a major problem in membrane separation process due to accumulation of substances on the membrane surface and/or within the membrane pore structure resulting to aggravation of the membrane performance. In this study, membranes about 5.2 m^2 size were recovered from 3 stages of an RO unit fed with pretreated surface water from Dae-ho River in Chungnam, Korea to analyze biofouling condition. Viable microbial count was found to be 10^4-10^6 colony forming units per cm^2 of membrane sample. Out of the 515 culturable microbes isolated in R2A media, 184 microbes (35.7%) were found to show chemical signals that can mediate population density-dependent (quorum-sensing) gene expression using *Agrobacterium tumefaciens* NTL4/pZLR4 as acylated homoserine lactone (AHL)-responsive reporter strain. AHL activity and quorum-sensing gene expression have been considered as important physiological components of biofilms. PCR-terminal restriction fragment length polymorphism (TRFLP) was used to monitor the microbial community composition. From the results, it was observed that there were only little differences in the microbial community and that major T-RFs were almost similar. In addition, substrate utilizations of microbes were determined thru community-level physiological profiling (CLPP) analysis. Total substrate utilizations in stage 1 and 3 were almost the same and were higher then the total substrate utilization in stage 2.

Keywords: Biofouling, Biofilm, Membrane

B005

Preconcentration and Optical Detection of Copper (II) Using a Copper Chelating Bead-Packed Microfluidic Device with a Fluorescent Chemosensor

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Copper is an essential element needed to keep the skeletal, reproductive, and nervous systems healthy. However it causes gastrointestinal symptoms such as nausea, abdominal pain, diarrhea, and vomiting in human when copper concentration reaches 4.0 – 6.0 milligrams per liter (mg/L) in drinking water. Thus, the detection method of copper concentration is one of the prerequisite to control of Cu^{2+} in drinking water. We have previously reported a fluorescent chemosensor which can effectively sense Cu^{2+} in solutions such as buffer and biological samples (0.5-50μM, Tetrahedron Letters 47, 2006). In this study, for the practical applications of the fluorescent chemosensor, a microfluidic plaform has been developed for the fluorescent detection of Cu^{2+} in ppm range. In details, a microfluidic system was fabricated with a glass slide and PDMS (polydimethyl siloxane), and copper-chelating alginate beads (50~250μm, BioScicence) were immobilized in microfabricated weir structures of the device. The beads in the device were used to preconcentrate Cu^{2+}, and the preconcentrated Cu^{2+} were later optically detected by eluting the preconcentrated metal ions with the fluorescent chemosensor. Based on the results, it is suggested that the microfluidic platform is ready to develop for an optical biosensor which can preconcentrate and detect Cu^{2+} in water and/or other environmental samples.

Keywords: alginate beads, copper chemosensor, microfluidic device

B006

Screening of Halophilic Bacteria Degradable Food Waste

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Since 1995, According to operation of the volume-rate garbage disposal system, the most problem is food waste treatment in Korea. Because food waste cause stink and leachate through the high percentage of water content and corruptible, it is possible causing serious environmental pollution when reclamation treat. The present, most region self-governing system have been treating food waste as composting, feed stuff and removal of food waste after separate garbage collection. Some of them, composting of food waste is receiving proper eco-friendly attention, because this treatment dispose waste matter and make use of valuable resource. This study, After survival microorganisms in environment containing NaCl 10% isolated from variety salted sample and its carried out organic limit of resolution test. First, we made an experiment salinity decrease ability, and then screening of protein and fat, starch, cellulose degradation enzyme. Good-organic limit of resolution strain identified using by API kit and FAMEs, 16S ribosomal DNA.

Keywords: food waste, halophilic

B007

Analysis of Microorganisms Structure Community Isolated from Mt. Paekdu

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Because the majority of the intact bacteria in soil are unable to grow on traditional research method, both bacteria which grow up in unknown environment and nonculturable bacteria have to be studied together on that point of resources conjugation.In this study, we collected soil at nearby the summit of Cheon-moon and the Jang-baek falls in Mt. Paek-du of all 5 spot for analysis of bacterial diversity that were investigated by phylogenetic analysis of the partial 16S rDNA sequence.Out of 26 clones sequenced. all clones were from domain bacteria within the domain bacteria, several kingdoms were represented : the eubacteria(bacillus ; 12 clones, paenibacillus ; 6 clones, staphylococcus ; 2 clones), the high G + C content gram-positive group(actinobacteria, 3 clones) and γ-proteobacteria(2 clones), β-proteobacteria(1 clone)

Keywords: 16S rDNA, Mt. Paekdu, nonculturable bacteria, unknown environment

B008

Screening of Bacteria Degradable BTEX

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Inadequate administration of generation of organic waste and amount of organic solvent or subterranean from leakage and soil pollution presents important environmental problem because of increasing their amount with making a high level of industry.BTEX(benzene, toluene, ethylbenzene, o-, m-, p-xylene) that from over 80% percent of petroleum makes use of diversity form and increase amount with the chemical industry development from industrialization.This has caused pollution problem of soil and subterranean is on the rise in worldwide because of oilstoretank corrosion and leakage from damage, which should progress actively a research.In the case of our country, pollution of subterranean and soil that caused underground oilstortank were concerned with industrialization began to gather speed.BTEX is branch from a potential carcinogenic substance, and a main pollutant that is preferentially settled in Environmental Protection Agency(EPA). This method is undesiable disposal process on economical efficiency and environmental preservation aspects because of the increase of disposal cost from using chemical and additional requirement of disposal process secondary pollution induction.Hence, inthe present study, through the screening of microorganisms isolated from internal polluted water, we studied their abilities and characteristics on BTEX, and their distribution as well as their possibilities using as fundamentals on biological purification.

Keywords: BTEX, pollution, biological purification

B009

Comparative Study of Environmental and Nutritional Factors on the Mycelial Growth of Edible Mushrooms

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The culture condition for the mycelial growth of 444 strains under 10 edible mushrooms species was investigated. Temperature suitable for the mycelial growth was obtained at 25 ºC but optimal range of temperature was 20~30 ºC. Mushroom has a broad pH range (5~9) for their mycelial growth and mostly favorable growth found at pH value 6 and 7. Based on mycelial growth and density Hamada, Lilly, PDA and YM were the most suitable media. On the other hand, Czapek's, Hoppkins and Hennerberg were the most unfavorable media for radial growth of mushrooms. Among 10 different carbon sources, dextrin, fructose and sucrose were the best but lactose and galactose were the most unfavorable carbon sources. In every carbon source mycelial density was compact to somewhat compact. The most suitable nitrogen sources were glycine, ammonium acetate and calcium nitrate but most unsuitable were histidine and ammonium phosphate for mycelial growth on the culture media. In all of the nitrogen sources mycelial density was found somewhat thin to thin.

Keywords: Culture condition, Edible mushroom, Media, Mycelial growth, Nutrition

B010

Construction of Microarray-based Metagenome Library for Sequence-based Screening of Genes

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Traditional cultivation techniques have missed up to 99% of microbial diversity existing in nature. Thus, microbial community genome technology is being developed in attempts to identify novel genes in genome fragments involved in matter cycles. In fact, several important genes (archaeal amoA, proteorhodopsin, pufM, etc.,) in microbial ecology were found using this metagenomic approach. PCR has been employed for screening of each gene from large metagenomic libraries as a sequence-based approach. In order to improve this labor-intensive PCR screening approach, microarray format of metagenome library was developed for screening diverse genes repeatedly. Community genome libraries could be transferred on a slide and used for screening specific genes by hybridization using gene or oligonucleotide probes. Sensitivity and specificity of metagenome microarray were determined using templates of clones of fosmid library prepared from marine sediments. This approach would be applied to efficiently screen diverse genes in metagenome library for genome-based analysis of microbial communities.

Keywords: metagenome, microarray, community, genome

B011

Ubiquity and Diversity of a New Group of Sulfide-quinone Reductase Gene in Sediment Environments

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Hydrogen sulfide is the most reduced form of inorganic sulfur and generated from anaerobic environments by sulfate-reducing bacteria. Biological sulfide oxidation in oxic-anoxic interface is important in sulfur cycle. Among several systems of sulfide oxidation, sulfide-quinone reductase (sqr) gene were detected in wide heterotrophic and autotrophic microorganisms. Recently, sqr genes have been reported in several additional bacteria through genome sequencing projects. Based on phylogenetic study of sqr genes from database, we could classify sqr into 4 groups including archaeal group. Using group-specific PCR primers, we could detect a novel subgroup of sqr genes in a group comprised of cyanobacteria and proteobacteria. The new sqr genes are related to those of *Thiobacillus denitrificans* and *Polaromonas naphthalenivorans* of b-proteobacteria with about 70% amino acid similarity. Since we could detect this subgroup in all our marine sediments and freshwater sediments, this subgroup of sqr gene is suggested to be important in sulfide cycling in diverse oxic-anoxic interfaces.

Keywords: SQR, SRB(sulfate-reducing bacteria), SOB(sulfide-oxidizing bacteria), sulfur cycle

B012

Antimicrobial Activity of Medicinal Plant Extracts against *Staphylococcus aureus* ATCC 25923 and Methicillin-Resistant *Staphylococcus aureus* (MRSA) ATCC 43300

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This study was carried out to research antimicrobial agents from natural products, the ethanol extracts of 20 medicinal plants were tested for the antimicrobial activity against *Staphylococcus aureus* ATCC 25923 and methicillin-resistant *Staphylococcus aureus*(MRSA) ATCC 43300. Among them, the extracts of *Glycyrrhiza uralensis, node of Pinas densiflora* showed significant antimicrobial activity against *Staphylococcus aureus* ATCC 25923 and methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300.

Keywords: antimicrobial activity, Staphylococcus aureus, MRSA

B013

Molecular Phylogenetic Analysis on the Bacterial Communities in Activated Sludge from Municipal Waste Water Treatment Plant

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Seasonal diversity and transition of bacterial community in activated sludge were investigated by culture-independent molecular methods. Community DNAs were extracted from 24 samples collected from municipal waste water treatment plant (WWTP), and 16S rRNA genes were amplified by PCR. Fluorescently labeled PCR products were subjected to terminal restriction fragment length polymorphism (T-RFLP) analysis to group samples according to the similarities between community structures. Cluster analysis of T-RFLP patterns resulted in four major groups, which correspond to four seasons. 16S rDNA clone libraries were constructed from the seasonal representative samples. Total genetic variations (theta) in the 16S rDNA clone libraries for spring, summer, autumn, and winter samples were 136.27 ± 67.11, 136.27 ± 67.11, 115.29 ± 57.29, 171.90 ± 84.19, respectively. Majority of the cloned 16S rRNA sequences in all samples belonged to the division of *Proteobacteria* (spring, 77.78%; summer, 90%; fall, 91.30%; winter, 48.38%). While the members of *beta-proteobacterial* subdivision were observed with high proportion in all samples, *alpha-*, *beta-*, and *gamma-proteobacterial* subdivisions were the most dominant groups in summer, spring, and fall, respectively. In addition, previously uncultured bacterial group such as *Verrucomicrobia* was observed in summer and winter samples.

Keywords: 16S rDNA clone library, T-RFLP, diversity of microbial community, proteobacteria

B014

Morphological and Molecular Identification of *Aspergillus candidus* and *A. flavus* Isolated from Stored Rice

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In the previous study of fungal population and diversity in stored rice collected from 12 regions of Korea, two dominant isolates, Asp 317 and Asp 57, were observed and examined to identify. The characteristics of isolates were compared to the reference isolates *Aspergillus candidus* KACC 41846 and *A. flavus* KACC 40244 from Korean Agricultural Culture Collection (KACC), Suwon, Korea, respectively. Each isolate was inoculated on Czapek yeast agar, Czapek dox agar, malt extract agar, and Czapek yeast agar with 20% sucrose. Asp 317 showed pale yellow conidia and white mycelium which were similar to those of the reference isolate KACC 41846. However, Asp 57 showed deep green colored conidia and white mycelium which were similar to those of the reference isolate KACC 40244. Microscopic characteristics of Asp 317 seemed highly similar to those of the KACC 41846. Conidial heads are radiate, walls smooth, vesicles globose, conidia globose and smooth-walled. Isolate Asp 57 was observed to be similar to the KACC 40244 showing radiate to columnar conidial heads, globose conidia with smooth walls. For molecular identification of Asp 317, we used mitochondrial DNA *cytochrome b* gene. Primers targeting mitochondrial DNA *cytochrome b* gene amplified the same sequence between Asp 317 and KACC 41846. Therefore, these results showed that isolate Asp 317 could be identified as *A. candidus* while isolate Asp 57 may be *A. flavus*.

Keywords: Identification, rice, fungi, Aspergillus candidus, Aspergillus flavus

B015

Impact of Living Modified Rice on Belowground Soil Biota Community Structure

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Many kinds of Living Modified Organisms (LMOs) have been developed for various purposes including increased crop production. However, the impact of LMOs on natural ecosystem has been assessed a little, in particular with respect to belowground biota. We investigated the effect of 2 kinds of LM rice (ABC and protox) on dynamics of belowground biota community structure including bacteria, fungi, and nematode in paddy soils using terminal restriction fragment length polymorphism (T-RFLP) analysis. 16S rRNA gene for bacteria, Internal transcribe space (ITS) region for fungi, and 18S rRNA gene for nematode were targeted for PCR, respectively. The T-RFLP profiles revealed that the below-ground biota community structure was not significantly different between LM rice and non-LM rice planted soil samples, while community structure was observed to be shifted with time. The result suggests that the impact of LM rice on below-ground biota community would be small in a short period and long term monitoring is needed to assess the risk of LM rice on natural ecosystem in precise (This work was supported by grant from the Korea Science & Engineering Foundation)

Keywords: LM rice, bacteria, fungi, nematode, T-RFLP, soil

B016

Phylogenetic Diversity and Global Distribution Patterns of *Acidobacteria*

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Culture-dependent and culture-independent molecular phylogenetic surveys were carried out on the bacterial community in chestnut rhizosphere soil. Clone libraries were constructed from the 16S rRNA genes amplified from the soil DNA and ribosomal RNA by PCR and reverse transcription (RT)-PCR, respectively. While most of cultured bacteria were phylogenetically affiliated with division of *Firmicutes*, the cloned 16S rRNA gene sequences were primarily affiliated with one of three groups: division of *Fibrobacter* (ca. 60%), *Proteobacteria* (ca. 20%), and *Firmicutes* (ca. 5%). *Acidobacteria*-related sequences belonging to *Fibrobacter* division were the dominant member of the clone library generated by PCR as well as RT-PCR. In addition, we studied the global distribution patterns of *Acidobacterium sp.* using subgroup- specific primers (31F, A, Y, O, G). Soil DNAs were extracted from global soil samples and acidobacterial 16S rRNA genes were amplified, and analyzed by terminal restriction fragment length polymorphism analysis (T-RFLP). Twenty five samples (89%) showed positive PCR results for genus *Acidobacterium*, and acidobacterial subgroup A. T-RFLP patterns were consistent with the PCR results, and unpredicted T-RFs, which represent as-yet-unpublished acidobacterial 16S rRNA gene sequences, were also found. This study showed that the genus *Acidobacterum* and *Acidobacterum*-like bacteria are ubiquitous in soil, and suggested that unrevealed high diversity resides in this bacterial group.

Keywords: Acidobacteria, phylogenetic analysis, diversity, global distribution

B017

Use of Stable Istope Probing to Explore Time-Dependent Dynamics of PCB-Degradative Population Dynamics in Biphenyl-Fed Soil Microbial Communities

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Very little is known about the influence of pollutant exposure history on community structure and population dynamics among biodegraders in soil. One of such reasons may be the currently limited capabiliy of microbial ecology tools for community functioning, i.e., functioanl community analysis. Here, we used stable isotope probing (SIP) in exploring time-dependent dynamics of biodegradative populations in biphenyl-fed soil microbial communties. After a clean soil from PCB contaminated area (Picatinny, NY) was fed with isotopic carbon labeled bipheny (^{13}C-biphenyl), only biphenyl- and PCB-degrading populaitons grew and their biomass was incorporated with the heavy carbon. At different peridos of incubation (7, 14, and 28days), DNA and RNA were extracted, and heavy (^{13}C-labeled) nucleic acids were isolated using ultracentrifugation and fractionization. The following 16S rRNA gene amplication and clone-sequening provided results to link phylogenetic with functional (biphenyl- and PCB-degradation) information. Although *Actinobacteria* were the predominant in the soil, *proteobacteria* populations became enriched in the biphenyl-fed microbial communities. The SIP results revealed that beta-*Proteobacteria* were not only ealry-time but also late-time specialists. Proably because of this heterogenuous nature, the biphenyl-degradative beta-*Proteotacteria* could become the predominant as a group.
Keywords: Stable isotope probing, Polychlorinated biphenyl (PCB), Functional community analysis

B018

Molecular Analysis of Denitrifying Baterial Communities in Paddy Soils Planted with Transgenic Rice Varieties

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The diversity and seasonal variations of denitrifying bacterial communities in rice field soils planted with genetically-modified (GM) rice varieties were studied by using both culture-dependent and DNA-based analyses. Most probable number (MPN) estimation showed that the seasonal levels of denitrifying bacterial populations ($4.8\times10^4 \sim 2.5\times10^5$ cells/g soil in GM subplots and $8.1\times10^4 \sim 5.8\times10^5$ cells/g soil in non-GM subplots) were similar to each other between GM and non-GM subplots. Both in the GM and non-GM subplots, dominant denitrifying bacteria isolated from the paddy soils over the year were the *nirS* type denitrifiers and belonged to the *Pseudomonas* and *Bacillus* species. Sequence diversity analysis of the *nirS* and *nirK* genes cloned from soil DNAs revealed that the community structures of the denitrifying bacteria were similar to each other between the GM and non-GM subplots in a given month, suggesting that there were no significant differences in the structures of denitirifying microbial populations between GM and non-GM rice paddy soils during the experiment. However, the denitrifying bacterial community structures appeared to change seasonally as shown by different DGGE DNA banding patterns over the year. The results of this study suggested that the denitrifying bacterial community structures of the experimental rice field were changed with time, but that they were not significantly affected by cultivation of GM rice plants.
Keywords: denitrifying bacteria, denitrification, nir gene, rice field, transgenic plants, DGGE

B019

Cultivation of 19 Novel Bacterial Species from a Eutrophic Freshwater Pond, Inkyong

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Although a dilution-to-extinction culturing has shown a great potential to isolate previously uncultured bacteria, it has a little shortcoming in the typical bacterial classification system because standard biochemical characterization requires much quantity of biomass. To isolate and classify previously uncultured freshwater bacteria, a standard dilution plating method was applied to a eutrophic freshwater, Inkyoung pond, located within Inha University. A total of 60 strains, 15 strains per each culture medium, were obtained using four different kinds of culture media, including R2A, 1/10R2A, PCA, and 1/10PCA. Among the 60 strains isolated, 27 strains showed less than 97% 16S rRNA gene sequence similarities to validly published species, and thus they are considered to comprise 19 novel species. Of the 27 strains assigned to the novel species, the majority of the strains (20 strains) were affiliated with the *Alphaproteobacteria* and *Betaproteobacteria*. The remaining 7 strains were affiliated with the *Gammaproteobacteria*, *Firmicutes*, *Actinobacteria*, and *Deinococci*. Remarkably, 11 novel strains assigned to the *Betaproteobacteria* comprised 9 novel species. Because we have isolated 19 novel species from a usual freshwater pond using a conventional culturing technique, our results suggest that an unexplored ecosystem, even if it looks like a common ecosystem found elsewhere, harbors diverse unidentified microbes, which will be definitely further characterized.
Keywords: cultivation, freshwater, novel species, 16S rRNA gene, bacterial diversity

B020

Effects of pH on Microbial Communities of Activated Sludge Performing Enhanced Biological Phosphorus Removal in a Sequencing Batch Reactor

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The effects of pH on microbial communities of activated sludge performing enhanced biological phosphorus removal (EBPR) were investigated in an anaerobic/aerobic sequencing batch reactor (SBR) supplied with acetate as a sole carbon source. Almost complete P-removal could be achieved at all pH, meaning that pH changes did not effect on phosphorus removal efficiencies. However, terminal restriction fragment length polymorphism (T-RFLP) and 16S rRNA gene sequencing analyses showed that microbial communities were changed dramatically by pH changes. At high pH (No pH control, ~ pH 8.4) *Pseudomonas*-related bacteria were present predominantly, but *Rhodocyclus*-related bacteria that have been known as typical phosphorus accumulating organisms (PAOs) were absent. The population of *Pseudomonas*-related bacteria decreased by pH decrease and at low pH (pH 6.5) *Rhodocyclus*-related bacteria became a major group. This suggested that *Pseudomonas*-related bacteria group and *Rhodocyclus*-related group might switch their roles as Polyphosphate Accumulating Organisms (PAO) at different pH in an anaerobic/aerobic sequencing batch reactor (SBR) supplied with acetate. We isolated *Pseudomonas*-related bacterium and confirmed that the isolate contained polyphosphate kinase (ppk) and polyhydroxy alkanoic acid synthase (phaC). Additionally in this study sequence analysis of ppk and phaC genes and possibility of *Pseudomonas*-related bacteria as a PAO candidate will be discussed.
Keywords: Enhance biological phosphorus removal, Microbial community, pH

B021

Isolation of Biofilm Forming Bacteria from the Marine Biofilm

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We harvested biofilms on three surfaces (acryl, glass, and steel) exposed to seawater for 9 hours. The collected samples were diluted in sterilized seawater, and incubated on marine agar plates at 20℃. We isolated more than 150 strains from samples, and screened 26 strains by using amplified 16S ribosomal DNA restriction analysis (ARDRA). Finally, 7 distinct strains were selected by 16S rDNA sequence analysis. These strains belong to Pseudoalteromonas, Rhodobacter, Acinetobacter, and Vibrio. The development of biofilm during the cultivation of these strains was observed by using cristal violet staining. The amount of biofilm of *P. elyakovii*, *P. tetraodonis* and *R. litoralis* was 5~12 times higher than that of other strains. [Supported by grants from MarineBio21]

Keywords: 16S rRNA, ARDRA, Biofilm, Pseudoalteromonas

B022

The Responses of Sulfate Reducing Bacteria and Denitrifier Communities to Elevated CO_2 and/or Salinity in a Salt Marsh

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In the future, it is anticipated that coastal marshes will experience dramatic environmental changes due both to elevated CO_2 and salinity changes. Considering the fact that costal marshes are an ecosystem with extremely high primary production, changes in environmental conditions may have substantial influences on local as well as global biogeochemical cycles. In particular, the effects of elevated CO_2 and salinity on soil microbial community will have important implications for nutrients cycling and trace gas emission. We investigated the structure of sulfate reducing bacteria (SRB) and denitrifier communities in a salt marsh that had been exposed to elevated CO_2 and/or salinity. We analyzed the community structures using T-RFLP of *DSR* and *nirS* genes. Elevated CO_2 did not affect the community structure of denitrifiers and SRB. However, salinity increased the diversity of denitirfiers significantly (P<0.01). As a result of MRPP testing, significant differences due to salinity were observed in SRB and denitifiers, but not due to elevated CO_2. Ten *DSR* T-RFs that differed significantly among treatments (P<0.05), based on indicator species analysis, existed as indicator fragments for salinity, meanwhile there was no one for elevated CO_2 concentration. As for *nirS* genes, 5 and 1 indicator species were observed for salinity and CO_2 concentration, respectively. We suggest that SRB and denitrifiers would be more vulnerable to changes in salinity compared to elevated CO_2.

Keywords: elevated CO_2, salinity, salt marsh, sulfate reducing

B023

Culture Conditions and Antifungal Activity of Isolated *Streptomyces* sp. AM50 against *Phytophthora capsici*

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Chemical pesticide applied to increase crop production is a major input in agriculture. However they are costly, can cause environmental ruin and may induce pathogen resistance. Therefore, biopesticide have become an important approach to sustainable agriculture. The cellulose and chitin having a β-(1,4)structure, they are an abundant renewable sources obtained from insects, algae and fungal cell walls. Our studies showed that chitinase and cellulase producing microorganisms were screened from soil samples and its production condition. Isolated a strain which produces against plant pathogen fungi effects of *P. capsici* from the sawmill in Korea. Crude fungicide obtained from the culture broth of chitinase and cellulase strains grown in a defined medium containing 0.4% wet colloidal chitin and 0.5% carboxymethyl cellulose(CMC). Subsequently, enrichment culture in a medium containing 0.4% wet colloidal chitin a sole carbon source or a kind of carbon sources. Chitinase assay was done based on the estimation of reducing sugar released during the hydrolysis of colloidal chitin. Yellow halo was identified when the culture media supernatant was loaded onto 0.5% CMC agar plate. Antifungal activities against of plant pathogen fungi using paper disc method. As a result of the 16S rRNA gene sequencing identified of *Streptomyces* sp.. The results of *Streptomyces* sp. AM50 were high units of chitinase produce appreciable amounts of both enzymes and showed growth inhibition of *P. capsici*.

Keywords: Antifungal activity, Streptomyces sp., Chitinase, Cellulase

B024

Cultured Representatives of Uncultivated Marine Bacterial Gene Clusters Obtained from the East Sea and Yellow Sea

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Recently ecologically important representatives of marine prokaryotic gene clusters, such as SAR11 and marine group I *Crenarchaeota* have been successfully isolated, most of which don't form colonies on standard agar plates. The dilution-to-extinction cultivation approaches, however, have also shown that many of marine oligotrophic bacteria such as the OMG clade can form microcolonies after successive adaptation. In this study, we have mainly focused on isolating microcolony-forming marine bacteria to facilitate further valid publication of the marine isolates. Seawater samples were collected from three areas of the East Sea and Yellow sea. Either serial-dilution plating or dilution-to-extinction culturing followed by microcolonies observation was employed. The 16S rDNA sequences obtained from a total of 703 colonies were aligned in the ARB database and phylogenetic analyses were carried out. Representatives belonging to previously uncultured and cosmopolitan alphaproteobacterial lineages including SAR116, RCA, and OM75 clusters, unidentified gammaproteobacterial lineages such as CHAB-III-7, OM241, HOC21, ZD0117, *Candidatus* 'Endobugula', ARKICE-74, and agg47 clades, and *Verrucomicrobia* subgroup IV-2 clade were successfully cultivated. This study shows that the combination of dilution-to-extinction and microcolony observation was successful to isolate ecologically important cosmopolitan bacteria, although the routine cultivation method using solid agar plates is out of fashion.

Keywords: microcolony, dilution to extinction, uncultured, 16S rDNA, Seawater

B025

Effects of Genetically Modified Chinese Cabbage (*Brassica rapa* subsp. *pekinensis*) on the Rhizosphere Microbiota

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The genetically modified crops have raised question of environmental effects on non target organisms such as soil bacteria. The effect of genetically modified crop (*Brassica rapa* subsp. *pekinensis*) on the rhizosphere microbiota was examimed in the this study. Culture-dependent and -independent methodologies were used to estimate differences of microbial community between genetically modified and conventinal Chinese cabbages. Generally, plate count method observed no differences occurred but high CFU (4.01×10^6) at the flowering stage of Suwon sample. Community level physiological profiles were significant differences between genetically modified and conventinal Chinese cabbages at Yesan sample. And clone library are no significant differences between genetically modified Chinese cabbage and no genetically modified Chinese cabbage.. Therefore, difference in the soil microbial community is temporary and dependent on the presence of the transgenic plants.
Keywords: GM crop, microbial community

B026

Characterization of Streptogramin-resistant *Enterococcus faecium* Isolated from Pig Farm and its Environment in Korea

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In this study, we investigated the prevalence of Quinupristin/ Dalfopristin (Q/D) resistance in pig farm and its environment and characterized the streptogramin-resistant *E. faecium* (SREF) isolates.During February and June 2006, *E. faecium* isolates derived from 94 environmental samples of 8 pig farms were screen for resistance to Q/D and virginiamycin by E test and agar dilution method, respectively. A total of 31 SREF (MICs for Q/D: 4 to >32 μg/ml) were isolated from animal feces, feed, slatted floor, fence, soil, ventilation and nipple drinker : 20 from slatted floor, 5 from feces, 6 from different environmental sites. All streptogramin-resistant isolates were also resistant to virginiamycin (MICs: 4 to 64 μg/ml). The resistant determinant *vatE* and *erm B* gene were detected in 63% and 100%, respectively. The streptogramin resistant determinants were transferable by filter mating in seven of the 31 isolates frequencies ranging from 1.52×10^{-2} to 5.98×10^{-4} transconjugant per donors. PFGE revealed that the SREF isolates were genetically diverse but some isolates originated from same source showed the identical PFGE patterns.This study showed that the presence of SREF in pigs and pig farm environment of *E. faecium* that resistant to Q/D is cause for concern.
Keywords: Enterococcus faecium, streptogramin, environment

B027

Variation of Microbial and Biochemical Factors During Composting of Food Waste

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The variations of bacterial population, fungal population, and biochemical factors were surveyed during composting of food waste. Bacterial and fungal population decreaced until 60 day's fermentation, and maintained afterward. Biochemical factors varied similary. The compost made in this study showed good effect on plant growth.
Keywords: Compost, Food waste, Microorganisms, Biochemical factor

B028

Effect of Endophytic Fungi and Arbuscular Mycorrhizal Fungi on Plant Community Structure.

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This study was conducted to investigate effects of foliar endophytic fungi and arbuscular mycorrhizal (AM) fungi on plant community structure in experimental microcosms containing an assemblage of six species of plant (*Oenothera odorata, Plantago asiatica, Trifolium repens, Isodon japonicus, Aster yomena, Iris pallasii)*. Three species of AM fungal spores were extracted from pure cultures and the mixture of the four species was inoculated. Leaves of *Sasa borealis, Potentilla fragarioides, Viola mandshurica, Arundinella hirta* were collected in Chungbuk. Endophytic fungi were isolated from the surface sterilized leaves were identified to species using molecular and morphological techniques. Six isolates of the endophytic fungi were inoculated to the leaves of host plants. After four months of growth in green house, effects of both symbiotic fungi on plant species diversity, community composition and productivity were examined. The plant species diversity and composition showed significant differences with inoculation of the symbiotic fungi. Results indicate that both symbiotic fungi may significantly affect plant community structure.
Keywords: arbuscular mycorrhizal (AM) fungi, endophytic fungi, plant community structure, microcosm

B029

Algicidal Effect on *Cochlodinium polykrikoides* Using a TiO_2 Photocatalytic Reactor

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TiO_2 photocatalyst aluminar bead were prepared by sol-gel method using TTIP (Titanium Tetra Iso-Propoxyde) precutsor. The aim of this study was to design a high efficiency photocatalytic reactor and its application to kill *Cochlodinium polykrikoides*. The photocatalytic reaction was carried out with various flow rate of cell suspension and ultraviolet(UV)-A illumination time. In the photokilling efficiency of photocatalytic reactor on *Cochlodinium polykrikoides*, it was confirmed that UV-A illumination time and flow rate affected the cell killing activity. As the UV-A illumination time increased, algicidal effect increased. After 40min UV-A illumination time, killing activity of *Cochlodinium polykrikoides* was more than 80%.
Keywords: Algicidal, Cochlodinium polykrikoides, TiO2, photocatalytic reactor

B030

Biodegradation of Endocrine Disruptors by Several White Rot Fungi

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In recent years, endocrine disruptors have attracted a public attention owing to ubiquity in the environments and endocrine-disrupting activities in various animals. We investigated the biodegradation of endocrine disruptors by several white rot fungi such as *Irpex lacteus, Schizophyllum commune, Trametes versicolor* and transformants of *Trametes versicolor* (strain MrP 1 and MrP 13). Endocrine disruptors tested were bisphenol A, nonylphenol, and phthalates. *I. lacteus* degraded 100% of 50 mg/L^{-1} bisphenol A and nonylphenol in 1 day incubation, which was the highest degradation rate among 5 fungal strains tested. *T. versicolor* degraded 97% of 50 mg/L^{-1} bisphenol A and 35% of nonylphenol in 1 day incubation. The degradation of endocrine disruptors was accompanied by the peroxidase activity. We examined the effects of carbon and nitrogen source on biodegradation of endocrine disruptors by white rot fungi. The removal mechanism in terms of biodegradation and biosorption was also investigated.
Keywords: endocrine disruptor, white rot fungi, biodegradation, bisphenol A, nonylphenol

B031

Plant Growth Promoting Capability of Some Rhizobacteria and Photosynthetic Bacteria

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This study was conducted with some rhizobacteria and photosynthetic bacteria for the plant growth promotion. Some rhizobacteria and photosynthetic bacteria are able to exert beneficial effects upon plant growth. Several soil bacteria and purple nonsulfur bacteria were isolated from rhizosphere and river sediment. Purple nonsulfur bacterium, KL9 was applied to the germination test of tomato seed, and shoot and root length of germinated tomato after 9days were 13.1, 38.3 mm, respectively, which were longer than those of control. Production of indole acetic acid and aminolevulinic acid was 70 and 7.7 mg/L^{-1} by KL9, respectively. Rhizobacterium PS2 could solubilize 98.8% of 0.5% tricalcium phosphate in 3 days. In addition, PS2 inhibited the growth of pathogenic fungi, such as *F. oxysporum* and *P. infestans*. It also produced siderophore, hydrogen cyanide and ß-1, 3 glucanase, all of which can be helpful for plant growth. The combination of KL9 and PS2 may be used efficiently to enhance plant growth.
Keywords: plant growth promotion, seed germination, purple nonsulfur bacteria, rhizobacteria

B032

Microbial Community Structure Analysis of Domestic and French Polluted Aquatic Environments Using PCR-DGGE and Self-Organizing Map (SOM)

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Structures of microbial communities were analyzed using SOM to understand how the community structures responded to pollution status and gradient of streams polluted with domestic, agricultural, forest and industrial origins at Korea and France. Microbial communities separated and analyzed using PCR-DGGE. Using self-organizing map (SOM), one hundred and seventy eubacterial species of the microbial communities from 196 different samples in total were used as input data for the SOM analysis. The samples were closely grouped according to sampling dates within each cluster. This may indicate that microbial community structures could be more affected by the seasonal environmental factors (temperature, daylight, and kinds of nutrients derived from pollutant degradation, etc.) rather than local site differences. It was, however, shown that several *Clostridium* sp. were grouped together at the SOM map and commonly present in both streams of agricultural sites in France (July, 2004) and streams polluted with domestic wastewaters in Korea (Feb., 2005). This is interesting because the obligate anaerobic populations can be grown in certain places regardless of their sampling sites and dates. This may reflect that some specific sites could provide some specific microbial populations with an optimal habitat condition. It was suggested that these species could work as an indicator species for this kind of habitat.
Keywords: Microbial community, Self-organizing map (SOM), Indicator species, PCR-DGGE

B033

Vertical Distribution of Sulfate Reducing Bacteria in Sediment of Lake Sihwa

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For scrutinizing the distribution of sulfate reducing bacteria in natural ecosystem, FISH method was developed. Aprat from the conventional methods, such as cultivation and MPN, FISH was applied. The SRB385 probe which is specific to *Desulfovibrionaceae* and SRB 385 Db to *Desulfobacteriaceae* were chosen for FISH. Both probes have no signal with negative control and have high intensity with positive control after confirming of incubation temperature, duration and concnetration of washing solution. After confirming the usefulness of probes and process, the vertical distribution of SRB in sediment of Lake Sihwa was measured. SRB numbers in sediment of Lake Sihaw were varied from $3.0x10^6$ -$1.8x10^7$ cells/g ,and high peak was appearing at surface layer. After 6 cm depth, the number of SRB was constant, about $4.0x10^6$ cells/g.

Keywords: Sulfate reducing bacteria, Lake Siwha, sediment, FISH

B034

A Fungal Secondary Metabolite Increases Motility of *Paenibacillus polymyxa*

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P*aenibacillus polymyxa* is gram-positive bacterium can be isolated ubiquitously in rhizosphere, has been known as biocontrol agent enhancing plant growth, potential antagonizing bacterial or fungal plant pathogens. We observed that the motility of *P. polymyxa* E681 was increased by *Penicillium citrinum* on TSA. The main compound involving in increasing motility of strain E681 was identified as citrinin by LC/MS analysis. RT-PCR data showed that citrinin could increase transcriptional level of *sigD* (transcriptional sigma factor) and *hag* (encoding flagellin) of *P. polymyxa* E681. The result of flagella staining supported RT-PCR data that the flagella were propagated dose dependent by citrinin. Our results presented here provide an insight of a fungal metabolite that mycotoxin citrinin increased bacterial motility by up-regulation of transcriptional sigma factor and flagella synthesis.

Keywords: Paenibacillus polymyxa, Citrinin, Bacterial motility, mycotoxin

B035

Diversity Analysis of PHA Synthase (*phaC*) Genes in Activated Sludge Performing Enhanced Biological Phosphorus Removal

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Developments in molecular biology have led to rapid and reliable tools to characterize microbial community structures and to monitor their dynamics under *in situ* conditions. However, there has been a distinct lack of emphasis on monitoring the functional diversity in the environment. Genes encoding polyhydroxy alkanoic acid (PHA) synthase (*pha*C), as a key enzyme of enhanced biological phosphorus removal (EBPR), were analyzed to assess the gene diversity of activated sludge performing EBPR in sequencing batch reactor supplied with acetate as a sole carbon source. PCR primer sets (gp1, gp2, gp3, gp4) were designed from alignments of 31 known phaC gene sequences and tested with generally well-characterized strains. Among them, two primer sets, gp1 and gp3, amplified partial *pha*C genes successfully and their clone libraries were constructed. One hundred four clones with a partial *pha*C insert were analyzed by Restriction Fragment Length Polymorphism (RFLP) using *Hae*III and *Hha*I double digestion and were grouped into 39 distinct groups with same fragment patterns. The representatives of the 39 groups were sequenced and analyzed phylogenetically. The results showed that activated sludge performing EBPR had high diversities of *pha*C genes. Additionally in this study sequence analysis of *pha*C gene related *Rhodocyclus*-related bacteria will be discussed.

Keywords: Enhanced biological phosphorus removal, PHA, Diversity analysis

B036

Plant Growth Promoting Activity of Endophytic Fungi Isolated from Medicinal Plants in Korea

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Twelve Plant Growth Promoting Fungi (PGPF) isolates were selected *in vitro* from 321 endophytic fungi which were isolated from different medicinal plants in Korea. On the basis of their molecular characteristics 12 isolates were identified as *Cladosporium* (n=1), *Diaporthe* (n=1), *Leptosphaeria* (n=2), *Polyporus* (n=1), *Phomopsis* (n=1), *Spirosphaera* (n=1) and unidentified species (n=5). Germination rate of seed treated with isolate CNU050703 increased 5.3% more compared to the control. Three isolates significantly promoted the growth of rice *in vitro*. Out of selected twelve endophytic isolates, five showed cucumber growth promotion *in vivo*. Isolate CNU 050732 significantly increased the growth of cucumber followed by CNU050726 *in vivo*. Little growth promotion was found by isolates CNU050605, CNU050685 and CNU050804.

Keywords: PGPF, Endophytic Fungi, Medicinal Plants

B037

Isolation of TBTC (tributyltin chloride)-resistant Bacteria from Soil and Water

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Tributyltin chloride (TBTC) resistant bacteria were isolated from soil and water samples. The samples were collected from Shiwha industry complex and Ulsan shipbuilding yard. Using nutrient broth medium (pH 7.0) containing various concentration of TBTC (0-100 mg/l), the each sample was incubated and examined its viability at 30℃. In the case of the medium containing 100 mg/l of TBT, 5 morphologically different TBTC resistant strains were isolated. When the cultured media were monitored using stripping voltammetric method, variations in TBTC concentration were observed. The isolates and the analytic method of TBT herein can be further utilized to design effective bioremediation method for endocrine disruptors including TBT.

Keywords: Tributyltin, stripping voltammetry, TBT resistant bacteria

B038

Development of a New Biological Pesticide to Control Rice-Blast Using Anti-Fungal Bacteria and Plant Extracts

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It is well known that *Bacillus* sp., a bacteria usually used as a biological pesticide, possesses anti-fungal activity. But the anti-fungal activity of this bacteria strictly depends upon the environmental conditions. In order to complement its disadvantage, we tried to mix plant extracts having anti-fungal activity with the bacteria, and investigated the possibility that this mixture was available for the control of rice-blast. Through the screening of useful bacteria and plant extracts, we selected two strains of *Bacillus subtilis* (AE 111 and AE 1322) and cornhust as anti-fungal bacteria and plant extract, respectively. The mixed form of biological pesticide (referred to ARA) against rice-blast was made up with the culture supernatant of the anti-fungal bacteria (20%), the cornhust extract (20%), sodium alginate (3%), Tween-20 (5%), stablilizer (10%), and carrier materials (42%). When we carried out a field test in a farm located in Okcheon, Chungbuk, we found that the spraying of 100-fold diluted ARA markedly suppressed rice-blast (62% inhibition). These data indicates that ARA is a promising candidate available for the control of rice-blast. Further study to enhance the effect of ARA is currently performed.

Keywords: rice-blast, bacteria, plant, biological pesticide

B039

Community Structure of δ-*Proteobacteria* Associated with Pathways of Microbial Respiration in Vegetated and Unvegetated Intertidal Mudflats

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The objectives of this study were to relate the major pathways of anaerobic microbial respiration to molecular phylogenetic evidence in the unvegetated (UMF) and vegetated mudflat (VMF) of the intertidal sediments. Depth integrated C oxidation rates were 5 times higher at VMF (351 mmol C m^{-2} d^{-1}) in comparison to UMF (73 mmol C m^{-2} d^{-1}). Dissimilatory sulfate- and Fe(III) reduction accounted for 49.3% and 35.6%, respectively, of the C oxidation at UMF site, but represented 19.9% and 37.3%, respectively at VMF site. The results indicated that both sulfate- and Fe(III) reduction were a significant C oxidation pathways in UMF site, whereas Fe(III) reduction was more significant at VMF site. Clones related to δ-*Proteobacteria* accounted for 25.2% of 539 clones sequenced for 16S rRNA genes, which was a good agreement with the results of T-RFLP analysis. Phylotypes affiliated with known Fe(III) or S^{o} reducing bacteria (*Desulfuromonas/ Pelobacter*) were abundant in clone libraries from UMF (32.4%) and VMF (48.6%) sediments at deeper depths, whereas sequences close to sulfate reducers (*Desulfobacterium* & *Desulfobulbus*) were retrieved from all depths of the sediments sampled. Interestingly, the majority (56.2%) of the uncultured *Desulfuromonas/Pelobacter* group was observed in the rhizosphere of VMF (VMF-5 cm co). Therefore, the enhanced significance of Fe(III) reduction to C oxidation at the VMF site paralleled with the phylogenetic analysis of the δ-*Proteobacteria*.

Keywords: Intertidal mudflats, C oxidation pathway, Fe(III) reduction, Sulfate reduction, Delta-Proteobacteria, Suaeda japonica, Rhizoshpere

B040

Iron Zinc Superoxide Dismutase of *Streptomyces subrutilus* P5 and Its Contribution to Heavy Metal Tolerance

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Streptomyces subrutilus P5 produced an extracellular superoxide dismutase containing iron and zinc (FeZnSOD) that could absorb heavy metal ions (lead ions) as much as 1,000 gram-atom/mol subunit. Therefore, we produced the gene of FeZn SOD by PCR and expressed the insert DNA in *E. coli* strain M15[pREP4], using a commercial expression vector (pQE-30). Although the protein produced should have a N terminal 6x His tag and formed inclusion bodies in *E. coli*, it showed SOD enzyme activity that could be distinguished from the SODs of the recipient *E. coli*. In the presence of 0.1 mM of lead ions, the *E. coli* strain producing the exogenous SOD showed increased survival rates than the non-induced strain. Therefore, we could confirm that the role of FeZn SOD as a heavy metal sink which might confer the survival advantage in heavy metal polluted environment.

Keywords: FeZn SOD, Heavy metal tolerance

B041

Antimicrobial Resistance of 114 Swine Isolates of *Campylobacter coli*

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Campylobacter species were isolated from 24 porcine farms in 10 different regions in Korea, and were assayed with regard to their antimicrobial susceptibility to 8 antimicrobial agents. A total of 114 *Campylobacter* isolates from 572 intestinal samples were all identified as *C. coli* via both classical methods and molecular methods, including 16S rDNA sequence analysis and PCR using a specific primer set for the hippurate gene, designed to differentiate *C. coli* from *C. jejuni*. When minimal inhibitory concentrations to eight antimicrobial agents were determined via agar dilution, the MIC_{90}s were 64 μg/ml for ampicillin, ≥128 μg/ml for cephalothin, 8 μg/ml for chloramphenicol, 64 μg/ml for ciprofloxacin, 16 μg/ml for enrofloxcin, ≥128 μg/ml for erythromycin, ≥128 μg/ml for gentamicin, and ≥128 μg/ml for tetracycline. Resistance rates to each antimicrobial agent were as follows: 28.9% for ampicillin, 95.6% for cephalothin, 2.6% for chloramphenicol, 84.2% for ciprofloxacin, 83.3% for enrofloxacin, 46.5% for erythromycin, 20.2% for gentamicin, and 56.1% for tetracycline. All 114 of the isolates were found to be resistant to at least one antimicrobial agent, and 104 of the isolates were found to be multi-drug resistant to more than three antimicrobial agents in different classes.

Keywords: *Campylobacter coli*, swine, antimicrobial resistance

B042

Microbial Diversity in the Soil of Bigeum Island at Shinan

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The culturability of bacteria in the soil of farming field(Bigeum island at Shinan, Korea) containing germanium component was investigated by using diluted R2A agra and Humic acid-vitamine agar as the growth medium. 350 isolates were obtained from plate counting experiments and were identified by comparative analysis of partial 16S rRNA gene sequences. 98 species of these isolates were distributed group of soil bacteria within the divisions *Actinobacteria*, *Proteobacteria*, and *Bacteridetes*. One third of the isolates showed high similarities with diverse uncultured bacterium clones. Diluted R2A agra and Humic acid-vitamine agar medium was very effective to isolate the uncultured bacteria. This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, Ministry of Science & Technology, Republic of Korea

Keywords: Diversity, Isolation, Uncultured bacteria

B043

Immuno-$TCID_{50}$, a New Method for Rapid Detection and Quantitation of Enteric Viruses in Water

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Total culturable virus assay (TCVA), established by US EPA, is a method with standard operation procedure for detection of enteric viruses from water samples. However, the experimental procedure of the TCVA is labor-intensive and time-consuming. We suggested a new method, Immuno-$TCID_{50}$ that combined immunostaining and PCR for rapid detection and quantitation of the contaminated viruses. Poliovirus was used as a target virus in this study. The result of TCVA is displayed as most probable numbers which is calculated from the number of plates with cytopathic effect. In the Immuno-$TCID_{50}$, multi-well plates were inoculated with filter-concentrated water samples, incubated for 48 h, and immunostained with specific antibodies to target viruses. Thus, $TCID_{50}$ of the inoculum can be estimated by positive immunostaining of the viral proteins with cocktailed antibodies with specific chromogens, and primary identification of the contaminated viruses will be made simultaneously through the specific signals of antibodies used. After immuno-$TCID_{50}$, in addition, we used RT-PCR for qualitative analysis of replicated viruses in supernatant to confirm the types of the infectious viruses. As a result, it was shown that 1 to 10 infectious poliovirus particles can be detectable within 48 h when the results of immuno-$TCID_{50}$ was compared with those of TCVA. In conclusion, this new approach enables a fast monitoring of enteric viruses existing in environmental water samples.

Keywords: TCVA, immunostaining, $TCID_{50}$, water sample, enteric virus

B044

Influence of Electric Pulse on Bacterial Community Growing in Soil

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Titanium plates were equipped in square-type flowerpot to induce electric pulse between electrodes. Electric pulse was induced by reciprocal exchange of anode and cathode at the intervals of 30 seconds. Red peppers were growing between anode and cathode and whole cells of *Ralstonia solanicearum* cultivated in LB medium for 48 hr was inoculated into around the red pepper plants. Distance between anode and cathode was adjusted to about 26 cm and distance between electrodes and hot pepper was adjusted to about 13 cm. Electric potential charged between anode and cathode was DC 20 volts and maximal current at the second of electric poles exchange was about 8 mA but minimal current right before electric poles were exchanged was 0.5 mA. Bacterial cells were counted at the intervals of one week and *R. solanicearum* was separately counted from other bacterial communities by using selective medium containing indicator (2,4-d-triphenyltetrazolium chloride). Bacterial cell numbers in the electric pulse were lower than those in conventional flowerpot. Bacterial cell numbers around electrode were lower than those around roots of red pepper plants. However, the red pepper plant normally grew in the electric pulse in comparison with the red pepper plant grown in normal condition. This is a reasonable evidence that the bacterial community may be influenced by the electric pulse but the plant did not.

Keywords: electric pulse, red pepper plant, Ralstonia solanicearum

B045

Develpoment of ICC-SS-PCR for Fast-Track Strategy for the Detection and Quantitation of Enteric Viruses in Water

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Production and supplying qualified water become an important national object of every country throughout the world. Total culturable virus assay (TCVA), developed by U.S. EPA, has been suggested and used as the most reliable method to detect the virus in the water in many countries. Despite many advantages in application of TCVA, it claims rather longer assay period, about four weeks or longer, which often hinder rapid response to hazardous virus contamination in public water source. In this regard, we suggested a new strategy that enables rapid detection of enteric viruses in water samples. ICC-strand-specific PCR (ICC-SS-PCR) is a modified method of conventional strand-specific PCR. The ICC-SS-PCR includes an advantage that reduces the total assay time with an acceptible sensitivity of PCR. Poliovirus, as a target for detection, was inoculated to BGM cell, and negative strands, that can be present for evidence of infectivity, was amplified by strand-specific PCR. MPN based on the PCR results of inoculated plates can be obtained within 12 h and also shown to be comparable to the MPN from TCVA. In conclusion, the ICC-SS-PCR, which is a combined method of PCR sensitivity and TCVA reliability at minimal assay time consumption, can be substituted for TCVA at an emergency condition.

Keywords: TCVA, negative strand, water sample, enteric virus, ICC, strand-specific

B046

Effect of Ectomycorrhizal Fungal Diversity on Growth of *Pinus densiflora*

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This study was conducted to investigate effect of ectomycorrhizal (ECM) fungal diversity on *Pinus densiflora* seedlings. Total five species of ectomycorrhizal fungi were isolated from root tips of pine seedlings collected from Mt. Songni and used as inocula: *Phialocephala fortinii, Cenococcum geophilum, Mollisia cinerea, Leptodontidium elatius* and *Lachnum pygmaeum*. Single species and a mixture of five ECM fungal species were inoculated to the pots containing pine seedlings. ECM was formed on all of the seedlings except for control plants. Growth of pine seedlings responded differently on different ECM fungal species. Also, Pine seedlings inoculated mixture of five ECM species showed the highest biomass. Results suggest that colonization of more diverse species of ECM fungi would increase plant productivity and the selection of suitable ECM fungal species could be important factor for the plant growth.

Keywords: Ectomycorrhizal, Diversity

B047

Diversity of Ectomycorrhizal Fungi from *Pinus densiflora* Seedlings in Disturbed Forests

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The purpose of this research is to investigate diversity of ectomycorrhizal fungi (ECM) colonizing pine seedlings for the selection of suitable ECM fungi to restoration of disturbed forests in Korea. Three disturbed forests were selected in Korea and the pine seedlings with a ECM fungi were randomly collected at disturbed and undisturbed sites in a forest to compare ECM fungal community between sites. Tips of the roots were observed under microscope and the identity of ECM fungal taxa was determined based on morphotyping and molecular analysis through PCR with a fungal specific primer pair ITS1F/ITS4 and sequence analysis. Diversity of ECM fungal mophotype and species composition of ECM fungal communities were various in disturbed sites. The major ECM fungal species in disturbed sites were Araricales, Pezizales, Thelephorales. In conclusion, the composition of ECM fungal communities of a disturbed site was significantly different from a undisturbed site. A possible use of ECM fungi identified in this study for the revegetation of disturbed forest was discussed.

Keywords: Pine, Ectomycorrhizal, ECM, Diversity, disturbed

B048

Biochemical and Molecular Characterization of a Phthalate-degradative Pathway in Marine Bacterium *Chromohalobacter* sp. Strain HS-1

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The new marine bacterium *Chromohalobacter* sp. strain HS-1 was isolated for the ability to grow on phthalate as the sole carbon and energy source, and characterized to be moderately halophilic with an optimal NaCl concentration of 10%. Although HS-1 cannot grow on two phthalate isomers (iso- and terephthalate), it is capable of growth on several aromatic carboxylates such as benzoate, *m*- and *p*-hydroxybenzoate. By colorometric analysis for phthalate intermediates, it is proposed that HS-1 initiates the phthalate degradation through dioxygenation and decarboxylation forming 4,5-dihydro-4,5-dihydroxyphthalate and protocatechuate, respectively, which is degraded by a *meta*-ring cleavage dioxygenase. To study the phthalate degradation pathway in molecular level, PCR amplification was carried out with HS-1 chromosome and a degenerate primer set, which was designed for aromatic oxygenase genes. Successfully, a PCR product containing a gene fragment encoding multi-component aromatic dioxygenase was amplified, and then analyzed to be similar (65% identity) to a benzoate dioxygenase large subunit from a rhodococal strain. Now, colony blot experiments with a HS-1 cosmid library and the PCR product as a probe are in progress to obtain a phthalate-degradative gene cluster from HS-1.

Keywords: Chromohalobacter, Halophile, Phthalate degradation, Dioxygenase

B049

Tolerance of Ectomycorrhizal Fungi from Coal Mining Spoil to Heavy Metals

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In this study, tolerance of four species of ectomycorrhizal (ECM) fungi to heavy metals, Cu, Zn were investigated. Two species of ECM fungi, Cenococcum geophilum and Phialocephala fortinii were isolated from pine seedlings in an abandoned coal mine spoils in Korea, contaminated with high levels of heavy metals. The other two species of ECM, Pisolithus tinctorinus and Rhizoscyphus ericae were isolated from pine seedlings in soil with normal concentration of heavy metals. After 4 weeks of growth on the MMN media containing 25, 50, 75 and 100 ppm of CuSO4 and ZnSO4, tolerance of the ECM fungi was measured as inhibition of radial growth. All four ECM fungal species showed significantly higher tolerance to both heavy metals than other fungi. However, native fungal species in coal mine spoils showed higher tolerance to the heavy metals than the other ECM fungi. The results suggest that use of native ECM fungi would be an important factor in success in establishing seedlings in heavy metal contaminated soil such as mine spoils.

Keywords: tolerance, ectomycorrhizal (ECM) fungi, heavy metal, coal mine spoils

B050

Genetic Diversity in Iprobenfos-Sensitive and Resistant Isolates of *Pyricularia grisea* Using Random Amplified Polymorphic DNA Analysis

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In order to study genetic variation among populations of 50 sensitive and 50 resistant isolates of *Pyricularia grisea* (= *P. oryzae*) to iprobenfos, random amplified polymorphic DNA (RAPD)-based DNA fingerprinting of *P. grisea* was used with four primers (OPA-04, OPA-13, OPJ-06, and OPN-03) and produced 44 RAPD polymorphic bands. Cluster analysis on these bands revealed the presence of high genotypic diversity and continuous DNA fingerprint variation in the fungal population. The correlation was observed between RAPD patterns and fungicide resistance to iprobenfos. Unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis shows the presence of five main clusters. A similar pattern was obtained when principal coordinate analysis (PCO) was applied. Within clusters, similarity was higher than 70% except for the cluster V. When these clusters were compared in mycelial growth and sporulation as fitness components, there were closer relationships between RAPD-derived clusters and fungal fitness components. These results may indicate that isolates resistant to iprobenfos generally have higher ability in the fitness components with genetic variation.

Keywords: Pyricularia grisea, genetic diversity, fungicide resistance, random amplified polymorphic DNA analysis

B051

Visual Detection of *E. coli* from Environmental Samples Using the Nanogold Labeled DNA in a Microfluidic Chip

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Water is undoubtedly one of the major natural resources necessary to the maintenance and well being of human life. The use of indicator organisms, in particular *E.coli*, as a means of assessing the potential presence of pathogens in environmental samples that could result in the contamination of human water supplies has been pivotal in protecting public health. A few number of studies (Taton et al. 2000; Ye-Fu Wang et al. 2003) have reported methods of DNA detection based on a two-probe sandwich hybridization/nanoparticle amplification coloring technique. Based on it, this study presents how a biosensor works based on two probes-hybridization system using gold nanoparticles in a microfluidic chip to detect and monitor *E.coli* from environmental samples. Gold nanoparticle-supported detection probe and capturing probe, covalently immobilized on a slide glass, are hybridized to PCR-amplified target 16s rRNA fragment of *E.coli*, followed by silver enhancement for hybridization signal amplification. Employment of gold nanoparticle and silver enhancement increases sensitivity and decreases the time since black staining can be easily observed with light microscopy or naked eyes. Target 16s is detected on a glass slide by visual inspection. This decreases the cost since no costly detection equipment such as fluorescence scanner is needed for reading hybridization signals.

Keywords: Nanogold, silver enhancement, Microfluidic

B052

Role of *rpoS* Gene from *Pseudomonas* sp. KL28 in Alkylphenol Tolerance and Colony Morphology

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We have isolated a Tn5 transposon mutant of an alkylphenol degrader, *Pseudomonas* sp. KL28 that showed an increased resistance to cresol on agar plate. Mutation was found in *rpoS*, encoding a stationary phase sigma factor. The deduced amino acid sequence of *rpoS* has 96% identity to that of other *Pseudomonas* such as *P. putida* KT2440 and *P. entomophila* L4. The *rpoS* gene is flanked by *nlpD*, encoding a membrane-bound lipoprotein, and *rsmZ*, encoding a regulatory small RNA. This genetic organization is typically found in other *Pseudomonas*. Compared to the complementary strain, the *rpoS* mutant showed enhanced wrinkles on agar plate and also showed faster spreading on soft agar. In addition, the *rpoS* mutant exhibited an enhanced biofilm formation that was not stimulated by self-producing diffusible signals. These results imply that enhanced biofilm formation might afford the *rpoS* mutant to spread faster on solid surface and to give more resistance to cresol.

Keywords: Pseudomonas, rpoS, biofilm, chemical toxicity, alkylphenol

B053

Role of the GacA/GacS Two-Component Regulatory System from an Alkylphenol Degrader *Pseudomonas* sp. KL28 on Biofilm Formation

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Pseudomonas sp. KL28 is a soil isolate that can grow on 4-*n*-alkylphenol (C1-C5) and *m*-cresol and 3-ethylphenol. This bacterium was shown to have a unique *lap* catabolic genetic organization which is distinctive from those found in *Pseudomonas* that degrade phenol and methylphenols. In addition to the catabolic capability that a degrader has, the physiology of the strain would be important for biodegradation. Thus, we were interesting in the genes that modulate biofilm formation by strain KL28. In this study we have isolated Tn5 transposon mutants that have mutation on *gacA* and *gacS* and we have studied the role of the GacA/GacS two-component regulatory system on biofilm formation. Both mutants showed reduced surface translocation and also exhibited a reduction in biofilm formation in microtiter plate wells and pellicle formation in standing tube culture relative to wild type KL28. However, no significant difference was observed in the planktonic growth rates between the two mutants and KL28. Providing *gacA* in trans on the multicopy vector pBBR1MCS-5 to the *gacA* mutant restored normal biofilm formation. This study establishes GacA/GacS as important regulatory elements in *Pseudomonas* sp. KL28 biofilm formation.

Keywords: Pseudomonas, gacA, gacS, biofilm, alkylphenol

B054

Analysis of Bacterial Community Structure of Earthworm (*Eisenia fetida*) Intestine by DGGE

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A bacterial community structure of earthworm (*Eisenia fetida*) intestine was investigated based on 16S rDNA analysis. A polymerase chain reaction (PCR) - denaturing gradient gel electrophoresis (DGGE) method was used for analyzing 16S rDNA of the earthworm intestine microflora. Three universal primers for 16S rDNA of bacteria were first evaluated by PCR-DGGE using genomic DNAs of 6 pure culture strains of aerobic and anaerobic bacteria. The DGGE analysis was possible with 341F-GC and 518R primer pair. When this primer pair was used for analysis of microflora of earthworm intestine, good separation and quality of patterns were obtained in DGGE analysis. DNA fragments were excised from the DGGE gel and their sequences were determined.

Keywords: DGGE, earthworm

B055

Biodegradation of Endosulfan by Mixed Strains Immobilized on Active Carbon

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Immobilization system was used for the bioremediation of simulated pesticide contaminated groundwater and tested with recalcitrant compounds in batch and continuous regimes. In laboratory-scale study, the development of supporting materials with activated carbon for microbial immobilization. Also the biodegradation of endosulfan by immobilized *Klebsiella oxytoca* KE-8 and *Pseudomonas* sp. KS-2P in batch shake flask culture was conducted. A tested materials, granular activated carbon(GAC) 8~30mesh was the best attachable material and microorganisms were attached 4mg/g(dry weight) at surface in 24hr. we tested the biodegradation capacity of immobilized bacteria, different parameters such as substrate concentration, pH, temperatures, storage stability ,Recirculation and continuous contact with reaction medium that affect endosulfan biodegradation were investigated under batch system. [This work was supported by a grant (Code:20050301-034-421-006-01-00) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.]

Keywords: Endosulfan, Immobilization, Biodegradation

B056

Biodegradation Pathway of Vinclozolin by *Rhodococcus* sp. T1-1

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Recent concern about the possible effects of endocrine disrupting chemicals on humans and wildlife has resulted in considerable interest in environmental contaminants, such as pesticides, that effect aspects of reproduction and early development. Dichloroanilines (DCAs) are degradation products formed in natural environments by the degradation of various phenylcarbamate, phenylurea, and acylanilide herbicides, including vinclozolin. Previous reported that DCAs were cytotoxic *in vitro* to rat renal cortical and hepatic slices and had identified heterogeneity in the severity of renal toxicity among the various structural isomers of DCA. 3,5-DCA as the VCZ degradation products are more toxic and persistent than the parent VCZ. In this study, we report the concurrent degrading and propose of degrading pathway on VCZ and 3,5-DCA. To explore the applicability of this strain for bioremediation to control environmental VCZ and its metabolite's pollution, its degradation potential at a 200 ㎍/㎖ concentration was examined in a mineral medium devoid of carbon source. The strain represented the degradation efficiency of 25 ㎍/㎖ /day in minimal media containing VCZ and degraded about 80% of 3,5-DCA after 6 days. And we suggested VCZ degradation pathway by *Rhodococcus* sp. T1-1. [This work was supported by a grant (Code: 20050301-034-421-006-01-00) from BioGreen 21 Program, Rural Development Administration, Republic of Korea]

Keywords: Vinclozolin, 3,5-Dichloroaniline, Biodegradation, Detoxification

B057

Solubilization of Biological Waste Sludge by an Alkalophilic *Exiguobacterium* sp. YS1

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Conventional activated sludge process is an economical and effective biooxidation process although a large amount of excess sludge is necessarily generated. In this study, a new approch to the solubilization of excess activated sludge by a combined treatment of NaOH and alkaline enzymes of *Exiguobacterium* sp. YS1 was investigated with the aim of recycling the solubilized material back to the wastewater treatment plant for biodegradation. The operating conditions for this solubilization process were maintained at 30℃, reaction time of 24 h, total solids (TS) in the feed of 0.8-1.4%, and NaOH dosages of 15-20 ml/L. At pH=10 and TS=1.0% and after 10 h of reaction time, the concentration of soluble chemical oxygen demand (SCOD) reached maximally up to 6,200 mg/L. Under a continuous aerobic solubilization process, where operating conditions were maintained at 30℃, pH=10 and TS=3.0% and 48hr of hydraulic retention time, SCOD concentration of more than 3000 mg/L remained almost constant throughout the continuous process for over two weeks. On the other hand, under a continuous anaerobic solubilization process, SCOD concentration was continuously kept around 6000mg/L. Compared to the conventional alkaline treatment method it provided an increase of the final SCOD by about 15~20%. From these experimental results it was demonstrated that the application of *Exiguobacterium* sp. with alkaline treatment has potential as an effective procedure for processing of excess waste sludge.
Keywords: activated sludge, sludge solubilization, alkalophilic enzyme

B058

Distribution of Microorganisms and Physico-Chemical Characteristics in the Chagwi-Do Coastal Waters, Jeju Island

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To investigate the variations of physico-chemical factors and microbial population, in ten stations at water region of coastal area of Chagwi-Do, Nutritive salts, water temperature, transparency, suspended solid, salinity, COD, DO, pH, heterotrophic bacteria, coliform group and Vibrio spp. were analysed three times in September, November in 2004 and February in 2005. Heterotrophic bacteria in surface water was 3.5×10^{1}～1.16×10^{3} cfu/ml, 1.0×10^{2}～5.2×10^{1} cfu/ml, 2.0×10^{1}～7.6×10^{1} and bottom water counted 7.0×10^{2}～1.0×10^{3} cfu/ml, 1.4×10^{1}～2.5×10^{2} cfu/ml, 2.0×10^{2}～4.2×10^{1} cfu/ml in September, November in 2004 and February in2005, respectively. The cell number of total coliform bacteria in the surface water amounted to 0～4.3×10^{2} cfu/ml, 0～6.0×10^{1} cfu/ml, 0～1.0×10^{1} cfu/ml and bottom water amounted 0～2.2×10^{2} cfu/ml, 0～5.4×10^{2} cfu/ml, 0～2.0×10^{1} cfu/ml in September, November in 2004 and February in 2005, respectively. As for Vibrio spp., the cell number in the surface water was 1.0×10^{1}～2.5×10^{2} cfu/ml, 1.0×10^{1}～2.0×10^{1} cfu/ml, 0 cfu/ml and bottom water counted 1.0×10^{1}～5.2×10^{2} cfu/ml, 0 cfu/ml, 2.0×10^{1} cfu/ml in September, November in 2004 and February in 2005, respectively.
Keywords: Heterotrophic bacteria, COD, suspended solid, coliform group, Chagwi-Do

B059

The Optimal Culture Conditions for Mycelial Growth of *Lentinus giganteus*

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Lentinus giganteus, one of edible and medicinal mushroom belongs to Tricholomataceae of Basidiomycota, has been known to exhibit outstanding inhibitory effect on the sarcoma 180 of mice. As one of preliminary experiment for producing fruiting-body of *L. giganteus*, the culture conditions for mycelial growth were carried out. The optimal conditions for mycelial growth were 25℃ and pH 6, respectively. The favorable mycelial growth of *L. giganteus* was obtained in the Mushroom complete medium. The carbon and nitrogen sources promoting for optimal mycelial growth of *L. giganteus* were fructose and glycine, respectively. The favorable C/N ratio was about 40 : 1 in case that 3% glucose was supplimented to the basal medium as a carbon source.
Keywords: Lentinus giganteus, edible mushroom, culture condition, mycelial growth

B060

Sequencing and Identification of DGGE - Bands for the Analysis of Bacterial Communities in the Culture Enriched with Heavy Metals

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For the development of consortium removing heavy metals, unknown microorganisms in the sediments from K lake were enriched in BS-1 medium containing different concentrations of heavy metals such as 10, 50 and 100 ppm of Cr^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+}, at temperate condition. Using PCR- DGGE as well as cloning and sequencing, the changes of bacterial communities in those cultures were examined. Twenty- four clones of 5 excised DGGE - bands were identified and the results showed that in the culture enriched with 10 ppm of heavy metals, the identified bacterial communities included *Burkholderia cepacia* strain ATCC 53795*, Lepilemur dorsalis, Pseudomonas reactans isolate B132* and *Burkholderia cepacia* strain ATCC 53795*,* and their homologies were 98-100%. In the culture enriched with 50 ppm of heavy metals, *Escherichia coli* strain ATCC 25922*, Burkholderia thailandensis* E264*, Lepilemur dorsalis,* Uncultured bacterium, Uncultured bacterium clone 4, Uncultured soil bacterium colone HBS NT21 and *Caulobacter crescentus* CB15 with 85-100% of homology were found. *Burkholderia cepacia* strain ATCC 53795*, Bacillus cereus* strain ATCC 2562*, Bacillus mycoides* strain Mali 461*, Bacillus cereus* strain CICC10185 and *Bacillus cereus* strain ATCC 33018 were found in the culture enriched with high concentration of heavy metals of 100 ppm, and their homologies were 99-100%.
Keywords: PCR-DGGE, identification and sequencing of DGGE bands, heavy metals, bacterial communities

B061

Molecular Characterization of Naphthalene-Degrading Bacterium *Pseudomonas* sp. Strain As1 Overexpressing Antioxidant Enzymes

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In our previous work(Kang *et al*., *J. Microbiol. Biotechnol.* In press), we demonstrated that naphthalene degradation could impose severe oxidative stress. To alleviate oxidative stress generated during naphthalene metabolism, Overexpression of two enzymes [fpr (ferredoxin-$NADP^+$ reductase) and superoxide dismutase (sodA)] was conducted in naphthalene-degrading bacterium *Pseudomonas* sp. Strain As1, either under control of the native promoter [As1(fpr), As1(sodA)] or under control of an external promoter [As1(fpr-R), As1(sodA-R)]. Overproduction of each gene was confirmed by Northern blot analysis. Growth rate of all recombinant strains was higher than parental strain in the presence of naphthalene. HPLC analysis has shown that As1(fpr-R) degraded naphthalene effectively compared to all other strains including parental strain. Oxidative stress caused by naphthalene degradation in each strain was monitored by a green fluorescent protein (GFP)-based reporter using an oxidative stress-inducible promoter. The level of GFP expression of all strains harboring recombinant plasmid was much lower than that of parental strain. The data provides evidence that overexpression of antioxidant enzyme contributes to oxidative tolerance during naphthalene degradation in Strain As1. Molecular characterization on oxidative response in each recombinant strain will be discussed.(Supported by a NCRC grant R15-2003-012-02002-0)
Keywords: Naphthalene, ferredoxin-$NADP^+$ reductase, superoxide dismutase

B062

Detection of Phenylacetic Acid Using a Green Fluorescent Protein-Based Reporter Fusion in *Pseudomonas putida*

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Pseudomonas putida KT2440 has at least 15 genes that are involved Phenylacetic acid(PAA) catabolism and there are three putative promoters in PAA operons. Northern blot analysis has shown that the transcription of the first genes(*paaA*, *paaG*, and *paaL*) in each operon was highly induced when *P. putida* KT2440 was cultured in minimal medium containing PAA as a sole carbon source. However, expression of those genes was diminished when glucose was amended to medium containing PAA. We constructed a green fluorescent protein-based reporter fusion using the *paaA* gene to detect the presence of PAA in the field because PAA appears to be an environmental toxic chemical. *P. putida* KT2440 harboring the reporter plasmid exhibited an increased level of *gfp* expression in minimal medium containing PAA. In contrast to glucose effect, other carbon sources such as succinate and pyruvate did not lead to catabolic repression.Interestingly, overexpression of a *paaF* gene encoding PAA-CoA ligase leads to the induction of the *gfp* gene even in the presence of glucose. To minimize toxic effect of other chemicals such as copper in the medium, the reporter strain was encapsulated by Ca-alginate bead. Immobilization of the reporter strain was successfully used for detecting PAA under high concentration of copper (15mM). These data suggest that immobilization of the GFP-based reporter could be used for detecting toxic chemicals.(Supported by a NCRC grant R15-2003-012-02002-0)
Keywords: phenyl acetic acid, green fluorescent protein, immobilization

B063

Sulfate Reduction in Rice Paddy Soil, Reservoir Soil, and Foreshore Soil

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We studied sulfate reduction rate(SRR) using $^{35}S\text{-}SO_4^{2-}$, Soil components(moisture, ammonium, total nitrogen, total organic carbon, total carbon, total inorgainc phosphorus, total phosphorus, and sulfate), and the number of sulfate reducing bacteria(SRB) using most probable number(MPN) method in the organic/ conventional rice paddy soils(RPS), cleaned/polluted reservoir soils(RS) and cleaned/ polluted foreshore soils(FS) with the change of seasons. SRR(RPS, 2~47 nmol/g day; RS, 0.02~30 nmol/g day; FS, 1~31 nmol/g day) were more related to the number of SRB(RPS, 2.1×10^4~$2.39\text{x}10^5$ MPN/g wet soil; RS, 7.35×10^3~3.6×10^4 MPN/g wet soil; FS, $<3.61\times10^3$ MPN/g wet soil) than sulfate concentration(RPS, 2~5 mg/g soil; RS, 1~3 mg/g soil; FS, 28~50 mg/g soil), and it also related to other soil components including sulfate concentration if it have a similar number of SRB. And SRR recorded the highest pont in October soil samples. From these results, we can conclude that SRR of anaerobic environments was affected by the number of SRB, soil components and temperature.
Keywords: sulfate reduction rate, sulfate reducing bacteria, soil components, isotope, MPN

B064

Selection of Entomopathogenic Fungi for the Control of *Myzus persicae* and *Aphis gossypii*

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Various strains of entomopathogenic fungi such as *Lecanicillium lecanii*, *Paecinomyces farinosus*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Cordyceps scarabaeicola*, and *Nomuraea rileyi* were screened for the aphid control. Among all entomopathogenic fungi, *L. lecanii* 41185 showed the best virulent pathogenicity. The mortality of *Myzus persicae* and *Aphis gossypii* induced by *L. lecanii* 41185 was nearly 100% by 5 1 and 2 days after treatment with aerial conidia and the LT_{50} was 1.8 and 1.4 days, respectively. The mortality of aphid was drastically depended on the different concentrations of conidia 10^4-10^8 /ml but no significant difference was found between 10^7 and 10^8 conidia/ml in causing mortality. Almost of the tested strains of entomopathogenic fungi could grow at different temperatures at 15-30°C. L. lecanii strains showed the optimum growth at 25°C, whereas *B. bassiana* J57, *P. farinosus* J301 and *C. scarabaeicola* J94 exhibited optimum growth at 30°C, 20°C, and 30°C respectively. The aerial conidia of tested entomopathogenic fungi could germinate at 15-30°C but no observed germination was found at 35°C except *L. lecanii* 41185.
Keywords: Entomopathogenic fungi, aerial conidia, germination and vegetative growth, control value, Myzus persicae and Aphis gossypii

B065

Heavy Metal Adsorption Characteristics of Extracellular Polysaccharide Produced by Purple Nonsulfur Photosynthetic Bacteria Grown on Various Carbon Sources

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Extracellular polysaccharides, or exopolymeric substances (EPS) are produced by bacteria and have an important function in the removal of heavy metals from wastewater and natural waters. For instance, EPS produced by specific bacteria have been used as biosorbents for toxic metals. Five slime-forming photosynthetic bacteria (*Rhodopseudomonas palustris*, *Rhodobacter blasticus*, *Rhodobacter sphaeroides*, *Rhodobacter capsulatus* and *Rubrivivax gelatinosus*) used in this study. They obtained from Culture Collection for Environmental Industrial Microorganism and Genes. Photosynthetic bacteria were identified according to the classification keys of Bergey's Manual of Systematic Bacteriology Second Edition (Don J. Brenner *et al*., 2005) and 16S-rDNA sequence analysis. They were screened for EPS production in basal medium with various carbon sources, respectively. Only a few were found to support growth as the sole carbon source: *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* utilized succinate, malate and pyruvate, *Rhodobacter blasticus* utilized succinate, malate, pyruvate, glucose and fructose, *Rubrivivax gelatinosus* utilized succinate, citrate and malate. There were a change in the production and composition of EPS on various carbon sources. The various EPS composition were analyzed by gas Chromatography, and the heavy metal adsorption characteristics relative to a change in the composition were examined using an Inductively Coupled Plasma(ICP).
Keywords: exopolysaccharide, EPS, photosynthetic bacteria , Heavy metal

B066

Characterization of Two Key Enzymes for Ring-Cleavage of Aromatic Compounds in Multi-Drug Resistant and Pathogenic *Pseudomonas aeruginosa* DU102

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In this study, the multi-drug resistant *Pseudomonas aeruginosa* DU102, which was isolated from a patient, was tested for its biodegradability to evaluate its potential to survive in our surrounding environment frequently contaminated with aromatic compounds. The two key enzymes for ring-cleavages of aromatic compounds, catechol 1,2-dioxygenase (CD1,2) and protocatechuate 3,4-dioxygenase (PCD3,4) were assayed using cells grown on benzoate or *p*-hydroxybenzoate as a substrate, because they are among the most common aromatic compounds with benzenoid structures found in nature. Edman N-terminal sequencing and 2-DE/MS (MS/MS) analysis for the purified proteins expressed in response to the aromatic compounds such as benzoate and *p*-hydroxybenzoate revealed PcaG, PcaH, and CatA for ring-cleavages of aromatic compounds. Furthermore, many of them were among the enzymes responsible for the degradation of toluate, catechol and protocatechuate, which might be intermediates produced from the catabolic process of most aromatic compounds. This work was supported by Basic Research Program funded by the Korean Basic Science Institute (N26047).
Keywords: Multi-drug resistance, Biodegradation activity, Ring-cleavage enzymes, Pseudomonas aeruginosa

B067

Biodegradation of Benzene by Pure Bacterial Culture under Aerobic Conditions

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Monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (BTEX) are widespread contaminants in groundwater, soil and atmosphere, because it is often become pollutants include gasoline and other petroleum fuels, pesticides and wood-treating substances. (E.Jindrova, 2002) Since they suspected as being carcinogens (Bushnell, 1941), their release to the environment is strictly controlled and they are classified as priority environmental pollutants by USEPA. Long recognized as substrates supporting microbial growth, these hydrocarbons are both a energy source and a product of microbial metabolism.(Ehrlich, 1995) Studies have been carried out on the degradation of benzene in aerobic(Bayly, 1984), dinitrifying(Kuhn, 1988) and anaerobic(Vogel, 1986) conditions. Shirai (1986) used 1%(v/v) benzene in the vapour phase to isolate new strains of micro-organisms capable of producing catechol from benzene, and Winstanley *et al*.(1987) cultivated *Acinetobacter calcoaceticus* in 0.4%(v/v) liquid benzene. We examined aerobic degradation of Benzene at 0.05%(v/v) liquid phase by soil bacteria. We isolated 43 strains from oil contaminated soil in Korea. Among them, 12 isolates were grow up quickly with benzene as sole carbon source. Total 12 strains were screened for benzene removal, respectively. BJ10 showed the highest removal efficiency for benzene. In basal medium with 500 ㎎/ℓ benzene, BJ10 could degrade benzene over 95% and showed good biomass growth after 6h of incubation.
Keywords: Biodegradation, Benzene, BTEX

B068

Stimulation of Mycelial Growth and Yield Increase of *Lentinula edodes* Fruiting Body by Treatment of Wood Vinegar from *Quercus* Species

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In order to find out the proper concentrations and application methods for the practical use in *Lentinula edodes* cultivation, mycelial growth was compared on culture media supplemented with wood vinegar from *Quercus* species at the different concentrations. Mycelial growth was stimulated at the concentrations of 1 and 0.5 ㎍/㎖. Complete growth inhibition of the major pathogenic fungi such as *Trichoderma* spp. and *Diatrype stigma* was obtained at the concnetration of 2 ㎍/㎖. Fruiting body production of *L. edodes* from submerged wood logs under the wood vinegar solution was increased as compared to untreated control. Dry weight of harvested fruiting bodies from the logs submerged at the concentration of 10^{-2} dilution showed a significant differences. Storage test of fruiting bodies harvested from the logs submerged under the water adjusted with different concentrations, wrapped with vinyl film, and kept at 10℃ for 10 days showed the best results at the treatment of 0.4 ㎍/㎖ in color, shape, and freshness. Shear force value measured by texture analyzer also showed relatively higher value than others at the treatment of 0.4 ㎍/㎖ concentration. Application of wood vinegar in wood log cultivation of *L. edodes* seems to be very potential for increasing yield as well as freshness of fruiting bodies if it can be used at the proper concentrations.
Keywords: wood vinegar, Lentinula edodes, wood log cultivation, Trichoderma, Diatrype stigma, shear force value

B069

Electrochemical DNA Biosensor Using Nano Gold Particle for Detection of Waterborne Pathogens

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Water is essential to maintain the life of organisms in the ecosystem. Especially, humans are affected by drinking or contacting water. Although water is treated by advanced treatment methods, diseases resulted from waterborne pathogens remain still a big problem posing threats to public health. Among some infectious pathogens in water, *E. coli* is an important organism for water safety. Because *E. coli* is a natural inhabitant of the intestinal tract of humans and warm-blooded animals, it is an indicator organism reflecting fecal contaminant. Therefore, *E.coli* will be our model organisms for the development of electrochemical DNA biosensor.In this study, biosensor that works based on two probes-hybridization system using gold nanoparticles is detected by an electrochemical method. Reporter probes and capture probes are hybridized to PCR-amplified target 16S rRNA fragment of *E. coli*, followed by silver enhancement for hybridization signal amplication.To achieve the electrochemical detection, capture probes are immobilized on the gold electrode, and employment of gold nanoparticle and silver enhancement may produce electrical signal. Therefore, we can quantify *E. coli* by measuring the intensity of electrical signal produced by conducting nanoparticle gold and sliver enhancement.

Keywords: Biosensor, DNA, Nanogold-labeled, silver enhancement, electrochemical

B070

Biodegradation of Etylbenzene by Pure Cultures

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BTEX(benzene, toluene, ethylbenzene, and o-, m-, and p-xylene) compounds are an important family of organopollutants that are components of gasoline and aviation fuels and are widely used in industrial syntheses. Accidental spills and leaking underground storage tanks, as well as natural petroleum seeps, have released aromatic hydrocarbons into natural environments and led to abundant contamination of water resources. Aromatic hydrocarbons comprise one of the least reactive classes of organic molecules. Their relative inertness can be attributed to the absence of a functional group and the resonance energy stabilization of the aromatic ring. These properties hamper rapid biodegradation of alkylbenzenes in many environments (Hope A. Johnson *et al*., 2001). In this study, We isolated 17 strains degrading 500㎎/ℓ ethylbenzene from oil contaminated soil in Korea. 17 strains were screened for ethlybenzene removal, respectively. E49 was identified as *Pseudomonas putida* showed the highest removal efficiency for ethylbenzene. In basal medium with 500㎎/ℓ ethylbenzene, ethylbenzene was not detected in 9hours.

Keywords: Biodegradation, Etylbenzene

B072

Detection of Waterborne Pathogens by PCR-Reverse Blot Hybridization

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Contamination of drinking-water by microbial pathogens can cause disease outbreaks and contribute to background rates of disease. Currently, detection system for microbial parameters involves sampling and filtration of water followed by cultivation of the chosen microorganism on selective media, and subsequent colony counting or mere demonstration of growth. The present study was set up to develop a rapid and simple but yet specific and complete monitoring system for waterborne pathogens such as that may contaminate drinking water. For rapid and specific detection of waterborne pathogens, the use of molecular biological techniques such as polymerase chain reaction (PCR) and probe hybridization seemed to be adequate. In this study, we designed a new monitoring method by employing nested PCR and reverse blot hybridization(REBA). In order to develop REBA, we designed specific probe molecules to waterborne pathogens. In brief, the results from our study showed that reverse blot hybridization was successful to detect each species of waterborne pathogens at once. In conclusion, a sensitive and specific diagnostic PCR-reverse blot hybridization assay was developed for the simultaneous detection and identification of waterborne pathogens. This novel assay will offer a rapid, sensitive, and specific system for detection and identification of waterborne pathogens, and may be able to serve as a useful tool for monitoring of drinking water in near future.

Keywords: Waterborne pathogen, 16S rRNA, Nested PCR, Reverse blot hybridization(REBA)

B073

Generic Difference of Sponge-Associated Bacterial Isolates between Tropical Ocean, Chuuk Island, and Temperate Ocean, Taejongdae Korea

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Sponge-associated microorganisms has been highlighted due to their possibility of producing novel bioactive products. Many sponge-associated bacterial strains isolated from tropic area and temperate area for further utilization. Based on the 16S rRNA gene sequences, compositional difference between isolates from tropical area, Chuuk Island, Micronesia, and from temperate area, Taejongdae, Korea, was compared. Majority of isolates from temperate area belonging to the α-Proteobacteria distributed various genera include the genus *Pseudovibrio* in the family *Rhodobacteraceae* and in the family *Erythrobacteraceae*. However, majority of isolates from tropical area affiliated only into the genus *Pseudovibrio,* even more, produce different clade with that of temperate isolates in the same genus. Isolates belonging to the *γ-Proteobacteria* from temperate area affiliated into the genera *Bowmanella, Pseudoalteromonas, Shewanella* and *Vibrio*, however, isolates from tropical area affiliated into the genera *Acinetobacter, Halomonas, Microbulbifer* and *Pseudomonas*. In case of *Bacteroidetes*, isolates discriminated clearly as *Flavobacteria* (Temperate isolates) or *Sphingobacteria* (tropical isolates). The generic difference of sponge-associated was shown between Taejongdae isolates and Chuuk Island isolates, however, is it due to the different temperature or due to the site difference was unclear. [Supported by Marinbio 21 Program & KORDI]

Keywords: sponge-associated bacteria, generic difference, tropical ocean, temperate ocean

B075

Detection of Mn-Peroxidase from Soil and Wood-Rot Fungi Using Antibody against Mn-Peroxidase of *Trametes versicolor* KN9522

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We purified Mn-peroxidase isozyme family from *Trametes versicolor* KN9522. We also extracted total proteins from soil in the fired forest. Polyclonal antibody was prepared against the purified peroxidase in the rabbit. Mn-peroxidase could be detected from the samples and several types of wood-rot fungi using the antibody by western blot. From the above results, we could analyze Mn-peroxidase and the patterns of wood-rot fungi.

Keywords: Mn-peroxidase, Trametes versicolor, polyclonal antibody

B076

Analysis of Microbial Diversity in Shrimp- and Fish-Jeotgal, Traditional Korean Fermented Seafood, by Cultivation-Independent and Dependent Methods

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The extent of microbial community diversity in two different types of Jeotgals (Fish and Shrimp), traditional Korean fermented seafood sampled from Daejeon Fish market and Suwon Fish market, Korea were analyzed with culture-dependent "plating method" and culture-independent "Terminal-Restriction Fragment Length Polymorphism (T-RFLP)" and "random cloning of 16S rDNA PCR-amplified products" obtained from each sample. From culturing method, 200 strains of non-Lactic Acid Bacteria, 40 strains of Lactic Acid Bacteria and 10 strains of yeast were isolated. All the bacterial strains were identified on the bases of 16S rRNA gene sequences but yeast with 18S rDNA sequencing. From random cloning, 50 clones of yeast, 50 clones of LAB and almost 110 clones of non-LAB were prepared. We have very interesting data of bacterial diversity from these samples and it shows not only bacterial complex diversity but suggest some more precautions to handle this fermented food because it is not only used in daily routine in Korea but also abroad and it is directly related to human health. . From this analysis, it was concluded that microbial diversity depends on many factors and it varies from food to food and there is great need to analyze it completely with culture-independent and dependent methods.This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, Ministry of Science & Technology (Grant MG05-0101-4-0), Republic of Korea

Keywords: Microbial Diversity, Jeotgal, Cultivation-Independent method, cultivation method, T-RFLP.

B077

Single-Stranded Conformational Polymorphism (SSCP) Analysis of Human Noroviruses in River Waters

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We studied human norovirus diversity by the analysis of single-stranded conformational polymorphism (SSCP) in river waters. The samples were obtained every month for one year from four river water tributaries in Gyeonggi Province: Kyounan, Dukpoong, Wangsuk, and Sungnae. The viral particles were collected over membrane filters by the charge interaction with Mg^{2+}, eluted with a beef extract solution, and precipitated in polyethylene glycol. The noroviral RNA was detected by an RT-nested PCR. The patterns of norovirus-positive DNA fragments were analyzed by the SSCP analysis, and the single strands were excised and used as templates for the PCR amplification. The PCR-amplified DNA fragments were cloned and sequenced to confirm the nucleotide sequences. Of a total of 48 samples, 16 (33.3%) and 21 (44.8%) gave the positive results for norovirus genogroups I (GI) and II (GII), respectively. The distribution of GI did not show any seasonal variation, but strains of GII occurred prevalently in winter and spring. The SSCP analysis showed multiple single DNA strands in all norovirus-positive samples. By the comparative sequence analysis, the majority of them were classified into GI/1, GI/5, GII/3, GII/6, and GII/16. Our SSCP analysis was useful for knowing the norovirus diversity in the river tributaries, and for comparing the subjects with ecological parameters which vary in time and space. [Supported by KRF(2005-015-0409-20050042)]

Keywords: norovirus, SSCP

B078

Antibacterial Activites of Urushiol Derivatives

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For thousands of years the sap of lacquer tree has been used as an preservative surface coating material for wood. In terms of environmental aspect, the sap of lacquer can be developed as a good antifouling agent. It has been reported that urushiol components in the sap have antimicrobial activities. To develop antifouling agents, we extracted urushiol components from Korean Lacquer Tree and synthesized various urushiol derivatives. We analyzed the antibacterial activity of each component to examine the inhibitory effect on the biomembrane formation. To test the activity against bacteria, we incubated a representive gram positive bacterium, *Staphylococcus aureusa* and gram a negative bacterium, *Escherichia coli* in LB media and determinated the MIC (minimal inhibitory concentration) values for each component by Disc-plate method and serial 2-fold dilution method. The results showed that natural urushiol component and its derivatives have strong antibacterial activities.

Keywords: urushiol, antifouling agents, lacquer tree

B079

Cloning and Characterization of Plasmid-Borne Toluene Dioxygenase in Aniline Degrading Bacterium *Burkholderia* sp. HY1

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It has already been reported in our previous study that *Burkholderia* sp. HY1 was capable of degrading aniline as sole source of carbon and nigtrogen (JMB, 2000, 10:643-650). In order to determine the biodegradability of strain HY1 for monocyclic aromatic compounds with different functional groups (NH_2 in aniline instead of CH_3 in toluene), HY1 was tested with toluene which is the representative of volatile organic compounds. Metabolite analysis using HPLC and genetic study revealed that strain HY1 not only has the biodegradability for toluene, but harbors a plasmid DNA of which size is not determined yet. To elucidate the role of the plasmid gene, three *Eco*RI-digested plasmid DNA fragments were cloned into pBluescript SK+ vector and designated as pANOI, pANOII and pANOIII, respectively. Sequence analyses for the clones revealed that genes in the pANO clones were responsible for toluene degradation, which has over 95% similarity with toluene monooxygenase in Burkholderia sp. JS150. Interestingly, it was found that different metabolites were produced through HPLC analysis with toluene using both strains, HY1 and JS150. This work was supported by Korea Research Foundation Grant(KRF-2004-041-D00375).
Keywords: Aniline, Toluene, Plasmid-borne toluene dixoygenase, Burkholderia

B080

In *Situ* Biodegradability of Chlorinated Ethene Determined by Using Catabolic Genes Involved in Biotransformation

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This lab-scale study has been performed to develop a molecular means to determine biodegradation activity of soil in PCE/TCE-contaminated sites. To achieve the goal, two sets of experiment were set up as follows: i) Determination of biodegradation potential of a soil exposed to PCE by monitoring of the community members relating chlorinated-degrading bacteria, ii) Evaluation of the biodegradation processes by detecting catabolic genes involved in the degradation pathways. As a model ecosystem to approach a soil environment with PCE exposure, various soil microcosms were constructed in glass bottles and the bottles were anaerobically incubated at 30℃ while PCE vapor and H2 were provided continuously. After 120 days, bulk genomic DNA was analyzed by cloning, DGGE, and 16S-rDNA specific PCR amplification. Bacterial species showing significant homology to *δ-Proteobacteria*, *Clostridia*, *Dehalococcoidetes*, which are known to encompass PCE-degrading bacteria, were newly appeared. The phenotypic biodegradation activity was further evaluated by the detection of chlorinated catabolic genes using various reductase genes-specific primers including *vcrA*, *bvcA* genes, and *tceA* gene. As a result of specific PCR, *bvcA* gene was detected in the 60-day soil sample; *tceA* gene was detected in the 10 m-soil sample. We believe that this approach will overcome the pitfall and make a progress in molecular evaluation methodology.
Keywords: TCE, catabolic gene-specific PCR, molecular biomarker

B081

Horizontal Profiles of Abundance of Bacteria and Exoenzymatic Activities in Artificial Wetland

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For elucidating the microbial functions in artificial wetland, which was constructed at July 2005 for purification of slightly polluted stream water, the bacterial numbers by AODC method, active bacterial numbers by qDVC method and exoenzymatic activities(β-glucosidase and phosphatase) by MUF metho were anylyzed from 17. April. 2006 to 3. July. Pore waters of 0.2 m depth of 0, 10 and 20 m distance from input area were collected after planting Phragmites japonica. Bacterial numbers were ranging from 0.46 to 3.65 cells/ml, and showed decreasing trend with distance. Active bacterial numbers were 86.3% - 0.7% cells/ml and show different pattern of change with distance. The values of β-glucosidase activity were ranged from 103.87 to 1.04 Vmax($nML^{-1}hr^{-1}$) and shown decreasing pattern by distance. The values of phosphatase activity were ranged from 2.84 - 260.38 Vmax($nML^{-1}hr^{-1}$)and show different pattern of change with distance. These results suggest that the bacterial numbers and enzymatic activity can be used as evaluating items for wetland operation.
Keywords: artificial wetland, exoenzymatic activities

B082

Behavior of Microbial Community during Anaerobic Di-(2-ethylhexyl) Phthalate Degradation and Toxicity Analysis

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Di-(2-ethylhexyl) phthalate (DEHP), a well known endocrine disrupting chemical found abundantly in soil and aquatic environments. Few anaerobic soil bacteria are able to degrade DEHP in presence of certain electron acceptors. We investigated microbial community behavior during anaerobic degradation of DEHP by soil bacteria under nitrate or sulfate as electron acceptor. T-RFLP analysis using 16S rDNA displayed two diverse clusters each depended on either nitrate or sulfate as electron acceptor. In addition, a shift within each cluster of microbial community occurred which was dependent on different stages of DEHP breakdown due to accumulation of different intermediates. The toxicity imposed by metabolic products by exposure of culture extracts or authentic intermediates to bacterial biosensors was analyzed. Our results showed an initial five-day old culture extract did not show major toxicity. However, after five-days of incubation, the culture extract exhibited severe membrane damage followed by weak oxidative and DNA damages. This toxicity was lost after 35-days old culture filtrate, suggesting that the DEHP was anaerobically degraded to non-toxic intermediates during 5-35 days. Our results also indicate that the DEHP catabolic intermediates were more toxic than the parent DEHP itself.
Keywords: Di-(2-ethylhexyl) phthalate, Microbial community, Anaerobic degradation, Toxicity

B083

Analyses of Microbial Community in Hydrogen Producing Bioreactor by Culture-Independent and Culture-Dependent Approaches

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Hydrogen consuming reaction should be controlled in order to increase the hydrogen recovery in anaerobic fermentation. After inactivating methanogens through heat treatment and establishing the hydrogen production-collection method, the hydrogen consumption is suppressed by rapid removal of CO_2 used as electron acceptor of methanogen. We analyzed the microbial community changes in CO_2-removed and not-removed reactor. We searched main organisms which produce hydrogen and observed microbial community change over time by T-RFLP. Also we attempted to isolate hydrogen producing microorganisms which play an important role in reactor. From difference between T-RFs patterns and 16S rDNA clone analysis of CO_2-removed hydrogen production reactor and normal hydrogen production reactor, we expected community difference between both reactors. In the normal reactor, genus *Citrobacter*, *Escherichia*, and *Pantoea*, and *Clostridium butyricum* which is the well-known species in genus *Clostridium* were detected. On the other hand, in the CO_2-removed reactor, genus *Shigella* or *Salmonella*, and similar to species of genus *Clostridium* (95~97 % similarity) were detected, which suggested the existence of new uncultured bacterium producing H_2. Consequently, T-RFs pattern and 16S rDNA clone sequence analysis showed similarity in population, but not in species level in both reactor types. [This work was supported by grant of Korea Science and Engineering Foundation, MOST.]
Keywords: Biological hydrogen production, T-RFLP, 16S rRNA gene cloning, Bacterial community analysis

B084

Microbial Community in the Culture Performing the Removal of Biological Phosphorous in a Sequencing Batch Reactor

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Microbial communities of the sediment from K lake in an anaerobic/aerobic sequencing batch reactor (SBR) supplied with acetate as sole carbon source were analyzed to identify the microorganisms responsible for phosphorus-removal. This bioreactor were operated for more than one hundred twenty days. Average sixty five percent and maximum ninety seven percent removal of total phosphate were obtained by the enriched culture. The microbial community of the enriched culture was analyzed by DGGE (denaturing gradient gel electrophoresis). An aerobically grown pure culture isolated from a solid medium inoculated with the enriched culture-broth from the bioreactor was analyzed by 16S rRNA sequencing and identified as *Methylobacterium dichloromethanicum* with the sequence homology of 99%.
Keywords: Phosphorous-removal, SBR, enriched culture, DGGE, identification

B085

Effect of Nano-Silver Liquid (Nanover™) on the Mycelial Growth of Plant Pathologenic Fungi

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Among various materials, nano-silver has been known to inhibit effectively almost all microorganism under various different concentrations. Therefore, we have conducted in vitro experiments with nano-silver liquid (Nanover ™) for their effect against various plant pathogenic fungi. Different types of nano-silver liquid (Nanover ™), WA-CV-WA13B, WA-AT-WB13R, and WA-PR-WB13R were used. These are classified based on different manufacturing processes. The tested nano-silver liquid (Nanover ™) was produced by Bioplus Co., and provided by Korea Chem Tech., and the tested fungi were provided by KACC. As a result of the study, Nanover ™ WA-CV-WA13B showed the highest inhibitory effect against the tested fungi as compared to other Nanover ™ preparations. Nanover ™ WA-AT-WB13R showed the lowest inhibitory effect. Nanover ™ WA-PR-WB13R showed inhibitory effect better than WA-AT-WB13R. At 100ppm concentration of Nanover ™ WA-CV-WA13B, mycelial growth of F. oxysporum f. sp. cucumerinum, F. oxysporum f. sp. lypersici, F. oxysporum, F. solani, Fusarium sp., B. cinerea, and C. gloeosporioides were inhibited slowly at first. Nanover ™ WA-CV-WA13B inhibited the mycelial growth of Alternaria alternata, Alternaria brassicicola, Monosporascus cannonballus at concentrations of 25ppm or over. Nanover ™ WA- CV-WA13B inhibited the mycelial growth of Sclerotinia minor, Sclerotinia sclerotiorum, Stemphylium lycopersici, and Stemphylium solani at concentration of 10ppm or over.
Keywords: nano-silver, antifungal

B086

Effects of Co-Contaminating Heavy Metals on Microbial Reductive Dechlorination of Pollychlorinated Biphenyls

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Contaminated sludge from a PCB-contaminated site to determine the effects of various electron donors on rates of microbial reductive dechlorination. As a possible electron donor 20% hydrogen in CO_2 balance was added and compared with nitrogen headspace. Both originally contaminating PCBs and spiked PCB congeners and a mixture of Aroclor 1242 and 1254 were analyzed for 18 months. The originally existing PCBs were stable and no longer transformed during the incubation, but the spiked three tetrachlorobiphenyls were transformed through the dechlorination mainly at the *para*-positions. However, we did not observe any positive effects from the addition of methanol, fatty acids, or the surfactant except H_2 headspace. To investigate the roles of co-contaminating heavy metals present in the sludge on the results, a consortium able to dechlorinate 2,3,5,6-CB was incubated with the lagoon sludge, weak-acid-extractable metals, or individual pure metals. The results suggested that co-contaminating heavy metals had an inhibitory effect on PCB dechlorination. Zinc had a clear inhibitory effect, but the presence Cu, Co, and Cd increased the rate of dechlorination.
Keywords: heavy metal, biodegradation, PCB, dechlorination

B087

Effect of Soil pH on the Microbial Density and Diversity in Relation to the Degradation of Fallen Leaves

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To understand the role of microorganisms in the decay of fallen leave in this study we investigated the effect of soil pH on the diversity and population of bacteria and fungi in the soils where fallen leaves were subject to be degraded naturally. For the experiment, fallen leaves were put in gauze and placed on the surface of soils with pH 3, 3.5, 4.5, 5, and 5.5, respectively, in a hill at Hongneung, Seoul and let them decay for 2 years in nature. Two 2 yr later, soil samples were collected from the underneath of the decayed leaves and analyzed for microbial population and diversity. The density of bacteria seems to be increased according to the increase of pH in both the broad-leaves and needle-leaves. While, the density of fungi fluctuated and was relatively high in the soil samples of pH 3 and 5.5. Commonly dominantly isolated species from all the soil samples were *Burkholderia* spp., *Chryseobacterium* spp. and *Bacillus* spp. in bacteria and *Aspergillus* spp., *Penicillium* spp. and *Trichoderma* spp. in fungi. These dominant fungal species showed the ability of producing cellulase, cellobiohydrolase, and xylanase. However, there is no significant difference in the microbial diversity among soils of different pH. Overall, it seems that the degree of degradation of fallen leaves is related to the density of soil microorganisms rather than diversity of soil microorganisms.

Keywords: Soil pH, Microbial Density, Degradation of Fallen Leaves

B088

Diversity of Mercury Resistance Genes on Natural Condition in Korea

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Distributions of mercury resistant bacteria and *mer* genes were investigated in abandoned mine waters, sea water and sediments. Water samples in abandoned mines, sea water and sediments were tested to analyze the diversity of heterotrophic bacteria by 16S rRNA sequences, and to analysis the diversity of mercury resistance strains mer genes diversity by restriction fragment length polymorphism (RFLP). mer gene diversity of microorganism is important to water and soil in environment condition. *mer* resistance bacteria were distributed to environment condition of high heterotrophic bacteria size and flash water.

Keywords: diversity, *mer* gene

C001

Fruiting Bodies Formation of *Cordyceps bassiana* from *Beauveria* Isolate Using Single Conidial Isolation

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In general, *Beauveria* species produce only mycelia or synnemata *in vitro*. But, single conidial isolates of *Beauveria* sp. EFCC C-13188, preserved in Entomopathogenic Fungal Culture Collection (EFCC), Kangwon National University, fruiting bodies that had perithecial stromata. The specimen of *Beauveria* sp. EFCC C-13188 was collected from Yang-yang, Gangwon-Do in October 2005. Eight single conidial isolates derived from the original isolate of EFCC C-13188 were numbered from 13188-1 to 13188-8. Different combinations of single conidial isolates 13188-1x4, 13188-1x5, 13188-3x8, 13188-4x5, 13188-4x6, 13188-4x7 and 13188-5x8 produced perithecial stromata, whereas isolates 13188-2 and 13188-4 produced perithecial stromata without combination. Mycelial growth characteristics ands of perithecial stromata were like those of *Cordyceps*. The present study reports the formation of sexual stage of *Cordyceps bassiana* from *Beauveria* isolate. Further studies are necessary to confirm the relationship between the sexual and asexual life stages of *Cordyceps* species with *Beauveria* anamorphs.

Keywords: Beauveria, perithecial stromata., Cordyceps bassiana, sexual and asexual life stages

C002

Differential Expression of *pbpC* Encoding a Sporulation-Specific Penicillin-Binding Protein During Morphological Differentiationin *Streptomyces griseus*

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Six penicillin-binding proteins (PBPs) have been detected from membrane fractions of *Streptomyces griseus* when fluorescein-tagged β-lactam antibiotics are used to visualize penicillin-binding proteins. An 85 kDa PBP that preferentially binds to Flu-ACA accumulates during submerged sporulation. To understand the regulation of the septation machinery during sporulation in *S. griseus*, we investigated the expression of the *pbpC* gene. The 85 kDa PBP was prevented from binding to Flu-ACA by cefoxitin that inhibits sporulation septum formation. The *pbpC*-disrupted strain which did not accumulate the PBP was not defective in sporulation septation, implying that another sporulation septum-specific PBP is present in *S. griseus*. Computer analyses revealed the *pbpC* gene was clustered within developmental genes *mreB*, *mreC*, *mreD* (murein formation genes) and *sfr* (member of *SpoVE/FtsW/RodA* family) identified in other *Streptomyces*. S1 nuclease protection assays revealed that four *pbpC* transcripts were present during submerged sporulation, but not during vegetative growth. The transcripts reached a maximum at 16 hr of sporulation in the wild-type strain and accumulated much earlier and more abundantly in a group of nonsporulating mutants, *adpA* and *bldA* that prematurely form their sporulation septa from vegetative mycelia. These observations indicated that the *pbpC* gene encoding the 85 kDa PBP plays a role in forming sporulation septa.

Keywords: Streptomyces griseus, penicillin-binding protein, sporulation septa

C003

Studies on Catalase B Processing Responsible for Osmoprotection and Differentiation in *Streptomyces coelicolor*

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Streptomyces coelicolor produces at least three different catalases (catalases A, B, and C) under different conditions. Catalase B (CatB) is a developmentally regulated catalase, whose expression reaches its maximum during aerial mycelium formation and sporulation. The N-terminal 95 amino acids of CatB protein were cleaved off and the remaining 75 kDa-CatB protein was secreted to the medium. This post-translational regulation was coincident with the spore formation and independent of catalase activity. Deletion of N-terminal 95 amino acids impaired both the secretion of CatB protein and the proper differentiation. The proteolytic activity responsible for this processing was partially purified from both intra- and extracellular fractions, and characterized as a 35 kDa protein. And we identified CatB processing enzyme by MALDI-TOF analysis from partial purified fraction, which named "SmpA". (*Streptomyces coelicolor* Metalloprotease A). SmpA is developmentally regulated protease and responsible for processing of CatB N-terminal domain. *smpA* null mutant showed a delay in differentiation and growth defect on salt-containing NA solid media and minimal media. These result predicted the N-terminal domain of CatB is crucial for differentiation and osmo-protection and it supposed to be processed by SmpA protease that affecting on development and osmotic stress defence in *S. coelicolor*. *This work was supported by a grant from the KOSEF and an NRL grant from the Ministry of Science.*

Keywords: Streptomyces coelicolor, Catalase, protease, differentiation, osmotic stress

D001

Physicochemical Parameters Influencing the Activity of Fibrinolytic Enzyme Produced by *Bacillus subtilis*

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The relationships between activity of the fibrinolytic enzymes produced by *Bacillus subtilis* and several physicochemical parameters were monitored during 60 hours of incubation. Optimal temperature for the production and activity of the fibrinolytic enzyme was 45℃. At pH 8 of the cultures, the enzyme activity showed about 2.32 which was the highest enzyme activity in this pH studies. The effect of several additional C-sources (*e.g.*, glucose, fructose, lactose) on the enzyme activity was evaluated. As the result, the fibrinolytic acivity of the bacteria grown on the soybean media in the presence of 0.2% glucose was about 2.42 times compared to plasmin used as standard. The effect of supplemented N-sources [*e.g.*, $(NH_4)_2SO_4$, NH_4NO_3, KNO_3] on the enzyme activity was examined. Addition of NH_4NO_3 resulted higher enzyme activity which showed 2.8 times greater than that of plasmin. All maximum enzyme activities were achieved within 48 hours of inculation. In consequence, media composition for the production of fibrinolytic enzyme by *Bacillus subtilis* was optimized, and may apply for the mass production through scale-up. [This research was supported by grant from 2006 RIC]

Keywords: fibrinolytic enzyme, Bacillus subtilis

D002

Proteomic Analysis of TNT Degrading Bacterium, *Pseudomonas* sp. HK-6 in Different Media

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The purpose of this work was to investigate the proteomic analysis of *Pseudomonas* sp. HK-6 to explosive 2,4,6-trinitrotoluene (TNT) in two different media, basal salts (BS) medium and LB medium. Proteomic analysis for 2-dimensional electrophoresis of soluble protein fractions from the cultures of HK-6 grown on different media containing TNT demonstrated approximately 250 spots on the silver-stained gel ranging from pH 3 to pH 10. Among them, 9 spots induced and expressed in response to TNT were selected and analyzed. As the results, TNT-mediated stress shock proteins such as Tuf-1, Tuf-2, PotF-1, RibE-1 increasingly expressed under the condition of TNT-stress in BS medium. On the other hand, proteins involved in energy metabolism such as PotF-1 and Trx-1 were induced in LB medium containing TNT. Such proteins indicate that HK-6 cells grown in the media containing TNT induced for cell protection against harsh environmental conditions such as TNT stress. [This research was supported by grant No. R01-2005-000-106080 from the Basic Research Program of the Korea Science & Engineering Foundation.]

Keywords: TNT, Pseudomonas sp. HK-6

D003

The Role of Novel Thymidylate Synthase, ThyX, in Two Thymidylate Synthase Systems of *Corynebacterium glutamicum* ATCC 13032

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Corynebacterium glutamicum ATCC 13032 have both intact *dhfr/thyA* gene and a gene with strong similarity to *thyX* that may contribute to the synthesis of 1-carbon units for biosynthesis of thymidylate.To examine the expression of *thyA* and *thyX* under a variety of growth conditions, *thyA* and *thyX* from *C. glutamicum* were first cloned into *E. coli* (*thyA*-) mutant, and functionally complemented the growth defect of *E. coli* on minimal medium. Transcriptional level of *thyX* was enhanced in *C. glutamicum* as the concentration of trimethoprim increased. When *C. glutamicum* was grown in the medium containing sulfamethoxazole, the transcriptional level of *thyX* was similar to that of its growth in trimethoprim, which suggest that there might be a cross-talk between *dhfr/thyA* and *thyX*. Reverse transcription-PCR analysis also revealed that *thyX* transcribed in both minimal and rich medium while *thyA* transcribed only in rich medium, which indicate that *thyX* might rely on the lysine biosynthesis and thus increase its transcriptional level in minimal medium. These data show the role of ThyX on providing intracellular thymidylate by enhancing transcriptional level of *thyX* in parallel with the reduced activity of ThyA.

Keywords: Corynebacterium glutamicum, thyX, transcriptional regulation

D004

Purification and Characterization of Superoxide Dismutases in *Sphingomonas* sp. KS 301

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Sphingomonas sp. KS 301 was shown to have five different SODs (SOD Ⅰ, Ⅱ, Ⅲ, Ⅳ, Ⅴ) which can be separated by DEAE-Sepharose chromatography, and SODs Ⅲ, Ⅳ and Ⅴ were finally purified in this study. Specific activities of the SOD Ⅲ, Ⅳ and Ⅴ were higher than that of Mn type or Fe type SOD of *Escherichia coli* by 5 folds, 4 folds and 3 folds, respectively. The molecular weights of the SODs determined by SDS-PAGE were 23 kDa, 25.8 kDa and 25 kDa, and the apparent molecular weights of the native enzymes estimated by Superose-12 gel filtration chromatography were approximately 71 kDa, 70 kDa and 72 kDa, respectively. The optimum pH of all three SODs was 7.0, and the optimum temperature for SOD Ⅲ and Ⅳ was 20℃, while that of SOD Ⅴ was 20~30℃. To determine the type of SODs, inhibitory effects of NaN_3, H_2O_2, and KCN were examined. 10 mM NaN_3 was able to inhibit 56% of the SOD Ⅲ activity. On the other hand, SOD IV and V were more sensitive to the inhibition by NaN_3, and the same concentration of NaN_3 inhibited 92% of the SOD Ⅳ and 100% of the SOD Ⅴ activities. These results indicate that these SODs are Mn types. The amino acid composition of the purified SODs was different from those of other SODs from other organisms. Interestingly, SOD Ⅳ and SOD Ⅴ did not contain threonine at all, but relatively large amounts of proline, leucine, and glutamate were observed.

Keywords: SOD, Sphingomonas

D005

Regulation of Protein Synthesis by Redox in *Saccharomyces cerevisiae*

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Redox signaling is one of way to regulate growth and death of cell in response to change of redox of proteins. A number of reports have been documented about the target proteins interacting with thioredoxin during redox signaling. Fact that translation factors were one of the targets indicates that translation is one of way regulated by redox. As a preliminary attempt to search signaling pathway regulating translation, we asked whether the translation activity could be regulated by redox. Interestingly *in vitro* translation activity was increased up to 40% in the presence of dithiothreitol (DTT) when assay was performed under condition with or without DTT. Then we checked whether this positive effect by DTT was further accelerated by addition of thioredoxin (Trx). When a Trx purified from *Saccharomyces cerevisiae* was added to the *in vitro* translation extract, we observed a dose-dependent increase in translational activity. These results suggest the possibility of translation factors being redox-regulated via Trx in vivo. To identify whether glutaredoxin is another redox mediator to translation and/or whether endogenous reducing donor is NADH or NADPH, we purified glutaredoxin (Ttr1), thioredoxin reductase 1 (Trr1), thioredoxin 2 (Trr2), glutaredoxin (Grx1) and glutathione reductase 1 (Glr1) and are now under testing with those materials how the redox transmits to translation process.

Keywords: redox, translation, thioredoxin, glutaredoxin

D006

Isolation and Characterization of the Mutans Streptococci from Korean

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Mutans streptococci are considered the predominent pathogens in human dental caries. Therefore, accurate identification of mutans streptococci would be valuable for the pathogenic and epidemiological studies for the dental caries. The purpose of this study is to isolate and characterize the frequency of mutans streptococci from Korean dental plaque. The dental plaque samples were collected from the anterior and molar teeth of both of jaws in the 89 human subjects (aged 2 to 29.4 years, average age was 12.5 years). The mutans streptococci were cultured selectively on mitis-salivarius bacitracin (MSB) agar plate. The bacteria growing on the MSB plates were then identified with biochemical tests (for biotyping), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) targeting dextranase gene (dex), and 16S rRNA sequencing comparison method. The 82 strains of *S. mutans*, 17 strains of *S. sobrinus*, 4 strains of *S. downei* were isolated. The biotyping data showed that biotype Ⅰ, Ⅱ, Ⅳ and Ⅴ were 69, 2, 20 and 10 strains respectively. Interestingly, Two variant biotype, similar to biotype Ⅳ except the positive for arginine hydrolysis test, were isolated for two person, we named this variants type Ⅶ. Above results revealed that *S. mutans* and biotype Ⅰ were frequently detected in Korean.

Keywords: Mutans streptococci, dental plaque

D007

Antibiotic Susceptibility in *Fusobacterium nucleatum* Isolated from Korean

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The purposes of this study were to investigate the antimicrobial susceptibility of *Fusobacterium nucleatum* isolated from dental plaques of Koreans. The minimum inhibitory concentration of 8 antibiotics for 34 strains of *F. nucleatum* was tested by broth microdilution assay. The data showed that only 2 out of 34 strains were have resistance to penicillin G, amoxicillin, Augmentin® (amoxicillin + clavulanic acid, 5:1), and oxacillin. The other strains have sensitiveness to all of the antibiotics tested in this study. The MIC90 of all of the antibiotics against the 34 strains of *F. nucleatum* were within the range of sensitiveness. These results suggest that penicillin G or amoxicillin could be used in the first choice antibiotics for the target of *F. nucleatum* in Koreans.

Keywords: F. nucleatum, Antimicrobial susceptibility, MIC

D008

Enhanced Production of Marine Green Microalgae (*Chlorella ovalis* and *Dunaliella parva*) by Bacteria Mineral Water Including a Natural Substitute Chelator for EDTA

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The productivities of *Chlorella ovalis* and *Dunaliella parva* were influenced by the rates of medium compositions obtained from the fermented animal wastewater (BM: bacteria mineral water) including a natural substitute chelator for EDTA(etylenediaminetetraacetic acid). The most favorable medium was -E+50 adding 50% BM in f/2 medium instead of EDTA, a chemical chelator, which increased more 19-fold of cell density in *C. ovalis* and 7-fold in *D. parva* than cells cultured on f/2 medium as well as the enhancements of chlorophyll *a* (f/2-E: 0.26 g L^{-1}, -E+50: 1.5 g L^{-1} in *C. ovalis*; f/2-E: 2.7 g L^{-1}, -E+50: 15 g L^{-1} in *D. parva*) and the increase of maximal PSII quantum yields. These results were verified that the BM could play an important part as a natural chelator substituted for EDTA. In the fields of biotechnology, food organisms in fishery and eco-industry of CO_2 sequestration and nutrient removal in water, the natural chelator of BM could be applied to enhance the biomass of the other microalgae.

Keywords: Chlorella ovalis, Dunaliella parva, EDTA, BM, biomass, natural chelator

D009

Cultural Characteristics and Favorable Condition of Mycelial Growth by *Tricholoma matsutake*

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The main objectives of this research were to study the cultural and nutritional characteristics of *Tricholoma matsutake* and to establish its liquid culture system. Ectomycorrhizal formation of *T. matsutak*e was also observed by inoculating liquid spawn in the pine forest. The last objective of this research was to study the possibility of fruiting induction of *T. matsutake* by density control, sprinkle irrigation and liquid inoculation. The optimum culture media for mycelial growth of *T. matsutake* were MYA and TMM. The optimum temperature for the mycelial growth was 25℃. Growth and ectomycorrhizal formation of *T. matsutake* could be observed up to 2-5 months after liquid inoculation in pine forest. Difference in ectomycorrhizal morphology could not be observed between naturally existing and artificially inoculated mycelia of *T. matsutake*. It showed survival rate of 40~50% on inoculation region.

Keywords: Tricholoma matsutake, Ectomycorrhizal formation, inoculating liquid spawn, MYA, TMM

D010

Growth Inhibition of Plant Juices on the Oral Bacteria

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The fifteen bacterial strains were isolated and identified from human oral cavities. These strains were identified as genus *Moraxella* and *Neisseria*, etc. The plant materials such as garlics, gingers, leeks, onions, and welsh onions were used in order to search antimicrobial substances from natural plants. The effects of these plant juices on the growth of oral bacterial strains were investigated. Garlic juice inhibited on the growth of *Moraxella* sp., etc.

Keywords: antimicrobial substances, natural plants

D011

Analysis of the Replication Region of Shuttle Vector for Succinic Acid Producing Rumen Bacteria

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As plasmid in succinic acid producing rumen bacteria is a poorly studied with respect to replication, it is desirable to study a replication mechanism. Shuttle vector pMEx was constructed. Replicon sequence analysis and deletion analysis show that replication of pMEx is dependent on a plasmid-encoded replication protein (Rep), iterons and A+T rich region. Feature of replicon configuration results that the replication origin of pMEx is most similar to pPS10 which is a theta replicating natural plasmid of *Pseudomonas savastanoi*. Southern blot analysis indicates that pMEx did not generate single-stranded DNA, in other words, pMEx did not replicate by the rolling circle mode in *Mannheimia succiniciproducens*. Plasmid pMEx minimal replicon shares no homology with highly conserved ColEl-type replicons, which use Poll for initiation but do not encode a Rep, or with CoIE2 and CoIE3 replicons, which require Poll for replication and encode a Rep. We propose that pMEx and a number of other naturally occurring and closely related plasmids form a distinct plasmid class. [This work was supported by the Genome-based Integrated Bioprocess Project of the Ministry of Science and Technology. Further supports by the LG Chem Chair Professorship, IBM SUR program, and by the KOSEF through the Center for Ultramicrochemical Process Systems are appreciated.]
Keywords: Replicon, Shuttle vector, Rumen Bacteria

D012

Optimal Conditions for Chitosanase Production by *Bacillus cereus* D-11

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A chitosanase-producing bacterium, *B. cereus*. D-11, was isolated from Tanwan soil. From the SEM and 16S rRNA the strain D-11 belonged to the genus Bacillus and termed *Bacillus cereus* D-11. The bacterium constitutively produced chitosanase in a minimal medium containing colloidal chitosan as the sole carbon source. For production of chitosanase, the culture condition was optimized. The optimum temperature, pH, carbon sources, inoculation concentration, nitrogen sources, and various sorts were investigated. The optimal medium for the production of the enzyme was composed of 0.5% inoculation concentration (14 x 10^7 CFU/ml), 0.7% colloidal chitosan, 1% yeast extract, and 1% NaCl at initial pH 7.0. After cultivation at 30℃ for 3 day, maximal productivity reached to be 7.8 U/ml in flask cultures.

Keywords: Chitosanase, Medium optimization, Characterization, Bacillus cereus D-11, Culture condition, Optimal production

D013

Factors Involved in Regulation of Prodigiosin Biosynthesis of the Marine Bacterium *Hahella chejuensis*

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Hahella chejuensis, a gram-negative marine bacterium, produces a red pigment identified as prodigiosin that has the cytolytic activity against red-tide organisms. Genome sequence information and functional analysis revealed a genomic region of *H. chejuensis* that is sufficient for biosynthesis of the red pigment. This gene cluster was named as *hap*. To search for factors involved in regulation of the production of prodigiosin, a plasmid library of the *H. chejuensis* chromosome was constructed and transformed into *E. coli* clones carrying the *hap* gene cluster on a fosmid. By observing visual pigment production of the transformants, plasmids altering the prodigiosin production were isolated. The *hap*-bearing *E. coli* clones transformed with the same plasmids produced the pigment confirming their effects. These fifty-three plasmid clones were sequenced-seven clones contained *hap* genes or genes located closed to the *hap* cluster while six clones encoded genes involving in two-component signaling systems indicating that the thirteen clones are likely candidates for regulators of prodigiosin biosynthesis. Validation of the regulatory effects and specific roles of these genes in biosynthesis of prodigiosin is in progress. By uncovering factors involved in prodigiosin synthesis, not only can we give information on how prodigiosin synthesis pathway is controlled in *H. chejuensis*, but do generate over-expression of prodigiosin in a heterologous host like *E. coli* or in *H. chejuensis* itself.
Keywords: Hahella chejuensis, prodigiosin

D014

Benzoate Catabolite Repression of the Phthalate Degradation Pathway in *Rhodococcus* sp. Strain DK17

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The metabolically versatile *Rhodococcus* sp. strain DK17 exhibits a catabolite repression-like response when simultaneously provided with benzoate and phthalate as carbon and energy sources. Benzoate in the medium is completely depleted before utilization of phthalate begins. Transcription of the genes encoding benzoate and phthalate dioxygenase paralleled the substrate utilization profile. The benzoate-negative mutant strain KC710 was unable to utilize phthalate in the presence of benzoate although the strain grew normally on phthalate in the absence of benzoate.
Keywords: *Rhodococcus*, benzoate, phthalate, catabolite repression

D015

Oxidation of Acridine by Laccase and 3-HAA Produced from *Pycnoporus cinnabarinus*

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This work was performed to investigate whether acridine, a three-ring nitrogen heterocyclic compound that is a constituent of the anthracene fraction of coal tar, is oxidized by the laccase produced from *Pycnoporus cinnabarinus* SCH-3. When *P. cinnabarinus* SCH-3 was grown in the culture medium containing acridine, the laccase activity was gradually increased and acridine was stoichiometrically transformed to acridone on culture time. Transformation of acridine was not occurred when laccase purified from the culture medium of *P. cinnabarinus* SCH-3 was reacted with acridine in the sodium tartrate buffer (pH 3.0). However, when 3-Hydroxyanthranilic acid (3-HAA) was added to the reaction system of purified enzyme and acridine, transformation of acridine was increased. About 40% of the substrate was transformed in the presence of 1.0mM of 3-HAA after 24hrs of incubation with laccase. The experimental results suggest that 3-HAA plays a role of mediator in the transformation of acridine by laccase.
Keywords: Pycnoporus cinnabarinus, Acridine, Acridone, 3-HAA, Laccase

D016

Expression and Characterization of Fusion Antimicrobial Peptide in *Eschericia coli*

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Antimicrobial peptides are effector molecules of innate immune system. LL-37 is a human origin peptide belonging to the cathelicidin family of 37 residue cationic antimicrobial peptide. Gaegurin 4 (GGN4) is a 37-residue antimicrobial peptide isolated from the skin of a Korean frog, *Rana rugosa*. In this study, we constructed expression vector for fusioned antimicrobial peptide between GGN4 and LL-37. Then we expressed and purified the three of LL-37, GGN4 and GGN4-LL-37 (we designated as GL-37) proteins using pGEX-5X-3 vector with Glutathione S-transferase (GST) gene fusion system in *E. coli*. The expression of fusion proteins were induced by IPTG and were purified by affinity chromatography. GST fusion proteins were cleaved by factor Xa and the peptides were purified by HiTrap affinity columns. The purified protein was analyzed by SDS polyacrylamide gel electrophoresis and western blot analysis. We then investigated the antimicrobial activity using purified fused antimicrobial peptides against Gram negative and Gram positive bacteria.
Keywords: Expression, LL-37, GGN4, GST fusion protein, fusion antimicrobial peptide

D017

Burkholderia glumae Produces Multiple Extracellular Lipases Controlled by Different Signals

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Burkholderia glumae, a Gram-negative bacterium causing seedling and grain rot in rice, produces many extracellular enzymes that may serve as pathogenic determinants. The extracellular protein fractions contained a high lipase activity, when the activities were measured with *p*-nitrophenyl butyrate in a variety of media. Reduced levels of lipase activity and *lipA* mRNA were observed in the *tofI* mutant, which is defective in the biosynthesis of *N*-hexanonyl and *N*-octanoyl homoserine lactones functioning as signal molecules in quorum sensing. No lipase activity was detected the *lipA* mutant, which does not produce the extracellular lipase with a molecular mass of 33 kDa. These observations indicated that expression of the lipase is regulated by the *tofI*-dependent quorum sensing system. However, the wild-type strain and the *lipA* and *tofI* mutants secreted another fraction of lipase activity in minimal media containing olive oil. This result demonstrates that *B. glumae* possessed at least more than one extracellular lipases induced by olive oil.
Keywords: Burkholderia glumae, lipase

D018

Structural and Biochemical Characterization of Thioredoxin Reductase from *Dictyostelium discoideum*

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Thioredoxin reductase is a flavoprotein family of pyridine nucleotide-disulfide oxidoreductase. It has been proposed that there is a large conformational change during catalysis. The three-dimensional structure of thioredoxin reductase (Trr) from *Dictyostelium discoideum* was determined by X-ray diffraction at 2.3 Å resolution. In oxidized Trr, a redox active disulfide is located in the FAD domain and Cys147 contacts more closely isoalloxazine ring of FAD. The redox active cysteines (Cys144 and Cys147) were changed to serines by site-directed mutagenesis. Spectral analyses of Trr showed a typical oxidized flavin spectrum, with absorbance maxima at 382 nm and 459 nm. In contrast, a peak near 460 nm disappeared in spectrum of TrrC144S. After incubation with NADPH, Trr147S formed a flavin radical, characterized by absorption maxima at 570 and 605 nm. The free radical nature of this species was confirmed by its EPR spectrum. TrrC147S was only slightly fluorescent compared to native Trr. This significant quenching of the flavin fluorescence may be due to proximity of Ser147 to the flavin in the flavin-oxidizing (FO) conformation. Both mutant enzymes almost completely lost thioredoxin reductase activities, however they exhibited different quinone reductase activities. TrrC144S retained quinone reductase activity, whereas TrrC147S had no detectable activity. Thus, these results suggest that TrrC144S is in the flavin-reducing (FR) conformation and TrrC147S are in the FO conformation.
Keywords: thioredoxin reductase, Dictyostelium discoideum, site-directed mutagenesis, redox active cysteine, three-dimensional structure

D019

The Isolation of Bacteriocin Producing Bacteria from Kimchi and Effect of Bacteriocin in Mammalian Cells

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Bacteriocins are small peptides (3~7 kDa), with antimicrobial activity, that are produced by bacteria. Among several classes of bacteriocins , class I and the class II are preferable by small, heat stable peptides. From our research, the thirty strains of bacteriocin producing bacteria were isolated from naturally fermented Kimchi samples. These antimicrobial activity was especially strong against *Salmonella enteritidis* ATCC 13076 and *Escherichia coli* ATCC 10536. The optimum temperature for the growth of strains were 37℃; however, production was 30℃. The antimicrobial activity of some bacteriocin producing bacteria was found to be stable even at 100℃ for 30 min. and over a wide range of pH(2 to 10). Those bacteriocin was a protein by treatment of proteinase K. Also, we studied the characteristics of bacteriocin producing bacteria by Gram staining, KOH test and Catalase test. The pediocin PA-1 by *P.acidilactici* was used for the study of toxicity in mammalian cells. By the nucleotide sequence analysis of *P.acidilactici* showed the presence of four clustered genes (pedA, prepediocin PA-1; pedB, immunity protein; pedC, transport; pedD, processing and transporting). From the proliferation test with 293T and NIH3T3, we found that this pediocin had no toxicity in these cells.
Keywords: Bacteriocin, Kimchi, Bacteriocin class I and class II, Mammalian toxicity

D020

Crystal Structure of a New Type of NADPH-Dependent Quinone Oxidoreductase YtfG from *Escherichia coli*

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Escherichia coli YtfG, catalyzing two-electron reduction of methyl-1,4-benzoquinone, is a new type of soluble NAD(P)H-dependent quinone oxidoreductase with distinct primary sequence and oligomeric conformation from previously known quinone oxidoreductases. The crystal structures of native YtfG and YtfG-NADPH complex reveal that YtfG consists of two domains (N-domain and C-domain) resembling that of NmrA, a negative transcriptional regulator belonging to the short-chain dehydrogenase/reductase family. The N-domain adopting the Rossmann fold provides platform for the binding of NADPH, and the C-domain, containing a hydrophobic pocket connected to the NADPH-binding site, appears to play important roles in the substrate binding. Asn143 near the NADPH-binding site has been identified as a putative catalytic residue from structural and mutational analyses. Moreover, the disappearance of several enzymes implicated in carbon metabolism of *ytfG*-overexpressing strain and the growth retardation of the strain suggest that YtfG could play some physiological roles beyond the quinone-reducing activity
Keywords: NADPH, Quinone, NmrA, carbon metabolism

D021

Functional Expression and Purification of Class IIa Bacteriocin in *E. coli* System Using High pI Tagging Protein

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PedA, antimicrobial peptide of *Pediococcus pentosaceus*, is relatively small and highly positively charged peptides which composed of 31 amino acid residues and displays high pI of 8.85. Although bacteriocin has a potent antibacterial activity, we constructed easy purification and overproduction of active PedA in *E. coli* system. Considering its potential toxicity against expression host *E. coli* strain and high pI characteristics, we designed expression plasmid encoding mature PedA fused with N-terminally 5 histidine-tagged BldD DNA-binding domain has a high pI characteristics of 9.1 under *trc* strong promoter. To make liberate active bacteriocin effectively from the translational fusion, we also added sequences encoding a proline/glycine rich linker and enterokinase recognition/cleavage between each segments. The construct pHBNP was introduced into expression host *E. coli* BL21AI™. The fusion protein was overproduced after induction of L-arabinose and isopropyl-*b*-D-thiogalactopyranoside (IPTG) and purified by His-binding affinity chromatography. For the recovery of biologically active bacteriocin, the purified fusion protein was cleaved by enterokinase and the liberated bacteriocin was finally purified by passage on same column. The heterologously produced PedA from *E. coli* has a potent antimicrobial activity against pathogenic bacteria *Listeria*.

Keywords: bacteriocin, Pediococcus pentosaceus, tagging protein

D022

Glutathione Regulates the Transition from Growth to Development in *Dictyostelium discoideum*

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Glutathione is a ubiquitous tripeptide, found in most plants, microorganisms, and all mammalian tissues and most prevalent reducing thiol-containing compound in eukaryotic cells. Glutathione serves as cellular thiol redox buffer to maintain a thiol/disulfide redox potential, and also known to participate in many cellular processes, including the synthesis of proteins and DNA, transport, enzyme activity modulation and metabolism as well as detoxification of free radicals. GCS-null cells are not able to produce glutathione by disruption of the gene encoding ɣ-glutamylcysteine in *Dictyostelium discoideum*. When GCS-null cells that had grown in culture media without GSH developed on non-nutrient buffer, they did not differentiation. This was rescued by addition of GSH but not by dithiothreitol, *N*-acetylcysteine and ascorbic acid. GCS-null cells fail to decrease the expression of the growth-stage gene *cprD*, and do not induce the expression of *cAR1* (cAMP receptor), *acaA* (adenylyl cyclase A) and *lagC* (aggregation marker) that required for the earliest stages of development. These results suggest GCS-null cells are defective in production of the extracellular cAMP that serves as the extracellular chemoattractant and cAMP signal cascade in *D. discoideum* development.

Keywords: Glutathione, Development, Dictyostelium discoideum

D023

Erythroascorbate Peroxidase Is Involved in Cell Growth and Hyphal Formation in *Candida albicans*

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In general, ascorbate peroxidase catalyses the reduction of hydrogen peroxide to water by using ascorbate as an two electron donor in plants and fungi. There is a high affinity for ascorbate as reducing substrate. To investigate the functional role of erythroascorbate peroxidase (*EAPX1*) in *Candida albicans*, we disrupted and overexpressed the erhythroascobate peroxidase gene in *C.albicans*. The increase of the intracellular erythroascorbic acid in *EAPX1* null mutant suggests the erythroascorbic acid as the substrate of *EAPX1* in *C. albicans*. The *eapx1/eapx1* null mutants showed a growth retardation compared to the wild type in minimal medium. The *EAPX1* null mutant was more susceptible to the oxidative stress than the wild type strain. Accordingly, the *EAPX1*-overexpressing strain was more resistant against various oxidants such as hydrogen peroxide, menadione, diamide, and *t*-butyl hydroperoxide. Interestingly, *eapx1/eapx1* null mutant and even *EAPX1*-overexpressing strain showed severe defect in hyphal growth on solid media, respectively. So, we showed that RNA expression profiles of *eapx1/eapx1* null mutant and *EAPX1*-overexpressed strain were observed. In fact, the expression of hyphal-specific and virulence-specific genes was significantly reduced in the null mutant and *EAPX1*-overexpressing strain by down-regulating function. Taken together, our results demonstrate that erythroascorbate peroxidase is required for normal cell growth and involved in hyphal growth in *C. albicans*.

Keywords: Erythroascorbate peroxidase EAPX1 cell growth hyphal growth

D024

Band Formation and Fruiting Body Production of *Cordyceps militaris* from Various Regions of Single Spore Colonies

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2004, respectively, from Korea were used in the present study. Thick mycelial bands were formed between single ascospore isolates of EFCC 12025 and EFCC 12448, when inoculated on opposite sides of SDAY agar plates. Later, *in vitro* stromata were grown by inoculating mycelial discs from the meeting line and boundaries of the isolates. It was observed that band formation between the cultures had no direct relationship with the fruiting body formation. Fruiting bodies were also produced from the boundary of right and left sides (A), from the boundary of the median regions (B), from single mycelial disc (C) and from two mycelial discs (D) of meeting lines between EFCC 12448 isolates. It was again observed that the mycelial discs from the meeting line between the cultures produced fruiting bodies, but not from the boundary regions in most of the crosses

Keywords: mycelial discs, Cordyceps militaris, fruiting body formation, meeting line

D025

Functional Screening of Genes from Edible Mushroom *Pleurotus eryngii* in *Saccharomyces cerevisiae*

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Edible mushrooms including *Pleurotus eryngii, P. ostreatus, Flammulina velutips, Lentinus edodes* are some of the most important farm products worldwide. They share approx. $1 billion market in Korea already. Regardless of their commercial importance, however, few attentions have been paid to study basic features in mushroom biology such as mushroom cell division, cell-to-cell communication, fruiting body development, etc. In this work, we report construction of a genomic DNA library of *P. eryngii* and functional screening of mushroom genes involved in cell division cycle using various temperature-sensitive mutants of yeast *Saccharomyces cerevisiae*. To construct the genomic DNA library, we firstly extracted genomic DNA from the mushroom mycelia. DNA fragments corresponding to the size of 3~5kb were generated by the treatment of a restriction enzyme *Sau*3AI. The fragments were ligated into the *Bam*HI site of a yeast multicopy vector pRS425. The resulting genomic DNA library was introduced into yeast cell cycle mutant strains such as *cdc5-1, cdc15-2, dbf2-2*. The yeast cells outgrown at a restrictive temperature was screened. Subsequently, the recovered plasmids from the selected yeast cell cultures were subjected to sequence determination. Functional studies on the isolated genes in both *S. cerevisiae* and *P. eryngii* are under progress.

Keywords: Eryngii, Cell cycle, Yeast, Genomic DNA, Mushroom

D026

Growth of *Weissella kimchii* and *Lactococcus lactis* in the Electric Pulse

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W. kimchii and *L. lactis* were isolated from traditional Korean kimchi with modified MRS medium.Two species were identified with 16S-rDNA sequence and routinely cultivated in the modified MRS medium. Two bacterial cells were cultivated in electric pulse generated anode and cathode by reciprocal exchange of electric poles. Electric potential charged between anode and cathode was DC 2, 4, 6, 8 and 10 volts and test group without electric charge was control. The lactic acid bacterial growth was influenced by the electric pulse and substrate consumption and metabolite production by the bacterial cells was also influenced by the electric pulse. Generally, the bacterial growth and metabolite production was tendency to be proportional to the electric pulse charged between anode and cathode. However, the elecrophoresis pattern of soluble proteins extracted from the lactic acid bacteria grown in different electric pulse of 2 to 10 volt did not different each other. It shows a possibility that the lactic acid bacteria did not perceive the electric pulse as an environmental factor but the enzymes catalyzing lactic acid fermentation may be influenced by the electric pulse.

Keywords: Weissella kimchii, Lactococcus lactis, electric pulse, lactic acid production

D027

Study on the Physiology of *Mannheimia succiniciproducens* MBEL55E via *In Silico* Environmental Perturbation

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In this study, we undertook *in silico* experiments in order to dissect the physiology of the capnophilic rumen bacterium, *Mannheimia succiniciproducens*, under various environmental conditions. For this research, we utilized a genome-scale metabolic model of this organism, which we previously reconstructed. With this model, we performed constraints-based flux analysis to observe how this in silico organism behaves in response to changing uptake rates of glucose, H_2 and CO_2. We particularly focused on the rates of cell growth and succinic acid production under drastically varing environmental conditions because this microorganism was isolated for the purpose of succinic acid production. Both gases appeared to have profound effects on both rates of cell growth and succinic acid production. H_2 had a positive effect on both of them, leading to almost linear relationship between H_2 and those two rates. In the case of CO_2, it did not significantly affect the cell growth rate, but was very influential on the production rate of succinic acid. [This work was supported by the Genome-based Integrated Bioprocess Project of the Ministry of Science and Technology. Further supports by the LG Chem Chair Professorship, IBM SUR program, Microsoft, and by the KOSEF through the Center for Ultramicrochemical Process Systems are appreciated.].

Keywords: Mannheimia succiniciproducens MBEL55E, constraints-based flux analysis, environmental perturbation

D028

Influence of Microwave on Bacterial Inactivation and Medium Efficacy of *Weissella kimchii, Escherichia coli,* and *Saccharomyces cerevisiae*

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Weissella kimchii, Escherichia coli and Saccharomyces cerevisiae was cultivated in MRS, LB and YPG medium and harvested by centrifugation at 5,000 xg and 4°C for 30 min. The cells was washed with different buffer from pH 5, 7, 9 and resuspended into the same buffer. The resuspended bacterial cells and yeast cells were treated with microwave oven for domestic using at 2,400 MHz for 10 to 60 seconds. The survival number of bacterial cells and yeast cells was reciprocally proportional to the treatment time and completely inactivated in the condition treated for 60 seconds. On the basis of these results, 100 ml MRS, LB and YPG was sterilized by autoclave or microwave treatment for 120 seconds. *W. kimchii, E. coli and S. cerevisiae* were cultivated in the medium prepared by autoclave and microwave treatment. Growth of, substrate consumption and metabolite production by *W. kimchii* and *S. cerevisiae* in the medium differently sterilized were compared. Growth, metabolite production and substrate consumption were relatively higher in the medium sterilized with microwave than by autoclave. On the basis of these results, microwave oven will be applied to the sterilization of yeast culture medium to improve the medium efficacy. [Acknowledgement: This work was supported by a grant (Code # 20050401-034-750-142-04-00) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.]

Keywords: Microwave sterilization, ethanol production, Saccharomyces cerevisiae

D029

Physiological Characterization of Starch-Utilizing Yeasts Isolated from Long-Term Ripened Kimchi

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Three yeast cells were isolated from kimchi ripened for more than 6 month and routinely cultivated in the medium containing 5g/L yeast extract, 5g/L peptone and 10g/L soluble starch. The growth of isolate was relatively higher at 20°C than 10°C and 30°C. The yeast cells were identified by API kit (BioMeriux, France) as *Candida biodinii, Candida albicans* and *Rhodotorula mucilaginosa*. The isolates were maximally grown at pH 3 to 4 and minimally grown at pH 5 to 6, however, the growth at pH 7 was higher than that at pH 5 to 6. The isolate was not grown at pH 8 to 9. The growth of isolate in medium with glucose was greatly higher than that in medium with starch and the isolates did not produce ethanol in the medium with starch but produce ethanol in the medium with glucose. In aerobic growth condition, the isolates grew in the medium containing acetate, lactate and glycerol instead of glucose. This is different character from *Saccharomyces cerevisiae* incapable of growing in starch. [Acknowledgement: This work was supported by a grant (Code # 20050401-034-750-142-04-00) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.]
Keywords: Kimchi yeast, Candida sp, starch-utilizing yeast

D030

Physiological Characterization of Lactic Acid Bacteria Isolated from Fresh Kimchi

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Six lactic acid bacteria were isolated from fresh kimchi ripened less than one week. The isolates were identified with 16S-rDNA sequence as *Weissella* sp and *Leuconostoc* sp. Two of six were *Weissella* sp and four of six were *Leuconostoc* sp. Growth of isolates were higher at 30°C than 20°C, which is different from growth tendency of other *Leuconostoc* sp. The optimal pH for growth of the isolated was 7 to 8 but the bacterial cells were a little grown at pH 3. In consideration of NaCl contained in the kimchi, salinity tolerance of the isolates was tested. The most of isolate were grown in the medium containing NaCl from maximum 4 to 7 %. The growth in salinity environment was greatly inhibited but the substrate consumption was highly increased. This shows that disadvantageous environment may induce the bacterial cell to consume more energy to resist against the environment. On the basis of these results, we will design a bioreactor for lactic acid bacteria to induce more lactic acid production and less biomass growth.
Keywords: Weissella sp, Leuconostoc sp, Kimchi lactic acid bacteria

D031

The Growth of Anaerobic Bacteria on the Brain Heart Infusion Agar

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Several obligate anaerobic bacteria were successfully cultured on the brain heart infusion agar within 48 hours. The strict anaerobic condition was created by the disposable anaerobic gas pack, which was made by this author, and BBL anaerobic jar. Brain heart infusion agar was prereduced for 24 hours in the anaerobic jar. The list of the anaerobic bacteria are as follows: *Bacteroides fragilis* (ATCC 25285), *Bacteroides vulgatus* (KCTC 2639), *Bifidobacterium bifidum* (KCTC 3281), *Clostridium difficile* (KCTC 5009), *Clostridium perfringens* (KCTC 3269), *Eubacterium limosum* (KCTC 3266), *Fusobacterium nucleatum subs. polymorphum* (KCTC 2488), *Mobiluncus mulieris* (ATCC 35239), *Peptostreptococcus asaccharolyticus* (KCTC 3321), *Porphyromonas gingivalis* (ATCC 33277), *Propionibacterium acnes* (KCTC 3314), *Veillonella criceti* (ATCC 17747).
Keywords: Anaerobic bacteria, Brain heart infusion

D032

Characterization of an Apple Polygalacturonase-Inhibiting Protein (PGIP) that Specifically Inhibits an Endopolygalacturonase (PG) Purified from Apple Fruits Infected with *Botryosphaeria dothidea*

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An apple polygalacturonase- inhibiting protein (PGIP), which specifically inhibits endopolygalacturonase (PG, EC 3.2.1.15) from *Botryosphaeria dothidea*, was purified from *Botryosphaeria dothidea* infected apple (*Malus domestica* cv. Fuji) fruits. The purified apple PGIP had a molecular mass of 40 kDa. The N- terminal amino acid sequence of the purified protein showed high homologies to those of PGIP from pear (100%), tomato (70%), and bean (65%). We also purified polygalacturonase (PG) from *B. dothidea*. The PG hydrolyses pectic components of plant cell walls. When the extracted apple pectic cell wall material was treated with purified apple PGIP and *B. dothidea* PG, the amount of uronic acid released was lower than that treated only with *B. dothidea* PG alone. This result demonstrates that PGIP functions specifically by inhibiting cell wall maceration of *B. dothidea* PG.. Furthermore, we characterized the *de novo* function of the PGIP against PG on the solubilization and depolymerization of polyuronides from cell wall of apple fruits inoculated with *B. dothidea*. This result demonstrated that PGIP of plant exhibits one of direct defense mechanisms against the pathogen attack by inhibiting PGs which are released from pathogens to hydrolysis of cell wall components of plants.
Keywords: Polygalacturonase-inhibiting protein (PGIP), Polygalacturonase (PG), Apple, Malus domestica, Glomere

D033

New Synthetic Medium for Germinating Fungi, *Mycena osmundicola*

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Mycena osmundicola one of the seed germinating fungi, was cultured on oak leave for induction of seed germinating of *Gastodia elata*. There was some difficulty to grow the fungi properly in petri dish for long observation in laboratory condition. To overcome such a difficulty in laboratory, new culture media were established. The medium was prepared using oak leaves with mixture of rice bran(10g/liter). The oak leaves were dried in oven and after that it were ground in mortar. One gram of the ground leaf material was added to PDA. The rate of new media were the same as seed germination rate comparing with leaves culture method and was much handy to spread seed on the agar plate.

Keywords: Mycena osmundicola, germinating fungi

D034

Quorum Sensing System in *Sphingopyxis alaskensis* RB2256

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The marine ultramicrobacterium *Sphingopyxis alaskensis* RB2256 is an oligotroph that is remarkably resistant to a range of stress-inducing agent; reactive oxygen intermediates, ethanol, heat, antibiotics and so on. In this experiment we found homoserine lactone type quorum sensing signal molecule(SAI) from *S. alaskensis* RB2256 culture broth. According to R_f of location and spot pattern of SAI on TLC, it was found similar to N-(3-hyroxyoctanoyl)-L-Homoserine lactone. We obtained two LuxI homologue sequence(*sLasI*, *sLuxI*) from *S. alaskensis* RB2256 genome sequence database. (http://genome.jgi-psf.org/draft_microbes/sphal/sphal.home.html) We cloned this two LuxI homologue genes into *E. coli* BL21(DE3) strain. The purified sLasI and sLuxI proteins catalyzed the synthesis of acyl homoserine lactones. [Supported by the Driving Force Project for the Next Generation of Gyeonggi Provincial Government in Republic of Korea]

Keywords: Quorum sensing, Sphingopyxis alaskensis RB2256, Acyl Homoserine lactone

D035

Functional Analysis of *Sedo*-Heptulose 7-Phosphate Cyclase (SalQ) in Salbostatin Biosynthetic Pathway

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Salbostatin was discovered as a metabolite of *Streptomyces albus* ATCC 21838, and its structure has a valienamine that is the common structure of α-glucosidase inhibitor such as Acarbose. Salbostatin biosynthetic gene cluster was cloned to cosmid vector from *Streptomyces albus* KCTC 9015, using *acbC* sequence as the prove. The sequence of Salbostatin biosynthetic gene cluster was similar to that of Acarbose biosynthetic gene cluster. Each subcloning of Salbostatin biosynthetic genes were performed by PCR using cloned genes of Salbostatin biosynthetic pathway. The multistep conversion of *sedo*-heptulose 7-phosphate to the final cyclitol moiety was extensively studied in Acarbose biosynthetic steps. Compared to Acarbose biosynthetic genes, each genes were expected as *salQ*, *salL*, *salO*, *salM* and *salN*. A possible five-step reaction mechanism by these five enzymes was proposed for the cyclization reaction. For confirmation of the mechanism, SalQ protein was purified as soluble form. SalQ activity was detected by TLC and reaction product was analyzed by GC-MS. SalL protein, the next reaction component in biosynthetic mechanism, was purified and the activity detected by TLC. Other proteins, SalO, SalM and SalN, were also induced using *E. coli* expression system. [Supported by the Driving Force Project for the Next Generation of Gyeonggi Provincial Government in Republic of Korea].

Keywords: sedo-heptulose 7-phosphate cyclase, Salbostatin, Streptomyces albus

D036

Attenuation of *N*-3-Oxododecanoyl Homoserine Lactone-Induced Apoptosis by an Acyl Homoserine Lactone Acylase from *Streptomyces* sp. M664

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The occurrence of virulence in many opportunistic pathogenic bacteria, such as *Pseudomonas aeruginosa*, is closely related to the quorum-sensing systems. *P. aeruginosa* is well known to produce *N*-3-oxododecanoyl homoserine lactone (3-oxo-C_{12}-HSL) as a quorum-sensing molecule, for cell to cell communication. In this study, the ability of an acyl homoserine lactone acylase (AHL acylase) from *Streptomyces* sp. M664 to attenuate 3-oxo-C_{12}-HSL-induced apoptosis in human macrophage cells was evaluated. When the cells were incubated in the presence of 200 μM 3-oxo-C_{12}-HSL, their viability was gradually decreased together with morphological changes in a time-dependant manner, implying that the molecule specifically promotes the induction of apoptosis in the human macrophage cells. However, the negative effect of 3-oxo-C_{12}-HSL on cell viability was found to be significantly reduced when cells were co-treated with the AHL acylase. The cell proliferation caused by co-treating the AHL acylase from *Streptomyces* sp. M664 in the presence of 3-oxo-C_{12}-HSL strongly suggests that the enzyme can be very useful for the control of AHL-induced apoptosis as an effective therapeutic agent.

Keywords: N-3-oxododecanoyl homoserine lactone, acyl homoserine lactone acylase, Apoptosis attenuation, Streptomyces sp. M664

D037

Cyclo (L-Phe-L-Pro) (cFP) Affects the Environment-Dependent Modulation of *rpoS*-Involved Stress Response in *Vibrio vulnificus* MO6 24/O

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We have identified cyclo (L-Phe-L-Pro) (cFP) in *Vibrio* spp. and showed that this molecule plays a role as a signal modulating the expression of *ompU* (Park *et al*., 2006). Analysis of DNA microarray showed that cFP modulates the transcriptions of sets of genes including those related with *rpoS*-involved stress response. As examined protein expression profiles and measured the transcriptional level using reporter fusion to *rpoS*, we found that cFP significantly enhances the expression of *rpoS* at exponential phase. The effect of cFP was more eminent at 37°C than at 28°C, and at the NaCl concentration of 0.9% than at 3.5%. We also found that the induction effect of the *rpoS* transcription by cFP was more increased upon amino acid starvation. In *V. vulnificus*, the stringent response effector molecule ppGpp was accumulated upon the amino acid starvation just as other bacteria such as *Escherichia coli*. However, the accumulation of ppGpp was abolished by the addition of cFP. Transcriptions of *relA* and *spoT* genes responsible for ppGpp synthesis are not significantly affected by cFP, suggesting that cFP affects the ppGpp accumulation in a post-transcriptional level. Taken together, these result suggest that cFP affects a signal transduction process associated with sensing environmental factors including temperature, salt concentration as well as nutritional conditions.* Reference Park *et al*.2006. Cyclo(Phe-Pro) Modulates the Expression of *ompU* in *Vibrio* spp. *J. Bacteriol*. 188: 2214-2221.

Keywords: Vibrio vulnificus, cyclo (L-Phe-L-Pro) (cFP), RpoS

D038

Glucose Transport Enzyme IIA^{glc} Regulates Insulin Degrading Enzyme in *Vibrio vulnificus*

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The bacterial phosphoenolpyruvate:sugar transferase system (PTS) participate in several physiological role besides phosphorylation coupled translocation of numerous sugars. Enzyme IIA^{glc}, soluble component of PTS, has multiple functions such as regulation of adenylate cyclase, fermentation/respiration switch, glycerol kinase activity and inducer exclusion in *Escherichia coli*. To elucidate regulatory roles of the *Vibrio vulnificus* PTS, we searched for a protein(s) interacting with enzyme IIA^{Glc}. We designed ligand fishing experiment using purified IIA^{glc} as bait and revealed that a novel peptidase belonging to the M16 Zn-metallopeptidase family and showing the highest sequence identity with mammalian insulin degrading enzyme (IDE) interacts specifically with IIA^{Glc}. This peptidase interacts with IIA^{Glc} independently of the phosphorylation state of IIA^{Glc}, but insulin-degrading activity of the peptidase was strictly dependent on the dephospho-form of IIA^{Glc}. We named this peptidase as insulin degrading enzyme (v-IDE). An insulin degrading enzyme-deficient mutant of *V. vulnificus* showed lower survival and pathogenicity than wild type in mice. We assume that the v-IDE-IIA^{Glc} complex is necessary to regulate host insulin level in pathogenesis.

Keywords: PTS, Insulin-degrading enzyme (IDE), enzyme IIA

D039

Pleiotrophic Effects of the Mutations Affecting Biofilm Formation in *Vibrio vulnificus*

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Biofilm formation of *Vibrio vulnificus* V1 strain on abiotic surfaces has been shown to be affected by phosphate concentration and carbon source, especially being repressed by glucose. Among numerous mutants showing variation in biofilm formation, three mutants M17, M41, and M20 were mapped at the phosphoribosylaminoimidazole succinocarboxamide synthase gene, the N-acetylglutamate synthase gene, and a homologue of *cqsA*, respectively. Both M17 and M41 formed much less biofilm than wild-type, while M20 formed much more on abiotic and biotic surfaces. Pleiotrophic effects of these mutations were investigated. M20 showed significantly decreased swarming phenotype on 2.5HI agar, while that of M17 increased. Motility of the mutants by flagella was affected in the same pattern. M41 did not show much change in both phenotypes. M20 produced more alkaline phosphatase activity, while the other two mutants produced less than wild type. Only M41 showed enhanced hemolytic activity on blood agar.

Keywords: Biofilm, V. vulnificus

D040

Cross-seeding Between Yeast Prions

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Yeast prions propagate in the host cell by protein aggregation and continuous seed generation. The propagation of yeast prions is independent of each other and this represents that each prion aggregate has strong sequence preference and should be made of only one cognate protein. The appearance of yeast prion, however, is obviously affected by other prions. The relation between [PSI+] and [RNQ+] clearly shows the interaction between prions. Overexpression of Sup35 induces the appearance of [PSI+] only in [RNQ+] strain. Cross-seeding model in which Rnq1 aggregate acts as an imperfect template on Sup35 was suggested. If this hypothesis is true, frequent collision between Rnq1 aggregate and Sup35, not a large amount of Sup35, will be sufficient to induce [PSI+] appearance. Based on this assumption, we have developed a new [PSI+] induction method which robustly supports the cross-seeding model. By using a new method, we also found that the cross-seeding between Sup35 and Rnq1 is mutual. We concluded that although it is less efficient than self-seeding, one yeast prion protein can cross-seed others and probably so do other amyloidogenic proteins in human.

Keywords: yeast prion, Sup35, Rnq1

D041

Systematic Generation of Functional Split Proteins and In-Frame Hybrids by DNA Transposition and Homologous Recombination

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Protein-fragment complementation assay (PCA) using split proteins are emerging as an universal technology to analyze molecular interactions under in vivo conditions. Moreover, in-frame hybrid proteins are being increasingly applied to the development of molecular biosensors. In these regards, we developed a system to produce Tn5-transposition libraries, which would produce split proteins and in-frame hybrids of individual proteins. First, a transposition reaction was performed in vitro to generate a random combinatorial library, and then TN5 insert was removed by restriction digestion, leaving 19-residue peptides as a scar. Next, the library with 19-residue scars was tested for the remaining activity of the original protein function. When the protocol was followed with GFP for the ease of analysis, twenty functional GFP variants with scars were isolated through fluorescence imaging of four thousand *E. coli* colonies on agar plates. Sequence analyses exhibited the location of scars are concentrated between residues 170-176, which are considered as an appropriate region for functional split of GFP. To evaluate the usefulness of obtained isolates, the scar-GFPs were reconstructed to bear a translation stop or interacting protein pairs, via general DNA manipulation or with a homologous recombination technology using Lambda red-recombinase. Detailed fluorescence characteristics and solubility of scar-, split-, in-frame hybrid GFPs will be discussed further in the poster.
Keywords: split protein, in-frame hybrid, transposition, recombination, PCA, GFP

D042

Genetic Circuitry as a Novel Screening System for Biocatalysts

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Increasing number of drug intermediates and industrial chemicals are manufactured by biocatalytic processes. A key factor for those processes is the availability of enzymes with the necessary activity, specificity, and stability under operational conditions. An approach to find desired activities from immense diversity of expressed DNA libraries are the application of genetic reporter system, which also called as the genetic circuitry. Generally, genetic circuits are composed of well-tuned transcriptional regulators to control promoters and the target genes to be expressed. In this work, we developed a genetic circuitry that recognize the product of a biocatalytic conversion through the transcriptional activation factor DmpR and respond by expressing green fluorescent protein or antibiotic resistance genes. The system responded very sensitively and quantitatively to the presence of phenol, 2-Cl-phenol, 3-Cl-phenol, 2-nitro-phenol, resorcinol, catechol, 3-methylcatechol, 2-methoxycatechol, and o-cresol in mM levels. When *E. coli* host with the genetic circuit was introduced with tyrosine phenol-lyase gene from *C. freundii*, green fluorescent colonies were evolved in the presence of L-tyrosine derivatives. By the same way, *E. coli* with the antibiotics-based circuit system was able to grow under selective conditions only when included TPL gene, resultantly enabling selection of biocatalytically active cells in large inactive populations.
Keywords: genetic reporter system, genetic circuit, DmpR, biocatalysts, phenolics

D043

Protein Engineering Supported by Homology Modeling and Docking Simulation

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Molecular modeling of protein structure and simulation of molecular docking is becoming popular in most field of biotechnology, including the development of engineered biocatalysts. Although evolutionary technologies based on diversified gene library have accomplished fabulous contribution in biocatalyst developments, the technologies have been compromised by the limited coverage of screening throughput, compared to the vast genetic diversity. At this point, homology modeling can reliably help to focus on a particular substructure for the improvement of biocatalyst function. Docking simulation could also be applied for the optimization of binding site if a detailed structural information is available. As a platform to support the enzyme engineering technology, we established a protein modeling and docking simulation facility based on DS Modeling 1.5 of Accelrys Software Inc. Our system and facility is able to support the functional analysis of primary sequences, the comprehensive estimation of the active site, homology modeling, and also the evaluation of binding free energy. Various catalytic enzymes, microbial binding proteins, and small molecule-protein interactions are under investigation to evaluate the DS modeling systems and calculation facility, based on our knowledge of structure, function, and evolutionary relationships between protein sequences. [Supported by Protein Engineering Innovation Center Program and KRIBB Research Initiative Program]
Keywords: homology modeling, docking simulation, protein engineering, directed evolution

D044

Development of Photo-Switch Proteins for Reversible Control of Fluorescent Protein by Using Cyanobacterial Phytochrome Cph1

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Phytochromes are photoreceptors of plants, fungi, and bacteria that allow these organisms to respond to environmental light conditions. *E. coli* cells turned to green when N-terminal [1-514] of phytochrome Cph1 of *Synechocystis* sp. PCC6803 was co-expressed with heme oxidase (HO1) and phycocyanobilin-ferredoxin oxidoreductase (PcyA). The two enzymes, HO1 and PcyA, were to produce phycocyanobilin in *E. coli* cells. Phytochromes exist in two spectroscopic states, Pr and Pfr that absorb respective red and far-red lights, which can be reversibly converted by light. The photo-conversion between Pr and Pfr is associated with very rapid isomerization of phycocyanobilin (PCB) followed by a slower recovery in the dark. The photo-conversion of Cph1 was introduced in this work as a photo-switch to regulate the emission intensity of yellow fluorescent proteins (YFP) that have spectral overlap with the absorption spectrum of Pr-form. The phytochrome switch could be applied to expand the present repertoire of genetically encoded fluorescent proteins, which are being used as a imaging probe in various fields of cell biology and drug screening. Here, four fusion proteins consisting of cyan fluorescent protein (CFP), Cph1, and YFP were constructed: CFP-Cph1, Cph1-YFP, CFP-Cph1-YFP, and CFP-Cph1-YFP. The fluorescent fusion proteins were purified from *E. coli* cells and investigated about fluorescence and color characteristics, after illuminated with red or far-red light.
Keywords: photo-switch, phytochrome, fluorescent proteins, Cph1, YFP

D045

Production of Tansgenic Potato (*Solanum tuberosum L.*) Harboring a Gene for Lectin in Korean Mislteoe (*Viscum album coloratum*)

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Mistletoe is a semi-parasitic plant that grows on different kinds of trees, like oak. Lectin obtained from the mistletoe has a variety of biological activities, such as cytotoxicity, antioncogenicity, and so on. The lectin is composed of two domains: A chain linked with B chain by a disulfide-bond. The Korean mistletoe lectin (KML) has higher cytotoxic activity than European mistletoe lectin (EML). The KML has various isoforms. We cloned a gene (*kml*) for lectin in Korean mistletoe obtained using reverse transcriptase polymerase chain reaction (RT-PCR) from total RNA. The kml was constructed by the application of pRSET A vector system. The gene is composed of 1,593 nucleotides coding 531 amino acids (aa) with a signal peptide of 33 aa (GenBank accession number DQ011864). The recombinant clone was well expressed in *E. coli* BL21(DE3). It's molecular weight was determined to be 58.3 kDa by SDS-PAGE. To produce a transgenic potato plant, the *kml* was ligated into a vector, pCAMBIA 3300, to generate pCAMBIA 3300/kml. The pCAMBIA 3300/kml was transfected into potato internodes by *Agrobacterium*-mediated transformation system. A number of transgenic potatoes have been generated from callus culture. They were resistant to Basta (1.5 ㎕/㎖) as expected. The transfected gene, *kml*, was confirmed by the Sanger.

Keywords: Korean mistletoe lectin, Transgenic potato, Agrobacterium-mediated transformation

D046

Production and Characterization of a Novel Thermostable Phospholipase D from *Streptomyces* cs 621

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Phospholipase D (PLD) class of enzymes catalyzes two types of reactions: hydrolysis of phosphatidylcholine(PC), a major substrate of PLD, to phosphatidic acid (PA) and choline(hydrolytic reaction), and transfer of polar head groups to others (transphosphatidylation reaction). The soil samples were screened for actinomycetes strains capable of producing thermostable phospholipase D, and one *Streptomyces* sp. CS 621 showing a high phospholipase D activity were isolated. One PLD in the culture supernatants from *Streptomyces* sp. CS621 was purified through ammonium sulfate fractionation, heat treatment, ultramembrane (YM30) filtration, Sepharose CL-6B column chromatography, and DEAE-sepharose CL-6B column chromatography. Biochemical and physicochemical properties including thermostability, substrate specificity and transphosphatidyation activities of phospholipase D from *Streptomyces* sp. were examined.

Keywords: Streptomyces sp. CS621, Phospholipase D, Purification, Characterization

D047

Identification of *Sreptomyces* cs 636 Strain Isolated Korean soil and Production and Characterization of a Phospholipase D from *Streptomyces* cs 636

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Sreptomycete cs 636 srain isolated Korean soil shows close phylogeny to genus *Streptomyces*. The morphological and 16S rDNA data shows suggest that this species may be *Streptomyces* sp.which produce thermostable extracellualr phospholipase D when grown in GYT medium containing glucose 2.0%, yeast extrac 1.5%, trypton 0.5%, CaCo3 0.1%, at 28℃. One form of extracellular phospholipase D was partially purified through ammonium sulfate fractionation, heat treatment, ultramembrane (YM30) filtration, Sepharose CL-6B column chromatography, DEAE-sepharose CL-6B column chromatography. Some biochemical and physicochemical properties including detergent effect of isolated phospholipase D were examined.

Keywords: Identification, Streptomyces cs 636, Production, Characterization, Phospholipase D, thermostable

D048

Purification and Biochemical Properties of a New Neutral Protease Produced by *Streptomyces* sp. CS624

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Streptomyces CS624 has been isolated in soil sample from, Ui island, located in the Jenneonam province, Korea, and produces high neutral extracellular protease. Culture broth of *Streptomyces* CS624 showed the highest protease activity at late exponential phase when grown in GOKN medium (glucose 1.5%, oatmeal 1.5%, K2HPO4 0.3%, NaH2PO4 0.3%) at 28℃. Protease was fractionated by Ultrogel AcA 54 column chromatography, and further purified through ammonium sulfate fractionation, ultramembrane filtration, and DEAE-sepharose CL-6B column chromatography. The optimum pH values of protease was shown to be 7.5. The optimum temperature for the activities of protease was 35℃. Some other biochemical properties including inhibition of protease inhibitor of protease from *Streptomyces* sp Cs624 were examined.

Keywords: Purification, biochemical properties, new neutral protease, streptomyces sp. CS624

D049

Cloning of Manganese Peroxidase and Laccase Gene and Transformation of *Trametes versicolor* to get Transformants with Extracopies of the Genes for Lignin Degrading Enzymes

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The white rot basidiomycete *Trametes versicolor* secretes laccases and peroxidases which are involved in the degradation of polymeric lignin. Using PCR and RACE-PCR approaches with various primers, we have cloned and sequenced cDNA gene of manganese peroxidase and laccase from *T. versicolor*(951007-22-5) isolated in Korea. cDNA genes of Mnp and laccase showed high homologies with those of other white-rot fungi. Genetic transformation of *T. versicolor* was successfully carried out by restriction enzyme mediated integration(REMI). We have constructed pBARGPMNP2 and pBARGPLAC which has the manganese cDNA(mnp) and laccase cDNA(lac) regulated by glyceraldehyde-3-phosphate dehydrogenase gene promoter. The integration of the plasmid in *T. versicolor* chromosomal DNA is confirmed by Southern blot analysis using probe containing bar gene.

Keywords: recalcitrant compounds, laccase, manganese peroxidase, *Trametes versicolor*

D050

Genetic Transformation of *Phlebia tremellosa* to Phosphinothricin Resistance by Restriction Enzyme-Mediated Integration

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As human life generates more wastes which can contaminate our environment, there should be a proper method to save our environment by removing the toxic chemicals. Phlebia tremellosa is a white-rot fungus showing high degrading activity against diverse recalcitrant compounds. Bioremediation using this fungus can be a good candidate, and it is highly required to introduce foreign genes into this fungus in order to increase the degrading capability. Genetic transformation method for this fungus was successfully carried out using the classical protoplast-$CaCl_2$ method. During the introduction of transforming vector by the restriction enzyme-mediated integration, the inserted gene generates many diverse mutants which show very stable phenotype even after five or more consecutive transfers under non-selective conditions. In order to performed REMI-transformation in P. tremellosa, pBARGEM was linearized by restriction enzyme EcoR Ⅰ and they were mixed with the fungal protoplast for integration of the vector into the fungal chromosome. The integration of the plasmid in *P. tremellosa* chromosomal DNA is confirmed by PCR with bar gene-specific primers.

Keywords: transformation, endocrine disrupting compounds, *Phlebia tremellosa*

D051

Electrochemical Study of Nitrate- and Ferric Iron-Reducing *Lactococcus garvieae*

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A lactic acid bacterium growing in soil, *Lactococcus garvieae* can reduce ferric iron and nitrate to ferrous iron and nitrite, respectively. The growth on basic medium was relatively lower than that on basic medium containing nitrate and ferric iron. When the isolate was cultivated in the basic medium containing both of nitrate and ferric iron, ferric iron reduction was not influenced by the nitrite and nitrate reduction also was not influenced by the ferric iron. It shows that the nitrate and ferric iron reduction system may be differently controlled. The bacterium can reduce ferric iron immobilized into graphite electrode instead of suspended ferric iron, by which electrons can be transferred from bacterial metabolism to electrode. On the basis of the bacterial character, we designed a biofuel cell. We applied the bacterium to the biofuel cell system equipped with the electrode modified with ferric iron to compare the electricity production in the condition with ferric iron or nitrate. Theoretically, redox potential of nitrate is higher than ferric iron, by which more electron may flow into nitrate than ferric iron in the medium containing mixture of nitrate and ferric iron. And the electricity production by the bacterium may be inhibited by nitrate and ferric iron added to the medium.

Keywords: ferric iron-reducing lactic acid bacterium, Biofuel cell, Lactococcus garvieae

D052

Morphological and Structural Characterization of Cold-Induced Spherical Biopolymer Shelters Made by *Pseudomonas fluorescens* BM07 and Study on the Implications to its Low-Temperature Viability

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Pseudomonas fluorescens BM07 is a psychrotroph. When the cells solid-agar grown at 30℃ were inoculated the cells grown in King's B medium or M1 medium containing 70 mM fructose at 10℃ and 30℃, respectively, only the cultures grown at 10℃ were found to secrete a lot of a biopolymer. The secretion of the extracellular biopolymer was assumed to be related with a living strategy of BM07 strain in colder environments to fight against any coldness by surrounding the cells with a certain shell of biopolymer. To prove the suggestion, the morphology of the shells and the structures of the component polymers were characterized to find any relationship between their secretion and low-temperature viabilities. According to the scanning electron microscopic data, *P. fluorescens* BM07 grown in M1 medium at 10℃ was found to make a characteristic spherical form whose apparent volume was several tens times larger than that of the cell volume. However, the characteristic shape was so fragile that it was broken down under the centrifugal shear force of several thousands of g-value to uniquely structured basic units of ~20 nm which were segregated during centrifugation to form fibers of 0.05 ~ 0.1 micron thickness. The whole and broken structures were characterized by two-dimensional X-ray diffractometry and differential scanning calorimetry. The primary basic component of the shells was identified as a glycosylated, very hydrophobic and water insoluble ~5 kDa polypeptide and the isolated freeze-dried polypeptide has a reversible melt transition at ~125℃. The low-temperature viability of *P. fluorescens* BM07 cells grown at 10℃ and 30℃, respectively, was measured after storing the cells at -20℃ and -70℃, respectively, depending on the storage time. The cells grown at 10℃ showed a higher freezing-viability than grown at 30℃. Thus, the excessive secretion of ~5 kDa polypeptide and construction of the characteristic spherical shells at low temperatures by *P. fluorescens* BM07 may be a means to resist any coldness to survive during winter. We are currently trying to find the related genes and their functional roles.

Keyword: biopolymer

D053

Role of the Substrate Binding Domain Ⅰ and Ⅱ in the Degradation of Polyhydroxyalkanoic Acid by *Pseudomonas stutzeri* BM190 Extracellular PHA Depolymerase

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Pseudomonas stutzeri BM190 extracellular PHA depolymerase is known to consist of four domains: the catalytic domain, linker domain, and two substrate binding domains. The enzymatic degradation, as a first order approximation, is believed to be initiated by binding of the substrate binding domains to PHA substrate to form a stable PHA substrate/enzyme complex. In a previous study, the depolymerase isolated from the wild-type *P. stutzeri* BM190 exhibited a decrease in its activity with an increase in the level of 3HV-unit in the *Hydrogenophaga pseudoflava* P(3HB-co-3HV) copolyester granule; when the level of 3HV-unit was increased upto 30mol%, no degradation was observed. The enzyme could not degrade P(3HV) homopolyester granule, either. Based on the first-order kinetics analysis of P(3HB-co-3HV) degradation, *P. stutzeri* BM190 depolymerase was found to be active against the copolymer substrate which has at least three 3HB-units in a sequence (trimer). When the copolymer contained less than 20mol% of 3HV-unit, the 3HV-unit in the copolymer was also recognized and degraded by the enzyme and released into medium, which was confirmed by HPLC analysis. For the characterization of each role of the two binding domains in terms of their possible effect on the local sequence specificities, we prepared three types of recombinant proteins, catalytic domain-linker-SBD Ⅰ, catalytic domain-linker-SBD Ⅱ, and catalytic domain-linker-SBD Ⅰ-SBD Ⅱ and studied their degradation kinetics and sequence specificities by analyzing the degradation products and the remaining polymers by high resolution NMR spectroscopy.

Keywords: PHB depolymerase, Pseudomonas stutzeri BM190

D054

Cloning of Extracellular and Intracellular Endoglucanase Genes Existing in Tandem from Hyperthermophilic *Thermotoga maritima*

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The genome of *Thermotoga maritima* MSB8 encodes thermostable endoglucanases genes cel5C and cel5D genes encoding athermostable endoglucanases, existing in tandem in Thermotoga maritima MSB8were cloned and expressed. A clone having of 2.3 kb fragment havingshowed cellulase activity, designated as pTM100, was sequenced and found to have two ORFs. The cel5C gene consists of 954 bp which encodes 317 amino acid residues with signal peptide of 21 amino acids. The other gene, cel5D, has 990 bp encoding a protein of 329amino acid residues. Two cellulases do not have cellulose-binding domain but have similarity with those of glycosyl hydrolase family 5. Cel5C which was extacellular cellulase whilewhere as Cel5D was intracellular cellulase. SDS-PAGE analysis of the purified Cel5C and Cel5D showed bands corresponding to 35 kDa and 39 kDa respectively. Both the enzymes were exhibiteding maximal activity at 80^{o}C, 60^{o}C and pH 5.0 ,but foundand stable over a period of at least 3 h at 60^{o}C. Cel5C retained 65% of its activity after 1 h at 80^{o}C.

Keywords: Hyperthermophilic, Thermotoga maritima MSB8, Endoglucanase, celCD gene

D055

Psychrophillic Bacteria Producing Extracellular Lipase Isolated from Arctic Sea Sediment

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Psychophillic or psychrotolerant lipase has increasing application potential in industry as well as fine chemical process. A low temperature adapted bacterium was isolated from the sea bottom sediment collected at the area around Spiztwergen island, Norway, Artica by screening from lipid containing agar plate showing a halo around colony. This strain, identified as *Pseudomonas* sp. L994 from the 16S rDNA sequence and other physiological properties produces extracellular lipase. The optimal condition of lipase production was found, such as 15 ℃, pH 8.0, in Sea water complete media with 40 % sea water content. The lipase was purified from the culture broth by ultrafiltration, ion exchange column chromatography and gel filtration chromatography.

Keywords: Psychophillic lipase, Arctic Pseudomonas

D056

Psychrophillic Bacteria Producing Extracellular Phospholipase A Isolated from Arctic Sea Sediment

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Psychophillic or psychrotolerant phospholipase A type has increasing application potential in fine chemical process such as phospholipid biotransformation, especially fatty acid transesterification. A low temperature adapted bacterium was isolated from the sea bottom sediment collected at the area around Spiztwergen island, Norway, Artica. This strain, identified as *Pseudomonas* sp.L157-2 produces extracellular phospholipase A. The optimal condition of phospholipase production, such as 15 ℃, pH 8.0, in Sea water complete media with 40 % sea water content were studied. The phospholipase was purified from the culture broth by ultrafiltration, ion exchange column chromatography and gel filtration chromatography.

Keywords: Phospholipase A, Arctic sediment

D057

Function of the Novel *YKL161C* Gene Product in Beta-Glucan Biosynthesis of *S. cerevisiae*

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β-Glucan has been known that it has non-specific immune-enhancing activity in animal and human beings and found in cell wall fractions of many kinds of fungi. *Saccharomyces cerevisiae* has exposed β-glucan in its cell wall as large amount. The zymolyase is a kind of β-glucanase that digests the β-glucan structure and finally inhibits the β-glucan synthesis of cell wall. High level percentage of β-glucan is quite resistant to zymolyase and we performed a β-glucanase sensitivity screening to find the genes which are involved in zymolyase resistance by over-expression of yeast genomic DNA library and genome-wide cDNA microarray analysis. We have found that over-expression of *YKL161C* showed the high resistance to the zymolyase treatment. To confirm the function of *YKL161C* gene product, we have constructed deletion strain of *YKL161C* and GFP-tagged version of *YKL161C*. The deletion strain of *YKL161C* was more sensitive than wild type to zymolyase treatment and the GFP tagged Ykl161c protein was found in the nuclear. The Yeast Genome Database (SGD) gives the information that Ykl161c protein may work as a kinase in the β-Glucan synthetic pathway and regulates the expression of many genes which are involved in β-Glucan synthetic pathway. Interestingly, we have found that the expression of septin gene was induced by several folds from cDNA microarray analysis and indicates the involvement of Ykl161c in cell wall integrity.

Keywords: S. cerevisiae, Beta-Glucan, Cell wall, Microarray, ymolyase

D058

Plate Assay of Cellobiohydrolase, Pectinase, and Xylanase in *Penicillium*

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Penicillium is very common as a food spoilage organism and also in indoor environments. Many *Penicillium* fungi have been isolated in Korea for several decades. But their ability of producing enzymes has not been evaluated yet. To evaluate their ability of producing enzyme in this study we performed a plate assay using a mixture of chromogenic substrates and media. A total of 119 *Pencillium* species including 347 isolaes from Korea were tested for their ability of degrading cellobiose, pectin, and xylan. Tests with 4 different chromogenic-dyes, congo red, phenol red, remazol brilliant blue and tryphan blue showed that congo-red was the most useful dye in the clear detection of cellobiohydrolase, pectinase, and xylanase. The activity of cellobiohydrolase in *Penicillium* was generally strong in all the *Penicillium* isolates tested. *P. citrinum*, *P. charlesii*, *P. manginii* and *P. aurantiacum* showed the higher ability of producing cellobiohydrolase than other tested species. Pectinase activity was detected in 59 *Penicillium* isolates. *P. paracanescens*, *P. sizovae*, *P. sartoryi*, *P. chrysogenum*, and *P. claviforme* showed strong pectinase activity. In xylanase assay 113 *Penicillium* isolates showed activity. Strong xylanase activity was detected from *P. megasporum*, *P. sartoryi*, *P. chrysogenum*, *P. glandicola*, *P. discolor*, and *P. coprophilum*. The degree of pectinase and xylanase activity varied depending on both *Penicillium* species and isolates.

Keywords: Penicillium, Chromogenic media, Cellobiohydrolase, Pectinase, Xylanase

E001

Determination of Antioxidative Potential of Antarctic Lichens *in vitro*

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The study was aimed at evaluating the antioxidant activities of 10 Antarctic lichen species such as *Cladonia furcata, Cladonia scabriuscula, Himantormia lugubris, Pseudephebe pubescens, Ramalina terebrata, Sphaerophorus globosus, Umbilicaria Antarctica, Usnea Antarctica, Usnea aurantiaco-atra* and *Xanthoria elegans*. The antioxidant activity against lipid peroxidation, reducing power, superoxide anion radical scavenging activity and free radical scavenging activity were examined. Methanol extract of some lichen species showed very strong antioxidant activity at the concentration of 0.2 to 2 mg/ml. For example, *Ramalina terebrata* scavenged more than 85% of DPPH at the concentration of 2 mg/ml. *Sphaerophorus globosus* also scavenged 98% of surperoxide anion at the same concentration. All these activities were found to be concentration-dependent. The result indicates that Antarctic lichens of *Himantormia lugubris, Ramalina terebrata, Sphaerophorus globosus, Usnea antarctica* and *Xanthoria elegans* have strong antioxidant activity and they can be used as a potential bioresource for novel natural antioxidant.

Keywords: Antarctic, antioxioant, bioresource, lichen, lichen-forming fungi

E002

Determination of Antioxidative Potential of Chinese Lichens *in vitro*

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Twenty species of Chinese lichens were examined to evaluate their antioxidant activity against lipid peroxidation, reducing power, superoxide anion radical scavenging activity and free radical scavenging activity. Methanol extract of lichen thalli were used at the concentration of 1 and 2 mg/ml. Among the 20 species, *Allocetraria madreporiformis, Cladonia rangiferina, Evernia mesomorpha, Peltigera canina, Thamnolia vermicularis and Umbilicaria* sp. exhibited stronger antioxidant activity than other species. Gradient concentrations (0.5, 1, 2 and 4 mg/ml) of these six lichens showed that all the tested activities were concentration-dependent. They effectively inhibited linoleic acid peroxidation and scavenged free radical DPPH and superoxide anion, whereas they were not effective in reducing power, compared to positive control. The result suggests these six species of Chinese lichens can be used as a potential bioresource for novel natural antioxidant.

Keywords: antioxidant, bioresource, China, lichen, lichen-forming fungi

E003

Anticancer Activity of *Lethariella zahlbruckneri* against Human Colon Carcinoma Cell Line HT-29 *in vitro*

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Highland Chinese lichen of *Lethariella zahlbruckneri* was firstly attempted to investigate biological activity of the lichen against human colon carcinoma cell line HT-29 *in vitro*. Effects of the lichen extract on cell apoptosis, cytotoxicity and cell growth were examined at the concentration of 10 to 200 μg/ml. Methanol and acetone extract of the lichen thalli effectively inhibited the cell growth and induced apoptosis of the cell in a dose-dependent manner. Acetone extract was more effective in cell growth inhibition than methanol extract, even at the low concentration (10 μg/ml). Acetone extract showed significant cytotoxicity ($IC_{50} \leqq 30$ μg/ml) against the cell line, whereas methanol extract showed moderate cytotoxicity ($IC_{50} \leqq 100$ μg/ml). In the cell cycle assay, neither of the extract showed significant activity, even at the high concentration (100 μg/ml). This study suggests that *L. zahlbruckneri* can be used as a potential bioresource to develop novel natural substances for colon cancer treatment.

Keywords: anticancer, bioresource, colon carcinoma, lichen, lichen-forming fungi

E004

Demineralization of Crab Shell by *Lactobacillus paracasei* subsp. *tolerans* KCTC-3074

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Demineralization (DM) and deproteinization (DP) from crab shells (CS) waste were carried out using a lactic acid producing bacterium *Lactobacillus paracasei* subsp. *tolerans* KCTC-3074 for 7days at 25, 30, and 35℃. pH declined from pH 8.0 to about pH 4 on the 7th day of culture, while TTA increased until 16% at 35℃. Residual dry weight of crab shell waste and pH decreased in accordance with culture temperature, but their rates are little different. DM rates were 89-92% and little affected by temperatures. DM was also done for 4 particle-sized shell samples (0.84-3.35, 3.35-10.0, 10-20 and 20-35 mm) with 10% inoculum, 5% shell, and 10% glucose at 30℃ and 180 rpm for 7 days, and found out that shell size was little affected on the rate of DM. Negative relationships were found between DM and residual dry weight (r^2=0.960), and between DM and pH (r^2=0.906). On the other hand, positive relationships were found between DM and medium protein (r^2=0.696), and between DM and TTA (r^2=0.630).

Keywords: Demineralization, Crab shell, Lactobacillus paracasei subsp. tolerans KCTC-3074, Particle-sized shell sample

E005

Analysis of Anthocyanins in Black Soybean during Fermentation with *Bacillus* spp. Isolated from Chungkookjang

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Anthocyanins are water-soluble, flavonoid pigments that have antioxidative activity. They generally occur in the plant as glycosides and acetylglycosides of anthocyanidins. Three major anthocyanins in black soybean are found as delphinidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucoside. In this study, anthocyanins in black soybean were analyzed during the fermentation with *Bacillus* spp. isolated from Chungkookjang. The molecular changes and the antioxidative activities were measured with HPLC and TLC, respectively. In the analysis with HPLC, the peaks of anthocyanidins were decreased and changed during fermentation. In TLC, spot for antioxidative activity was smeared in the control and several spots were appeared in the fermented products.
Keywords: Anthocyanins, Black Soybean, Bacillus spp., Chungkookjang

E006

MS Analysis of Isoflavones in Chungkookjang

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Chungkookjang, Korean fermented soybean contains isoflavones. They are believed to play important parts in human cellular signal transduction. Soybean was fermented by *Bacillus licheniformis* B1, and 90% ethanol extract of fermented soybean powder were prepared. MS were used to provide molecular mass information present within the extract of fermented soybean. Genistein (271 m/z), daidzein (255 m/z), and glycitein (285 m/z) were present. Genistin (433 m/z), daidzin (417 m/z), glycitin (447m/z) did not exist in the sample. This suggests that glucose attached to the isoflavones was fully cleaved by the glucosidase of the strain. Also modifed isoflavones such as 8-OH-genistein, 6-OH-daidzein, 8-OH-daidzein, and 8-hydroxyglycitein were not found.
Keywords: Chungkookjang, Isoflavones, Genistein, Daidzein

E007

Inhibition of Proliferation in Colon Cancer Cell Lines and Colonic Bacterial Enzymic Activities as Possible Factors in the Ethiology of Colon Cancer by *Bifidobacterium adolescentis* SPM 0212

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In this study, we investigated the effects of *Bifidobacterium adolescentis* SPM 0212 on the anti-cancer activity and inhibitory effects on fecal harmful enzymes of intestinal microflora. *B. adolescentis* SPM 0212 isolated from healthy Korean in the age of 20s was used in this experiment. In order to investigate the anti-cancer effects of *B. adolescentis* SPM 0212, three human colon cancer cell lines (HT-29, SW 480 and CaCO-2) were treated with *B. adolescentis* SPM 0212. XTT assay (sodium 3-[1-(phenylaminocarbonyl)-3, 4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate, Sigma) showed that *B. adolescentis* SPM 0212 inhibited the proliferation of HT-29, SW 480 and CaCO-2. The production of TNF-α was largely affected in dose-dependent manner in *B. adolescentis* SPM 0212 and cell morphological change was also largely affected by *B. adolescentis* SPM 0212.Fecal harmful enzymes such as ß-glucuronidase, ß-glucosidase, tryptophanase and urease, were effectively inhibited during the administration of the *B. adolescentis* SPM 0212. These results suggested that *B. adolescentis* SPM 0212 indicated anti-cancer effect and inhibition of fecal harmful enzymes.
Keywords: anti-cancer effect, Bifidobacterium adolescentis SPM0212, fecal harmful enzymes

E008

Effect of Lactic Acid Bacteria on D- and L-Lactic Acid Contents of *Kimchi*

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D-form of lactic acid is often found in fermented foods and excessive dietary intake of D-lactic acid may cause a metabolic stress in infants and patients. To determine the prevailing microorganisms involved in D-lactic acid accumulation in *kimchi*, D-/L-lactic acid synthesis profiles of the major lactic acid bacteria (LAB) in *kimchi* were analyzed and the relationship between D-/L- lactic acid contents and microbial populations was investigated during the fermentation. When cultured in a medium containing glucose, *Leuconostoc mesenteroides* and *Leuc. citreum* mainly synthesized D-lactate (75.28mM and 85.00mM, respectively) with small amount of L-form. *Leuc. gelidum* and *Leuc. inhae* showed a similar pattern with it. *Lactobacillus plantarum* and *Lb. brevis* converted glucose into a balanced mixture of D-/L-lactic acid (114.13/81.74 and 115.47/84.77 mM, respectively), while *Lb. casei* synthesized mainly L-lactic acid (185.36mM) and a very little amount of D-lactic acid (8.20mM). When *kimchi* was incubated at 8℃ or 22℃, the D-lactic acid (11-14mM) was over-produced than L-form (5-8mM), and leuconostocs was the leading genus during the period of D-lactic acid accumulation. Accordingly, it can be said that the main strains for D-lactic acid production in *kimchi* are leuconostocs and some of lactobacilli: leuconostoc is the major producer between the initial to mid-phase of fermentation and *Lb. plantarum* or *Lb. brevis* may boost D-lactic acid content at the late stage of acid accumulation.
Keywords: D-lactic acid, kimchi, acidosis, lactic acid bacteria, leuconostocs

E009

Character Analysis of Geubong and Campbell's Early Grapewine Produced by Commercial and Wild Yeasts

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Geubong and Campbell's Early grapewine were produced with the commercial yeast, EC1118 and the wild yeasts which are present in the grapes naturally. After wine making, the wine yields with Geubong and Campbell's Early grapes were 85% and 75%, respectively. Total acidities in the wines produced with wild yeasts were lower than those in the wines produced with the commercial yeast. Higher pH values and alcohol concentrations were obtained from the wines with wild yeasts. Alcohol concentrations in Geubong and Campbell's Early wines were 12.5~13.0° and 10.6~11.0°, repectively. In color analysis, lightness values (L^*) were 55.08~55.29 in Geubong wine and 54.01~54.25 in Campbell's Early wine. Redness values (a^*) and yellowness values (b^*) were 3.38~3.42 and -42.21~42.30 in Geubong's and 3.52~4.12 and -42.46~-42.72 in Campbell's Early's.

Keywords: Geubong, campbell's Early, wild yeast

E010

Production of Monacolin K and Citrinin on Various Media by *Monascus* Isolated from Various *Monascus* Fermentation Products

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Recently, *Monascus* is drawing many attentions from academia and industry because of its ability to produce bioactive compounds such as monacolin K and γ-butyric acid (GABA), etc. It also produces nephrotoxic and hepatptoxic citrinin, which concerns its application for commercial food production. To screen for the strains suitable for industrial production of functional foods, we isolated 17 *Monacus* sp. from various *Monascus* fermentation products and compared their productivity of monacolin K and citrinin on various media. Isolated starins produced different levels of monacolin K and citrinin on different media: 15.79~20.05 mg/L of monacolin K and ~255 ㎍/L of citrinin in submerged culture of monacolin K producing media, 1.27~2.69 mg/L and ~507.4 ㎍/L in YES medium, ~0.78 g/kg and ~9.7 mg/kg in *Monascus* red rice products prepared with isolated strains, and 0.21~0.27 g/kg and ~4.2 mg/kg on soy bean based media. In addition, the producibility of monacolin K and citrinin by each strain was dependent on media, suggesting that a strain for a certain application should be screened using the similar culture conditions of an application.

Keywords: Monascus, monacolin K, citrinin

E011

Myxochelin A, a Cytotoxic Compound from *Angiococcus disciformis* (Myxobacteria)

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In the course of screening for new anticancer antibiotics from myxobacteria, strain JW357 was found to produce a cytotoxic compound that was active against several human cancer cell lines. This strain was identified as *Angiococcus disciformis* by morphological and cultural characteristics. The cytotoxic compound was identified as myxochelin A, which was previously isolated as a siderophore from a culture broth of the same species of myxobacteria. This compound demonstrated significant cytotoxicity against certain human cancer cells with IC_{50} values ranging 2.85~5.84 μM. It was interestingly as active against mutidrug-resistant CL02 cells as against the sensitive parent cells (HCT15).

Keywords: Myxochelin A, cytotoxic, myxobacteria

E012

Purification and Chricterization of an Alginate Lyase from Marine Bacterium *Vibrio* sp.

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Alginate is produced as a structral polysaccharide by brown algae and as an extracelluar polysaccharide by ceartain bacteria. Alginate is an acidic. polysaccharide composed of 1-4 linked copolymer of alpha-D-mannuronic acid (M) and α -L guluronic acid(G). Alginate lyase is an enzyme that catalyzes the degradation of alginate by a beta-elimination mechanism at the new non reducing terminus, forming 4-deoxy-L-erythro-hex-4 enopyranosyluronate. In order to screen a strain that has alginate lyase activity, We have collected 20 samples from the south of Chonnam area and then samples were selected twice in modified M9 medium. Finally we isolated a microorganism which has the alginate lyase activity also identified this strain by 16s-rRNA sequence that named Vibrio. sp We optimized a culture condition of this strain ; M9 medium in 0.5% NaCl at 30℃. We purified and identified alginate lyase protein by SDS- PAGE to be a 40kD size protein and optimum condition of the enzyme is at pH 7 and 30℃ Temp 30℃. We analyzed alginate lyase products by TLC and counted lyases activity by DNS and TBA method.

Keywords: Alginate lyase, Vibrio.sp

E013

Purification and Characterization of an Extracellular Chitosanase Produced by *Bacillus* sp.

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For the enzymatic production of chitosan oligosaccharides from chitosan, a chitosanase-producing bacterium was pursued from the south and west sea shore mud. Microorganisms were screened in M9 medium plus chitosan and several colonies were isolated to have a capability to produce chitosan oligosaccharides. One sure strain was selected through more further screening processes. Analysis of products was carried out by thin layer chromatography. To estimate chitosanase activity the 3,6-dinitrophthalic acid method was adapted. The physiological conditions for a microorganism culture and chitosanase reaction were analyzed. Maximum enzyme activity was exhibited at pH 6.5 phosphate buffer including 40mM NaCl and 20mM $MgCl_2$ at 30℃. The enzyme activity increased by about 1.4-fold by the addition of 20mM $MgCl_2$. The best culture condition of this strain was 4-5 days incubation at 30℃ in a medium containing 0.5% chitosan, 1% tryptone, 1% NaCl in a rotary shaker. This strain was identified to the one of Bacillus sp. on the basis of the 16s-rRNA and gram positive test.
Keywords: chitosanase, Bacillus sp.

E014

The Characterization of *Kimchi* Fermented by *Lactobacillus sakei* IFT 002 as a Starter for Long Term Storage

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In this study, we developed the novel starter which could extend edible period of kimchi for one more month. Among the lactic acid bacteria which isolated from Kimchi, we selected the strain which had a superior characteristic as follows; high acid-tolerance, high salt-resistance, high growth rate at 20℃ and low growth rate at -1℃ and 4℃. The selective strain was identified as *Lactobacillus sakei* IFT 002. The inoculum size of *L. sakei* IFT 002 as a starter was 5×10^5 cfu/g in Kimchi and viable cells, acidity, pH were monitored during the fermentation at -1℃ for 3 months. As a result, the edible period of Kimchi fermented by *L. sakei* IFT002 was extended to month than Kimchi fermented by control strain(*Leuconostoc mesenteroides*). Therefore, *Lactobacillus sakei* IFT 002 could be suitable starter long term storage Kimchi fermentation.
Keywords: Kimchi, Lactobacillus, starter

E015

The Immune Activation of Fermented Rice Bran by the Novel Lactic Acid Bacteria, *Lactobacillus plantarum* IFT-K18

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Rice bran is composed of the aleurone layer of the rice kernel and some part of the endosperm and germ. It contains tocotrienols, γ-oryzanol, β-sitosterol, phytic acid, and myo-inositol. It has been known to stimulate immune responses. Fermented rice bran was obtained by Koji (*Aspergillus oryzae*)-saccharification, and then fermentation with *Lactobacillus plantarum* IFT-K18(IFT-K18). IFT-K18 was selected by screening for macrophage-activating lactic acid bacteria from 300 different cells isolated from Kimchi.In this study, we investgated the immune responses of fermented rice bran by the novel lactic acid bacteria, IFT-K18. By fermented rice bran treatment, the macrophage-activity and the production of nitric oxide, IL-10, and IL-12 were increased. The production of cytokines by fermented rice bran were increased to higher level than by saccharified rice bran treatment alone. In case of the nitric oxide production in RAW 264.7 cell, when stimulated LPS, nitric oxide level was lower by fermented rice bran treatment. It was thought that fermented rice bran modulated inflammatory responses.Macrophages are important regulatory and effector cells that play a central role in cell-mediated immunity. Fermented rice bran induced macrophage activation and the moderated production of its cytokines. This result suggests that fermented rice bran used in foods could have a beneficial effect in maintaining an immunological balance and increasing resistance to infections
Keywords: immune, Lactobacillus, Rice bran, fermentation

E016

Influence of Electric Pulse on Ethanol Production of Resting Cells of *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae was cultivated in the electric pulse and conventional condition of YPG medium containing 5g/L yeast extract, 5g/L peptone and 1M glucose for 72 hr. 72 hr old yeast cells were harvested by centrifugation at 5,000xg for 30 min and then washed with 25 mM phosphate buffer. The harvested cells were resuspended into 25 mM phosphate buffer, to which 1 M of glucose was added. Yeast cell density was adjusted to 1.6 as optical density at 660 nm. The yeast cells resuspended in reactor containing 25 mM phosphate buffer (pH 7.0) and 1 M glucose was cultivated in the electric pulse of DC 6 volts and conventional condition for 48 hr. The yeast pre-cultivate in the electric pulse of 6 volt produced relatively higher ethanol when cultivated in the electric pulse of 6 volt than the yeast cells grown in different conditions. It shows that the electric pulse may induce yeast cells to activate the metabolism of ethanol fermentation. Acknowledgement: This work was supported by a grant (Code # 20050401-034-750-142-04-00) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.
Keywords: Saccharomyces cerevisiae, electrochemical reactor, electric pulse, resting cell

E017

PHB Production from Carbon Dioxide with Anaerobic Bacterial Consortium Growing Electrochemical Bioreactor

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To produce PHB from carbon dioxide, anaerobic bacterial consortium was enriched in the medium containing 3g/L ammonium chloride, 10mM sodium bicarbonate, 2mM phosphate, 1ml trace mineral and 1g/L yeast extract. Carbon dioxide was recycled from reservoir to the bioreactor and bubbled into the bacterial culture to promote dissolving efficiency. No organic compound was added to the medium except yeast extract, which was used as a growth factor. During operation of the electrochemical bioreactor for 13 days, about 45 liter of carbon dioxide was assimilated into bacterial metabolism and the crotonic acid (PHB) extracted from the bacterial cells was 12 mg, which is not economic and possible that the PHB may be produced from carbon dioxide fixation. However, it is very possible that the PHB may be produced as a byproduct in consideration of carbon dioxide fixation for reduction of green house gas. The major goal of the electrochemical bioreactor is biological carbon dioxide fixation and minor goal is to produce polymerized compounds such as PHB, which can be left in store house without special container for a long time. However, the methane or acetic acid are difficult to be stored and required a special container for storage for a long time. We are trying to improve the bacterial growth, carbon dioxide fixation efficiency and PHB productivity.
Keywords: electrochemical bioreactor, carbon dioxide fixation, PHB, Crotonic acid

E018

Development of Miniaturized Cultivation Method of Filamentous *Aspergillus terreus* Cells for Mass Screening of Lovastatin High-yielding Mutants and Transformants

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For rapid improvement of the productivity of producer strains, it is crucial to develop a miniaturized cultivation method for a large screening of high-yielding mutants. From our previous experiments, it was found that maximizing the biosynthetic capability of lovastatin-producing fungal cells was very difficult in a miniaturized tube culture(i.e. 10ml tube-culture), because gas-liquid oxygen mass transfer rates always became significantly diminished due to the filamentous structure of the producing mycelium. In this paper, we are going to report a cultivation method that could facilitate oxygen transfer into the fermentation broth to a great extent, even in the miniaturized tube-cultures. It was observed that, for maximum production of lovastatin, the producers should be proliferated in condensed filamentous forms in miniaturized growth cultures, so that optimum amounts of highly active cells could be transferred to the production culture-tube as reproducible inoculums. Under this highly controlled fermentation conditions, compact-pelleted morphology of optimum size(less than 1mm in diameter) was successfully induced in the miniaturized 10ml tube of the production culture, which turned out prerequisite for the maximal utilization of the producers' physiology leading to significantly enhanced production of lovastatin.
Keywords: Miniaturized Cultivation Method, *Aspergillus terreus*, lovastatin, fermentation, morphology

E019

Studies on Production of Exopolysaccharides (EPS) and Simultaneous Accumulation of Antidiabetic Agent in Fermentation Broth in Suspended Cultures of *Schizophyllum commune* Mycelium

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Studies on the production of exopolysaccharide(EPS) in suspended cultures of Schizophyllum commune mycelium has been carried out. At the same time, we also investigated the possibility of the simultaneous accumulation in the culture broth of an antidiabetic agent contained in a specific component of a fermentation medium(PM). For the purpose of optimizing fermentation conditions for the enhanced production of EPS together with maximal accumulation of the antidiabetic agent, carbon/nitrogen ratio(C/N ratio), KH_2PO_4 concentration, and inoculum size were investigated in both shake-flask and bioreactor cultures. C/N ratio was found to have the most significant effect on the EPS biosynthesis, with the maximal EPS production of 8g/L at the C/N ratio of 20:1(i.e. glucose plus yeast extract vs. soytone peptone = 20:1). Disappointingly, however, at the C/N ratio of 20:1 above, a small amount of unmetabolized fructose was found to still remain in the fermentation broth, even though the antidiabetic agent was effectively concentrated in the fermentation broth. This phenomenon, which made it somewhat difficult to separate the antidiabetic agent from the fermentation broth, was successfully overcome by adopting a fed-batch fermentation process, resulting in a complete consumption of the residual fructose left in the batch operation mode above.
Keywords: EPS, Schizophyllum commune, Fed-batch, C/N ratio

E020

Anti-Angiogenesis and Cytotoxic Compounds from Marine-Derived Actinomycetes

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Angiogenesis is the formation of new capillary blood vessels from existent micro vessels and also involves differential recruitment of associated supporting cells to different segments of the vasculature. Because tumor angiogenesis caused by angiogenic inducers is the most critical factor in the growth of solid tumors, as well as their invasion and metastasis, early control of angiogenesis may be a promising therapeutic strategy for the related diseases. As part of our ongoing program to develop the biomedical potential of marine microorganisms, we have focused considerable attention on the marine actinomycetes. From the bioactive isolates several new actinomycetes have been identified and a variety of bioactive molecules were characterized. We present here the discovery of anti-angiogenesis and cytotoxic compounds from marine-derived actinomycetes. The isolation, structure determination, and anticancer activity of the compounds from marine actinomycetes will be presented.
Keywords: Angiogenesis, Cytotoxic, Actinomycetes, Anticancer activity

E021

The Research Regarding the Physiological Active Materials of Oceanic Microbe Origin

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For the reseach of the natural marine antioxidant, an antioxidnat-producing bacterium was isolated from seawater in Jeju island. The isolated stain, SC2-1 was colonies orange or yellow. The isolated strain, SC2-1 was Gram-positive, catalase positive, oxidase positive, motile and small rods. The strain utlized sucrose, dextrosefructose, mannitol and maltose as a sole carbon and energy source and Na+ required for growth. This bacterium was identified based on morphlogical, biochemical characteristics and 16S rDNA sequencing, cellular fatty acids analysis, and nemed *Exiguobacterium* sp. SC2-1. The radical scavenging activity of the culture supernatants was determined by DPPH method. It might be explained by stable radical (DPPH) scavenging effects and by weak activity of the inhibit superoxide radical scavenging ability. Hydroxyl radical activity of the supernatant of *Exiguobacterium* sp. SC2-1 was 73%. The optimum culture conditions for production of antioxidant were 25℃ and pH 7.8 and NaCl concentration were 4%. The modified optimal medium compositions were maltose 2.5% (w/v), yeast extract 1.5% (w/v) and KH_2PO_4 0.05% (w/v). Free radical scavenging activity of under optimal culture conditions were 93%.

Keywords: DPPH, 16S rDNA, Hydroxyl radical, Optimal condition

E022

Isolation and Identification of Antioxidant Producing Marine Sources Actinomycetes and Optimal Medium Condition

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For the research of the natural marine antioxidant, several bacteria were isolated from the coast of Je-ju in Korea. An actinomycetes strains, S-1, containing antioxidant component was isolated from sea sand and was identified to a genus level 16S ribosomal DNA sequence analysis. From these results and other characteristics described in the Bergey's Manual, this strain was identificated as a *Nocardiopsis* sp. Strain S-1 showed high activity of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging. The hydroxyl radical scavenging ability of *Nocardiopsis* sp. S-1 broth was 53%. Nutritional and cultral conditions for the production of antioxidant by this organism under shake-flask conditions have optimized. Similary initial medium pH 7.6, incubation temperature of 25℃, sodium chloride concentration 2.5% and incubation time of 8day were found to be optimal. The optimum conditions for the production of antioxidants for carbon, organic and inorganic nitrogen sources were galactose and yeast extract. The DPPH free radical scavenging ability of *Nocardiopsis* sp. S-1 cultural supernatant was 88% from optimum culture condition.

Keywords: Actinomycetes, 16S ribosomal DNA, Nocardiopsis

E023

Thioesterase II Activity Dependent Secretion of (R)-3-Hydroxydecanoic Acid by *Pseudomonas aeruginosa* BM114 and its Inhibition by 2-Bromooctanoic Acid

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Polyhydroxyalkanoic acid (PHA) synthesis related metabolites secreted by or in *Pseudomonas aeruginosa* BM114 cells grown in minimal medium were analyzed for the cells grown at 30 and 37°C, respectively. The cells grown on fructose at 30°C for 144 h accumulated PHA as much as 30.2wt% of the dry cells whereas the cells grown faster for 48 h at 37°C accumulated only 4.4wt%. An addition of 2 mM 2-bromooctanoic acid to the medium at 30°C suppressed PHA accumulation down to 6.2wt% as well as the secretion of (R)-3-hydroxydecanoic acid (3HD). The ratio of 3HD secreted to polymerized was found to be ~1 for fructose-grown cells whereas ~5 for decanoate-grown cells which accumulated a blend of 46mol% poly-3-hydroxybutyrate (PHB) and 54mol% medium-chain-length (MCL)-PHA at 30°C but secreted only 3HD as major metabolite. Thus, BM114 thioesterase II activity is considered higher in decanoate grown cells than in fructose grown cells. When BM114 cells were grown on decanoate, an addition of 1 mM 2-BrOA inhibited the formation of MCL-PHA by 70% but stimulated the formation of PHB 1.7 times. Thus the blocking of 3-hydroxyacyl-CoA's passing throuth PhaG activated another pathway to consume the acyl-CoA in different forms. However, 10 mM concentration was required to inhibit the secretion of 3HD by 70%, suggesting the higher activity of thioesterase II than in fructose grown cells. Other carboxylic acids such as octanoate, nonanoate and undecanoate also secreted 3HD as the major 3-hydroxyacid.

Keywords: polyhydroxyalkanoic acid (PHA), (R)-3-hydroxydecanoic acid (3HD), Thioesterase II, 2-Bromooctanoic acid (2-BrOA), PhaG, Pseudomonas aeruginosa BM114

E024

Production of α-L-Arabinofuranosidase by Fed-batch Fermentation of Recombinant *Escherichia coli*

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A gene encoding α-L-Arabinofuranosidase was cloned from the hyperthermophilic microorganism, *Thermotoga maritima,* and expressed in recombinant *E. coli*. This enzyme hydrolyzes arabinan or arabinoxylan to release L-arabinose, which is a food supplemental pentose sugar to be used with sucrose. In order to evaluate the expression efficiency at the time of induction, cells were induced with 0.1 mM IPTG at two different cell grow phases(initial or mid-exponential phase). The pHCETAF plasmid was also used as a constitutive expression tool to compare productivity with an induction system. When cells were induced at the initial exponential phase (8.4 g/L DCW), the dry cell weight and the maximal unit per gram (DCW), unit per liter (culture solution) were 40.8 g/L, 319 units/g and 12630 units/L, respectively. When cells were induced at the mid-exponential phase (29.7 g/L DCW), the biomass and enzyme activities were 45.1 g/L, 338 units/g and 14892 units/L, respectively. When a constitutive pHCETAF plasmid was used, the biomass and enzyme activities were 36.0 g/L, 262 units/g and 9152 units/L, respectively. As a conclusion, a fed-batch culture showed higher DCW and enzyme activity rather than a batch culture, an induction system showed higher DCW and enzyme activity than constitutive system and the mid-exponential induction was better than the early induction.

Keywords: α-L-Arabinofuranosidase, Fed-batch Fermentation, induction, arabinan, pHCETAF

E025

Optimal Condition of Poly-ɣ-glutamic acid Production by Poly-ɣ-glutamic acid Hyper-producing Mutant (PBP) isolated from Chungkookjang

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Chungkookjang is a famous traditional soybean product fermented by various Bacillus species. ɣ-polyglutamic acid(ɣ-PGA), an amino acid polymer that consists of only D-glutamic acid or D- and L-glutamic acid polymerized through ɣ-glutamyl bond, is produced by several Bacillus species contained in Chungkookjang. ɣ-PGA is biodegradable, edible and non-toxic toward human and the environment. It has applications in a broad range of fields including food, cosmetics, medicine and water-treatment. A mutant of Bacillus subtilis chungkookjang, PBP, was seleced with UV radiations and N-methyl-N'-nitro-N-nitrosoguanidine were selected. The productivity of ɣ-PGA by PBP were 3.5 times higher than B. subtilis CBP. In this study, optimization of culture conditions for ɣ-PGA production of PBP mutant was investigated in liquid culture. The optimum composition of medium for ɣ-PGA production was 7% sucrose, and 1.5% MSG, and the optimum pH was 7.5. And the optimum culture condition was at 37℃ for 60h under 200 rpm with shaking.
Keywords: poly-gamma-glutamic acid, Chungkookjang

E026

Characteristics of Citrus By-Product Ferment Using Probiotics as Starter

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In this study, we investigated the biological activity of antioxidant and antibacterial activity of citrus by-product ferment. Among the six probiotic bacteria, Bacillus subtilis and Saccharomyces cerevisiae had the highest antioxidant activity. Hot water extracts from citrus by-product of ferment were screened for antibacterial activity fish pathogenic bacteria by paper disc method. Among the various hot water extracts, the Bacillus subtilis, Saccharomyces cerevisiae, Pediococcus pentosaceus, Lactobacillus rhamnosus, Lactobacillus plantarum, Enterococcus faecium showed relatively strong antibacterial activities in the order. The reducing activity on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and O^{2-} and ·OH radical scavenging potential were sequentially screened, in search for antioxidant activities of citrus by-product ferment.
Keywords: Citrus by-product ferment, six probiotics, antioxidant activity, antibacterial activity

E027

Optimization of Gibberellin Production by *Fusarium prolifertum* KGL0401 and its Involvement in Waito-c Rice Growth

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Gibberellins(GAs) are a large family of isoprenoid plant hormones, that control many aspects of plant development, including seed germination, shoot elongation, flower formation and development, fruit-setting, seed development, sex determination, and the chlorophyll content. *Fusarium proliferatum* KGL0401 was previously isolated from *Physalis alkekengi* var. *francheti* plant roots and exhibited higher GA productivity than wild type *Gibberella fujikuroi*. The aim of this work was to find out an optimal culture condition for GA production. Various carbon(fructose, glucose, lactose, maltose, sucrose) and nitrogen(KNO_3, urea, glycine, $NaNO_3$, NH_4Cl) sources were used for this study. GAs activities were analysed by gas chromatography and mass spectrometry(GC-MS). The highest yield of GA_3 was found in the growth medium supplemented with sucrose as carbon source and NH_4Cl as nitrogen source. The optimum carbon-nitrogen concentration for GA_3 production was found to be 50mM:17mM. Supernatant was prepared from the culture fluid of *F. proliferatum* KGL0401 cultured for 7 days at 30℃ and the 10 μl supernatant was treated with 2 leaf-rice seedling.
Keywords: Gibberellins, Fusarium proliferatum KGL0401

E028

Screening of Antioxidative and Antibacterial Activity from Hot Water Extracts of Indigenous Plants, Jeju-Island

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In this study, we investigated the biological activity of antioxidant and antibacterial activity of Indigenous Plants, Jeju-Island., which, using water were extracted. The reducing activity on the 1,1-diphenyl-2-picrylhydrazyl(DPPH) radical and O^{2-} and ·OH radical scavenging potential, in search for antioxidation activities of Indigenous Plants, were sequentially screened. Among the ten plant parts, Prunella vulgaris var. aleutica Fernald. flower had the highest antioxidative activity. Hot water extracts of ten indigenous plants were screened for antibacterial activity 13 fish pathogenic bacteria by agar diffusion method. Among the various Hot water extracts, the Prunella vulgaris var. aleutica Fernald, Gleichenia japonica Spreng, Microlepia marginata(panzer) Christ., Perilla frutescens var. japonica Hara. showed relatively strong antibacterial activities in the order.
Keywords: antioxidant, antibacterial activity, indigenous plants, Hot water extracts

E029

Two New Pulvinatal Analogues Isolated from the Mycelial Culture Broth of Mushroom Strain *Cyathus stercoreus* and their Free Radical Scavenging Activities

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Free radical scavengers can be used for various purposes such as cosmeceuticals, nutraceuticals, and medicines, because of their scavenging abilities against free radicals. In our screening process of Korean wild mushrooms, the ethyl acetate soluble fraction of fermented *Cyathus stercoreus* exhibited significant free radical scavenging properties against ABTS+, DPPH and superoxide radicals. From this mushroom strain, we previously reported two new polyketide type free radical scavengers, pulvinatal(CS1) and demeoxylated-pulvinatal CS2). Further investigation of the active fractions of the EtOAc extract gave two more new pulvinatal analogues, CS3 and CS4. The structures of the compounds were determined by various spectroscopic methods including NMR and HREIMS. The molecular formulas of C3 and C4 were $C_{17}H_{14}O_8$ (HREIMS: cacld.346.0689, found. 346.0688) and $C_{20}H_{20}O_8$ (HREIMS: cacld. 388.1158, found. 388.1159), respectively. The chemical structures of CS3 and CS4 showed the replacement of methoxy group substituted to the C-8 of pulvinatal by hydroxyl and propanoxyl groups, respectively. In the DPPH and $ABTS^+$ radical scavenging activity assays, C3 and C4 showed higher radical scavenging activities than well-known antioxidants, trolox and BHA. Therefore, these new pulvinatal analogues are also expected to be potent antioxidants.
Keywords: Free radical scavengers, Mushroom, activity-guided fractionation, polyketide

E030

Development of Electrochemical Bioreactor for Continuous Culture of *Saccharomyces cerevisiae*

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A 1500 ml volume of electrochemical bioreactor was designed, in which two electrodes were parallel equipped on the bottom. Both electrodes were made of titanium plate of which diameter was 100 mm. Distance between two electrodes was adjusted to 10 mm. DC 6.0 volts of electric potential was charged to the electrodes and electric poles were reciprocally exchanged at the intervals of 60 seconds. The electric pulse was generated by the reciprocal exchange of electrode poles, by which the electrode corrosion was completely protected. Higher concentration of glucose was added to medium reservoir to remove substrate-limiting factor. The medium containing 200 g/L glucose, 5g/L yeast extract and 5g/L peptone was continuously added to the bioreactor at the speed of 40 ml/hr. The outflow was not recycled and the yeast cells were not additionally inoculated. The ethanol productivity was 1.3 – 1.5 times higher in the bioreactor to which electric potential was charged than the conventional bioreactor. Acknowledgement: This work was supported by a grant (Code # 20050401-034-750-142-04-00) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.
Keywords: Saccharomyces cerevisiae, elecrochemical bioreactor, Continuous culture, electric pulse

E031

Two Diketopiperazines as the Fermented Metabolites of *Stereum ostrea* and Their Tyrosinase Inhibitory Activity

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Melanin biosynthesis inhibitors are useful not only for the materials used in cosmetics as skin-whitening agents but also as a remedy for disturbances in pigmentation. Tyrosinase inhibitor has been the target of screening for anti-hyperpigmentation agents for depigmentation drugs and whitening cosmetics, because tyrosinase catalyzes the initial steps of melanin biosynthesis. In our screening process of Korean wild mushroom strains, the ethyl acetate soluble fraction of mycelial culture broth of *Stereum ostrea* showed strong tyrosinase inhibitory activities. From this mushroom, two compounds, SO1 and SO2 were isolated through silica gel c.c., Sephadex LH-20 c.c., and reverse- phased HPLC, sequentially. The structures of the two compounds were studied on the basis of NMR spectroscopic analyses and the chemical structures of these two compounds were identified as the type of diketopiperazines; these compounds showed the chemical structures resulted by the condensation reaction of two amino acids, tyrosine and proline for SO1(tyrosyl-prolyl diketopiperazine), and phenylalanine and proline hydroxylated in 4-carbon for SO2(phenylalanyl-hydroxyprolyl diketopiperazine). These compounds showed tyrosinase inhibitory activity against mushroom tyrosinase.
Keywords: Mushroom, Stereum ostrea, tyrosinase inhibitor, diketopiperazine

E032

Gene Dosage Effects of Glucose-6-phosphate Dehydrogenase and 6-Phosphogluconate Dehydrogenase Genes on NADPH Concentration in *S. lividans*

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The pentose phosphate pathway (PPP) and glycolysis comprise most central metabolic pathways in the primary metabolism. Most antibiotic biosynthetic pathways involve some reductive steps in which NADPH is necessary as a reducing power. NADPH is produced by two enzymes in the pentose phosphate pathway, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Thus enhancing the reducing power in the cell can be an effective way to induce overproduction of secondary metablites. From the genomic sequence data of *S. coelicolor*, the genes encoding the glucose-6-phosphate dehydrogenase (*zwf1*, *zwf2*) and the 6-phosphogluconate dehydrogenase (*zwf3*) were cloned by PCR. Various combinations of *zwf1, zwf2,* and *zwf3* gene were constructed in an *E. coli-Streptomyces* shuttle vector and introduced into *S. lividans*. The intracellular concentrations of NADPH of various transformants were analyzed by HPLC, which clearly showed that coexpression of *zwf1-zwf3* or *zwf2-zwf3* induced higher concentration of NADPH in the cells. [Supported by grant No.R01-2006-000-10860-0 from the Basic Research Program of the Korea Science & Engineering Foundation]
Keywords: Pentose phosphate pathway, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase

E033

A Manufacturing Method of Red Ginseng Oil and its Functional Study

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Panax ginseng C. A. Meyer is one of the most widely used herbal drugs and known to have a wide range of therapeutic and pharmacological uses. Phamacological effects of ginseng have been demonstrated in cardiovascular, immune system, antistress, and antioxidant activity. Ginseng contain many active components including ginsenosides, polysaccarides, peptides, polyacetylenic alcohols, and fatty acids(Lee FC, 1992). Moreover, Red ginseng is more phamacologically active than white ginseng due to the chemical constituents occuring during steaming treatment. The fat-soluble fraction of Red ginseng has more potent activities, such as antioxidant and antithrombosis. Thus, in this study we evaluated the method to develop Ginseng oil containing high content of phytochemicals by microbial enzymes. To select the optimum extraction process of Red ginseng with oils, the oxidation, rancidity, and antioxidant activities using different enzymes were measured. We found that Red ginseng/soybean oil extracted for 2 weeks at 40℃ after 0.5% cellulase treatment had higher antioxidant activity than the other conditions. It also showed no rancidity during the storage and an enhanced flavor. However, Red ginseng/olive oil and white ginseng oil had little functional activities. We also analysed vitamin E and A by GC and HPLC and found that Vt.E was increased by enzyme treatment in the oil. This is the first report that Red ginseng oil extracted by enzyme treatment has various beneficial effects.

Keywords: Red ginseng, microbial enzyme, antioxidant, oil

F001

Phenotypic and Genotypic Characterization of *Salmonella enterica* serovar. paratyphi B Isolates from Human Sources in Jakarta, Indonesia

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Salmonella enterica serovar Paratyphi B (O1,4,[5],12:Hb:1,2) can cause either paratyphoid fever or self-limited gastroenteritis in humans according to their ability of *d*-tartrate fermentation. The *d*-tartrate fermenting variant (dT^+) is called biotype Java and has been currently recognized as an emerging problem world wide. During the typhoid fever surveillance study at Jakarta, Indonesia between 2000 and 2002, forty-three *Salmonella* group B(O1,4,[5]) isolates were collected and discriminated 12 *S.* paratyphiB var. Java isolates by confirming traditional serotying method after screening of fliC(Hb)-specific PCR. Twenty-five isolates of 43 *Salmonella* group B isolates showed resistance to any of the 8 antibiotics and 4 of 12 *S.* paratyphiB var. Java isolates contained *Salmonella* genomic island 1 (SGI1), found in several *Salmonella* serovars and *E. coli*, which provides the multidrug-resistant phenotype (ACSSuT). Also genotypic analyses of 12 *S.* paratyphiB var. Java isolates were investigated by multilocus sequence typing (MLST) method using by 7 loci (*aroC, dnaE, hemD, hisD, purE, sucA* and *thrA*) but all isolates showed the same sequence type (ST) 43, reported as *S.* paratyphiB var. Java from Denmark. Among these 12 isolates, the genes encoding the effector proteins sopE1 and avrA, which are usually found in systemic pathovars, were not present.

Keyword: Salmonella

F002

The Identification of Novel *Pleurotus ostriatus* dsRNA Virus and Determination of the Distribution of Viruses in Mushroom Spores

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Double - stranded RNA containing fungal viruses have been reported in all kinds of fungal genera including yeast and mushrooms. Many different types of mycoviruses are appeared to be infected in Pleurotus species. In this paper we report that virus particles were isolated and partial cDNA clone was obtained from Peurotus Shin-Nong strain. We also examined the distribution of virus in spores by using specific primer from the partial cDNA sequence. Double - stranded RNA and virus particles were identified in Pleurotus ostriatus cultivar Shin-Nong in Korea. Isometric virus particles with a diameter of 33nm were purified, which are similar to other Pleurotus viruses reported previously. The particles contain 3 dsRNAs, designated RNA -1(2.5Kb), 2(2.0Kb), 3(1.8Kb). A non-encapsidated dsRNA about 8.0Kb also identified. partial cDNA from RNA-1 was cloned and sequence analysis revealed that this gene codes for RdRp. The comparison of the sequence from partial cDNA clone showed 35% amino acid homology with C-terminal end of RdRp gene of Heliocbasidum mompa virus and Rosalinia necatrix virus. Specific primers designed from partial sequences successfully amplified RT-PCR products from mycelium and single spore culture. We used this primer to determine the distribution of viruses in spores, among 93 different single spore cultures, specific RT-PCR products were identified in 25 cultures, indicating that about 27% of basidiospores contains viruses.

Keywords: Pleurotus virus, ds RNA, fungal virus, cDNA, virus distribution

F003

Transcriptional Organization of the *gum* Gene Cluster in *Xanthomonas oryzae* pathovar *oryzae*

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Genome sequence analysis of *Xanthomonas oryzae* pv. *oryzae* KACC10331 provides insight into the *X. oryzae gum* gene cluster that is composed of 14 open-reading frames (ORFs), designated *gumB*, *-C*, *-D*, *-E*, *-F*, *-G*, *-H*, *-I*, *-J*, *-K*, *-L*, *-M*, XOO3167, and *-N*. We analyzed the transcriptional linkage of the *X. oryzae gum* gene cluster by using areverse transcription-polymerase chain reaction (RT-PCR). Analyses of the *gum* gene cluster by RT-PCR with the wild-type and mutant strains, which carried a deletion of the promoter-like region upstream of *gumB* or an insertion of the *rrnB* transcriptional terminator into the *gumF* gene, revealed that the ORFs of this gene cluster were transcribed as polycistronic mRNA, from *gumB* to *gumN*, and the secondary promoter was located upstream of *gumG*. Taken together, these results suggest that the genes of this cluster constitute an operon expressed from overlapping transcripts.

Keywords: Xanthomonas oryzae pathovar oryzae, gum gene cluster, operon, RT-PCR

F004

Molecular Cloning and Characterization of MnP cDNAs from *Polyporus brumalis*

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The white-rot fungus, Polyporus brumalis(KFRI 20912) strain in Korea, has a resistance on the treatment with 250 uM DBP. In the medium containing 100 uM DBP, 90 % of DBP was degraded after 12 days of incubation. The MnP activity was gradually increased in the DBP treated groups. New 6 MnP genes have cloned from Polyporus brumalis for the purpose of understanding the physiological role of MnP during the degradation of DBP by white rot fungi. In MnP proteins deduced from cDNAs, all of them have similar properties in the size of ORF (360 to 365) and the pI (4.0 to 4.8), while identities of their amino acid sequences range from 62 to 96%. This suggests that the diversity in amino acid sequences may reflect differences in enzyme properties and biological functions although the analyzed MnPs are in the same families. In RT-PCR analysis all the MnP genes were highly expressed in the shallow stationary culture (SSC) liquid medium. Further analysis of the six MnP genes will be discussed in terms of the degradation of aromatic xenobiotics.

Keywords: Polyporus brumalis, white-rot fungus, MnP, DBP

F005

Retroviral Element of RNF19 Gene : Structure, Expression, and Evolutionary Conservation

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RNF19 gene located on human chromosome 8q22.2 has showed 4.4 kb transcript and expressed ubiquitously in various tissues. RNF19 gene coding dorfin protein that carry out E3 ubiquitin ligase, and highly conserved in pig, dog and cattle. RNF19 gene containing MaLR (mammalian LTR-retrotransposon) element in the first intron. Here we found its alternatively spliced transcript variants which derived from MaLR insertion. The MaLR-derived promoter transcripts are detected as two different types in all tissues examined, while breast tissue only showed three variant types. Reporter gene assay of the promoter activity of MaLR element on RNF19 gene indicated good activity in human colon carcinoma cells (HCT-116). These findings suggest that the MaLR element acquired the role of transcriptional regulation of RNF19 gene in various human tissues during primate evolution.
Keywords: RNF19, Dorfin, MaLR, LTR, HERV, retroelement

F006

Application of Realtime RT-PCR Analysis for the Dissection of HERV-W Env Elements

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HERVs (Human endogenous retroviruses) and LTR (long terminal repeat) - like elements are dispersed over 8% of the whole human genome. There are at least 22 independent HERV families within the human genome, which originated from germ-cell infection by the exogenous retrovirus during primate evolution. Elucidation of expression pattern in HERV elements should provide information about fundamental cellular activities and the pathogenesis of multifactorial diseases such as cancer and autoimmune disease. HERV-W env gene is related to multiple sclerosis, and has potential roles for normal differentiation of human villous cytotrophoblast into syncytiotrophoblast. HERV-W env gene was expressed differentiallyin human tissues. Especially, it was highly expressed in human placenta. This phenomenon indicates HERV-W env gene have the different roles in each tissues. Here, we applied realtime RT-PCR for detection of its expression in various human tissues. We also analysed such amplification using cancer cells and monkey tissues, and discussed in relation to physiological function.
Keywords: HERV, LTR, HERV-W, env gene, retrovirus, retroelement

F007

The Impact of Endogenous Retrovirus (ERVs) in Human Genome

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ERVs are the unique exterior elements which had been originated from germ line infection of ancient exogenous retroviruses. They had been regarded harmful elements for human genome. However, the results of human genome project gave rise to a question why human genome allowed many ERV elements compared to protein coding regions. To reveal the specific role of ERV elements, bioinformatic and evolutionary analyses were used. Totally, 67 genes were revealed that their transcript start site were provided by the ERV elements. Comparison of ERV gene and non-ERV gene showed the different trend of function and process by gene ontology analysis. Possible gene data sets of human, orangutan, macaca, rat, and mouse were compared for different usages of ERV elements. Different ERV elements were applied in different species for supplying the transcript start sites. From our analysis, we proposed the "remodeling hypothesis of ERV elements for host genome". During the species differentiation from common ancestor, many kinds of viral agents could invade the host genome. However, winner who survived from a fierce struggle for existence under the invasion of infective elements could acquire the privilege of using the outside resources for the promotion of their fitness through the remodeling of ERV elements.
Keywords: ERV, LTR, retroelement, HERV

F008

Bioinformatic Discovery of Transposable Elements Expression in Human Cancer

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Transposable elements are the most abundant interspersed sequences in human genome. It has been estimated that approximately 45% of the human genome comprises of transposable elements. Most of transposable elements are transcriptionally silent in human normal tissues, however, some of transposable elements have been found to be expressed in placenta tissues and cancer cell lines. Recent studies have shown that transposable elements could affect coding sequences, splicing patterns, and transcriptional regulation of human genes. In the present study, we investigated the transposable elements in relation to human cancer. Our analysis pipeline adopted for screening methods of the cancer specific expression from human expressed sequences. We developed a database for understanding the mechanism of cancer development in relation to transposable elements. Totally, 999 genes were identified to be integrated in their mRNA sequences by transposable element. We believe that our work might help many scientists who interested in cancer research to gain the insight of transposable element for understanding the human cancer.
Keywords: bioinformatics, cancer, Transposable element

F009

Functional Analysis of the *csp*-like Genes from *Corynebacterium glutamicum* Encoding Homologs of the *Escherichia coli* Major Cold-Shock Gene *cspA*

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Three *csp*-like genes were identified in the *Corynebacterium glutamicum* genome and designated *cspA, cspB,* and *cspA2*. The genes *cspA* and *cspA2* encode proteins comprising 67 amino acid residues, respectively, and they share 83% identity with each other. Identity of those proteins with *Escherichia coli* Csp proteins was near 50%. The *cspB* gene encodes a protein composed of 127 amino acids, which has 40 and 35% sequence identity with CspA and CspA2, respectively, especially at its N-terminal region. Analysis of the gene expression profiles using transcriptional *cat* fusion identified not only active expression of the three genes at physiological growth temperature of 30°C but also growth phase dependent expression with the highest activity at late log phase. The promoters of *cspA* and *cspA2* were more active than that of *cspB*. The expression of the two genes increased by 30% after a temperature downshift to 15°C and such stimulation was more evident in late growth phase. In addition, the *cspA* gene showed DNA-binding activity *in vivo* and the activity increased at lower temperature. Presence of *cspA* in multicopy hindered growth of the host *C. glutamicum* cells at 20°C, but not at 37°C. Taken together, these data suggest that *cspA, cspB,* and *cspA2* perform functions related to cold shock as well as normal cellular physiology. Moreover, CspA and its ortholog CspA2 may perform additional functions as a transcriptiona regulator.
Keywords: Corynebacterium glutamicum, csp, cold shock

F010

Expression of Heterologous Protein, β-galactosidase in *Leuconostoc citreum*

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Leuconostoc citreum isolated from kimchi proved to harbor a small cryptic plasmid pNS75. In order to develop an expression system for the heterologous genes in *Leuconostoc citreum*, we were previously reported *Leuconostoc - E. coli* shuttle vector system based on pLeuCM. The β-galactosidase gene(β-gal) of *Lactobacillus plantarum* was cloned in pLeuCM, constructed pLGal and expressed either in *E. coli* or *Leuconostoc citreum* 95. In *Leuconostoc citreum*, transformants carrying the pLGal were easily detectable by the appearance of a blue colony on a X-gal-containing medium and also by the growth on a medium containing lactose as a sole carbon source. These results show that the pLGal constructed in this study could be used as a selection marker for foreign-gene expression in *Leuconostoc* sp.
Keywords: Leuconostoc citreum, lactic acid bacteria, β-galactosidase, expression

F011

The Genetical Evidences of Gene Transfer Mechanisms Regulate by Polyphosphate Kinase and it's Relating Genes in *E. coli*

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Inorganic polyphosphate (polyP), a linear polymer of hundreds of phosphate residues linked by high-energy phosphoanhydride bonds, is having been found in all microbes, fungi, plants and animals examined. Several biological functions have been studied for phosphate and energy reservoirs with obvious advantage over Pi and ATP, a substitute for ATP for certain suger kinases and an association with poly-β-hydroxybutyrate and Ca^{2+} in a membrane domain found in transformation cell. In *E.coli* an enzyme responsible for polyP synthesis is the polyphosphate kinase(ppk), exopolyphosphatease(ppx), which hydrolysed the terminal residues of poly P, was discovered at same operon. In order to observed the genetical effect of ppk to the gene transfer mechanism(s), it was possible to determined transformation efficiencies not only to PPK deletion mutants but also the other various regulatory genes. This study suggests that, the *ssp*A(Stringent starvation protein A) and *hns* (histone-like protein) expression pattern altered ppk/ppx deletion mutant against wild type *E.coli* MG1655. Dependent on activities of ppk, the transcriptional activities of *ssp*A and *hns* altered significantly. Results proved that the gene transfer rate was also changed dependant on formation of inorganic polyphosphate. Accordingly to there genetical results, it is first time to show that the complex of poly P / PHB / Ca2+ may play an essential role to gene transfer *in vivo*
Keywords: polyphosphate kinase, PHB, Ca^{2+}, polyphosphate, transfermation

F012

Characterization of Replication-Competent Porcine Endogenous Retrovirus Class B Molecular Clone

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Xenotransplantation from pigs offers the chance to alleviate the shortage of organ supply from human being. PERV released from porcine cells infect human cells in vitro and PERVs are not eliminated due to their presence in germ line DNA. Three replication-competent, gammaretrovirus subgroups of PERV (PERV-A, -B, and –C) have been identified in the genomic DNA of pigs. PERV-A and PERV-B infect human cells, pig cells, and some other species, whereas PERV-C infection is mainly restricted to pig cells. A PERV-B molecular clone was constructed from bac clones containing PERVs genomes and its replication-competency was partially characterized. Pol region of PERV-B was detected in the genomic DNA of 293T cells transfected with the molecular clone B indicating that its DNA was integrated into the genomic DNA of 293T cells. PERB-B specific DNA also detected in the genomic DNA of 293T cells infected with media applied for culturing 293T cells transfected with molecular clone B. 293T cells transfected with molecular clone B were maintained and the tissue culture fluids were collected every five days for 80 days. PERV-B derived nucleic acid was most strongly detected at 62 days post-transfection in the collected media performed by RT-PCR and real time PCR. These results suggested that replication-competent PERV-B was produced from the 293T cells transfected with molecular clone B.
Keywords: xenotransplantation, PERV, molecular clone

F013

Characterization of an Inducible Laccase from *Cryphonectria parasitica*

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The chestnut blight fungus, *Cryphonectria parasitica*, and its hypovirus are a useful model system to study the mechanisms of hypoviral infection. To demonstrate value of laccase activity as a marker for CHV1 effects on *C. parasitica* , it was prompted to further investigate the laccases of this fungus. In particular, deletion of *Lac*1 revealed a seconed extracellular laccase (LAC3), a redundancy suggesting an important role for the extracellular laccase of this fungus. A novel gene, *lac*3, encoding an inducible laccase was cloned from a genomic library of *C. parasitica* using a probe which was prepared by PCR using degenerated primers based on the copper binding region of fungal laccase. The sequence of the *lac*3 gene revealed that it contained six introns, and the transcription initiation site was determined to be 214bp upstream of the start codon. The deduced amino acid sequence consisted a putative leader peptide of 18 amino acids long and a mature protein was estimated to be the molecular mass of 62 kDa containing four copper binding regions. In addition, the gene organization appeared to be highly similar to that of a four copper binding regions within fungal laccases. Northern blot analysis of the *lac*3 gene revealed that the *lac*3 was induced by copper and tannic acid but not by other well-known lignin-related inducers such as 2,5-xylidine and ferulic acid. Moreover, the *lac*3 was down-regulated by the presence of hypovirulence-causing dsRNA virus, CHV1.
Keywords: Cryphonectria parasitica, CHV1, Laccases, Copper, Tannic acid, lignin,2,5-Xylidine, Ferulic acid

F014

LAMMER Kinase, Lkh1, Regulates the Stability of RNA Binding Protein, Csx1, in Response to Oxidative Stress in Fission Yeast

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Our previous report showed that Lkh1 is involved in oxidative stress through stabilization *atf1*$^+$ mRNA (transcription factor), which regulates expression of the genes involved in protection to oxidative stress. Recently, it has been reported that Csx1 regulates the global gene expression in response to oxidative stress through stabilizating *atf1*$^+$ mRNA in fission yeast (Rodrige *et al.*, 2003). We examined the interaction between Lkh1 and Csx1 through Bacteral two-hybrid system. The Lkh1 and non-catalytic domain of Lkh1 (LN) showed strong interaction with Csx1, but not catalytic domain of the Lkh1 (Lcd). The Lkh1 phosphorylated the Csx1 more heavily than Lcd in in vitro kinase assay. The interaction between Lkh1 and Csx1 was dependent on oxidative stress *in vivo*, but independent on oxidative stress in spc1 deletion mutant. The *spc1Δcsx1Δ* and the *lkh1Δcsx1Δ* mutant cells were more sensitive to oxidative stress than single deletion mutants. Csx1 was degraded in response to oxidative stress in the *spc1 Δ*, the *lkh1Δ* and the *spc1Δlkh1Δ* mutant cells in consistent with *atf1*$^+$ mRNA degradation reported by Park *et al*. (2003) and Rodrige *et al*. (2003). Our data presented here indicate that the Lkh1 is involved in oxidative stress through regulating the stability of Csx1, RNA binding protein, in response to oxidative stress.
Keywords: LAMMER kinase, fission yeast, Lkh1, Csx1, oxidative stress

F015

LAMMER Kinase Homolog, Lkh1, Regulates the Gene Expression via Phosphorylation of Tup11 and Tup12 in *Schizosaccharomyces pombe*

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Previously we had reported the Lkh1 as a negative regulator for nonsexual flocculation of *S. pombe*. Through a pull-down assay with MBP-tagged Lkh1 and subsequent mass spectrometry, we identified Tup12 as an interacting protein. The interactions between the Lkh1 and Tup11 (or Tup12) were also confirmed by *in vitro* and *in vivo* binding assay. It was also confirmed that the Tup11 and the Tup12 were phosphorylated by the Lkh1 *in vivo*. Double deletion mutant of the *tup11*$^+$ and the *tup12*$^+$ showed flocculation of the yeast cells like the *lkh1*$^+$ deletion mutant. Assay with the *LacZ* fused to the downstream of the gene for fructose bisphosphatase, *fbp1*, or *fip1* promoter, which are negatively regulated by the Tup11 and the Tup12, showed that the β -galactosidase activity in the *kh1Δ* mutant was six-times and two-times higher than that of the wild type, respectively. The transcript analysis showed up-regulation of the *fbp1*$^+$ in the *lkh1Δ* and the *tup11Δtup12Δ* mutant cells. Microarray analysis also showed up-regulation of the genes in the *lkh1Δ* mutant, which are known to be repressed by Tup11 and the Tup12. Our data indicated that the activity of transcriptional repressors, the Tup11 and the Tup12, were activated by the LAMMER kinase homolog, Lkh1, through phosphorylation in *S. pombe*.
Keywords: LAMMER kinase, Lkh1, Tup11, Tup12, phosphorylation, S. pombe

F016

Preliminary Promoter Analysis of the Trimethylamine Dehydrogenase Gene (*tmd*) from *Methylophaga* sp. strain SK1

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Trimethylamine dehydrogenase (TMADH) is an iron-sulfur flavoprotein that catalyzes the oxidative demethylation of trimethylamine to dimethylamine and formaldehyde. *Methylophaga* sp. strain SK1, isolated from seawater at Mokpo, is able to grow aerobically on trimethylamine as a source of carbon and energy due to the TMADH. A 437 bp fragment of TMADH was generated by using degenerated PCR. A genomic fragment containing entire TMADH ORF (2983 bp, *tmd*)was isolated by colony hybridization with the 437 bp fragment as a probe against a SK1 genomic library. The 5' Rapid Amplification of cDNA Ends (RACE) system revealed the transcriptional start site "C". A ribosomal binding site (AGAAA) was found at 10 bp upstream of the *tmd* gene start cordon. A putative promoter region of *tmd* gene has the following conserved sequences: "GTATCA" sequence at -35bp and "TATACT" sequence at -10 bp from the transcription start site.
Keywords: Trimethylamine, tmd gene, Trimetylamine dehydrogenase, TMADH

F017

Promoter Activity of LTR Element of the Human FPRL2 Gene

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The human genome is estimated to consist of approximately 8% human endogenous retroviruses (HERVs) and related sequences. FPRL2 (fomyl peptide receptor-like 2) gene has a solitary LTR (long terminal repeat). The LTR is located between first exon and promoter region of the FPRL2 gene. The FPRL2 gene containing LTR element was expressed in various human tissues except fetal brain and cerebellum. The LTR element was detected in hominoid, Old World monkeys, and New World monkeys except for common marmoset, whereas LINE (long interspersed repetitive element) and SINE (short interspersed repetitive element) elements were detected in prosimian (ring-tailed lemur) and common marmoset. We also examined promoter activity of the LTR element in FPRL2 gene, and discussed its biological role. Taken together, the insertion of retroelements into primate genome could have different biological roles during primate evolution.
Keywords: LTR, LINE, SINE, HERV, Retrovirus

F018

ORF49 of Murine Gammaherpesvirus 68 Supports Efficient Virus Replication to Completion

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The regulation in the switch of life cycle in gammaherpesviruses, such as Epstein-Barr virus, Kaposi's Sarcoma-associated herpesvirus and murine gammaherpesvirus 68 (MHV-68), is critical to understand their pathogenesis. Replication and transcription activator (RTA) is an immediate-early gene that is mainly encoded by ORF50 during lytic replication and shown to play a prominent role in inducing the viral lytic replication in gammaherpesivurses. ORF49 resides next to ORF50 in a reverse orientation, but its function remains to be elucidated. We have generated a genome-wide, transposon-inserted mutant library, from which an ORF49^{null} virus showed attenuated growth in BHK21 cells, suggesting an important function of ORF49 in virus replication. In this study, we have mapped the 5' ends of the ORF49 transcripts using 5'-RACE and found that no splicing was involved in ORF49 expression. Co-transfection of ORF49 enhanced the ability of RTA to activate downstream early and early-late gene promoters, whereas ORF49 by itself did not activate these promoters. ORF49 increased virus DNA replication and gene expression from ORF50^{null} in the presence of RTA, indicating functional cooperation of ORF49 with RTA in the context of the virus genome. Interestingly, ORF49 alone was able to enhance DNA replication, gene expression and virus production from the wild type by increasing RTA expression, as measured by quantitative real-time PCR. *This work is supported by KOSEF R01-2006-000-11019.
Keywords: gammaherpesvirus, MHV-68, RTA, ORF49, gene expression, real-time PCR

F019

Human LTR Promoter in NOS3 Gene: Structure, Expression, Methylation and Evolution

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Endothelial nitric oxide synthase (NOS3) plays the important role of regulation of vascular wall homeostasis and regulation of vasomotor tone. Here we found new transcript variant that derived from LTR10A belonging to HERV-I family on human NOS3 gene. Previous studies found the HERV-I LTR elements were detected only in the hominoids and the Old World monkeys. The LTR10A element located on the upstream of the original promoter region of NOS3 gene seems to be inserted into primate genome approximately 33 Myr ago. We detect the LTR10A-derived promoter transcripts in placenta tissue only by RT-PCR amplification. Methylation study using the sodium bisulfied DNA sequencing demonstrates that LTR10A element of placenta tissue is occurred hypomethylation. Reporter gene assay of LTR10A element on NOS3 gene indicated good promoter activity of in human colon carcinoma cells (HCT-116). These findings suggest that the LTR10A element acquired the role of placenta-specific regulation of NOS3 gene during primate evolution.
Keywords: LTR10A element, methylation, promoter, primate evolution

F020

Identification of Genes Regulated by Kns1p in *Candida albicans*

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Previously we have reported the role of dual-specificity LAMMER kinase in yeast morphogenesis. In *Schizosaccharomyces pombe*, LAMMER kinase homolog, Lkh1p, acts as a negative regulator of filamentous growth and flocculation (Kim *et al*., 2001). Our unpublished results also showed the involvement of the *Saccharomyces cerevisiae* LAMMER kinase, Sc*Kns1*, in filamentous growth of the yeast cells. Based on these results, we tried to reveal the involvement of Kns1p in differentiation of the opportunistic human pathogen, *Candida albicans*. We cloned the Ca*KNS1* and constructed the single- and double-disruptant of the Ca*KNS1*. Interestingly, the Ca*KNS1* disruption showed significant alterations in hyphae and colony morphology in gene-dosage dependent manner on various culture conditions. In addition, the disruptants showed lower biofilm-forming activity and lower level of chlamydospore-production than the wild type. With the aid of the DEG-screening method, we screened genes regulated by Ca*KNS1* under the condition for the induction of filamentous growth and identified several genes including Ca*OSH4* for oxysterol binding protein. The cellular function of the Ca*KNS1*-regulated genes in relation to the morphogenesis of the *C. albicans* will be discussed.
Keywords: LAMMER kinase, Kns1, Candida albicans, gene screening, DEG-screening

F021

The Necessary and Sufficient Fragment of Promoter for a High Level Expression of the Cryparin Gene

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Cryparin is an abundant cell wall-associated hydrophobin of the chestnut blight fungus, *Cryphonectria parasitica*. Although cryparin is encoded as a single copy gene(Crp), it is the most abundant protein produced by this fungus when grown in liquid culture. Its accumulation is decreased remarkably, however, in *C. parastica* strains containing the double-stranded (ds) RNA virus Cryphonectria hypovirus 1. To characterize the transcriptional regulatory element(s) for strong expression and viral regulation, the transcription activity of a series of truncated cryparin promoters was measured using enhanced green fluorescent protein (EGFP) as a reporter gene. Promoter analysis indicated two positive cis-acting elements, one at nt -1,282 to -907 and the other at nt-640 to -427, and a repressor region between nt -427 to -181. The fragment between nt -188 and the translation start codon appeared to be a minimal but sufficient promoter element for the efficient expression of the cryparin gene. This -188 nt fragment contained a TATA box and a potential capping signal. To examine whether this small fragment is necessary and sufficient for a high level expression of the cryparin gene, two different chimeric reporter genes using ORF's of an inducible laccase (*lac3*) and hygromycin B resistance gene (*hph*) were tested. Nothern blot analysis and hygromycin B selection indicated that this -188 nt fragment is sufficient enough to show a strong transcriptional activity.

Keywords: Cryparin, ds RNA, EGFP, hygromycin B

F022

Analysis of Hypovirus-Regulated CpPK1 Substrates Using a Proteomic Analysis

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The cppk1 gene encodes the Ser/Thr protein kinase of *Cryphonectria parasitica* and is transcriptionally up-regulated by the presence of hypovirus CHV1-EP713. The cppk1 is important for coordinating growth with development and maintaining cell wall integrity. In order to identify CpPK1-dependent substrates, we conducted kinase-assisted two dimensional (2-D) polyacrylamide gel electrophoresis using partially purified CpPK1 and protein substrate. Among 70 fractions of cell-free extracts by HPLC using gel-filtration column, only one fraction have shown the presence of the CpPK1 protein by Western blot analysis. In order for the CpPK1-dependent substrates, fractions which were expected to contain the putative substrate of CpPK1 was obtained by HPLC using ion-exchange column. A kinase assay using CpPK1 fraction revealed CpPK1-dependently phosphorylated protein fractions containing putative substrates with estimated masses of 50 kDa and 40 kDa. In order to identify these two proteins, we conducted kinase-assisted two dimensional (2-D) polyacrylamide gel electrophoresis using partially purified CpPK1 and protein substrate and then the corresponding radioactive protein spots were further analyzed by using MALDI- and Q-TOF mass spectrophotometry.

Keywords: kinase assay, HPLC, cppk1

F023

Identification of Transcriptional Regulators Required for Fruiting Body Development of *Myxococcus xanthus*

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We have analyzed two new loci, *trmA* and *trmB*, that encode putative transcriptional regulators required for fruiting body development of *Myxococcus xanthus*. These loci were identified by genomic sequence analysis and directed mutagenesis. Sequence analysis indicates that *trmA* encodes a 459-amino-acid protein that consists of a fork head-associated (FHA) domain, an ATPase (AAA) domain, and a DNA binding domain. Meanwhile, *trmB* encodes a 474-amino acid protein that consists of a receiver (REC) domain, an ATPase (AAA) domain, and a DNA binding domain. Null mutations in the *trmA* gene and the *trmB* gene caused defects in fruiting body development. Since the TrmA and TrmB proteins contain signaling domains such as FHA and REC, it is suggested that they are parts of signal transduction systems regulating expression of genes required for fruiting body development.

Keywords: Myxobacteria, Myxococcus xanthus, Fruiting body development, Transcriptional regulator

F024

Identification and Characterization of PERV Elements in Different Pig Genome

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Porcine endogenous retrovirus (PERV) released from pig cells is a main risk factor associated with xenotransplantation from pig to human. Overcoming the main obstacle for xenotransplantation, the character of PERV element must be fully understood. From now, PERV have been identified to have three classes : PERV-A, PERV-B, PERV-C. PERV-A and PERV-B have been reported to have a infection ability for specific human cell line. However, PERV-C could not replicate their genome in human cell line. Using bioinformatic tools, several PERV long terminal repeat (LTR) elements were identified and analyzed from Genbank database sequences. Various subtype of PERV LTR elements were analyzed by the bioinformatic tools. Some of PERV LTR elements show a highly conserved sequences and insertional polymorphism. We cloned different subtype of LTR elements and analyzed the expression pattern from Yorkshire, domestic pig, and domestic wild pig.

Keywords: PERV, porcine, xenotransplantation

F025

Morphological Variation of Hybrids Developed from Different Mating Methods in *Pleurotus ostreatus*

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The expected international disputation about mushroom commercial strains has prompted us to develop for the native ones. The main objective of this study was to develop new hybrids of Pleulotus ostreatus. Since mushroom breeding takes a great deal of time and requires technical skills, there are only few experts in this field. To simplify hybrid breeding we studied the comparison between two reproduction methods i.e. mono-mono crossing and multi-sporous random mating. The multi-sporous random mating is as simple as anyone can apply to make his own favorable mushroom strain. This method also needs no monospore isolation, mating type determination, microscopic observation and crossing between monospores. Color mutants of Wonhyeong 1-ho were used for parental strains to calculate hybrids rate in progeny populations. Four different spore mixtures from white or dark gray fruitbody were tested to compare with mono-mono crossings. Production of dikaryons by multi-sporous random mating took only 10 days in comparison to 28 days required by mono-mono mating. Variation range on fruitbody morphology in dikaryons derived from two methods had little difference except on fruitbody color. Dikaryons from random mating showed 25%～37.7% of parental type and 62.3%～75% of hybrid type according to mixed combination. These results were confirmed using DNA polymorphism.

Keywords: Pleulotus ostreatus, hybrid, mono-mono crossing, multi-sporous random mating

F026

Characterization of a New Commercial Strain " Gold " in *Pleurotus cornucopiae* var. *citrinopileatus*

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A new commercial strain " Gold " of golden oyster mushroom was developed by hyphal anastomosis. It was improved with hybridization between monokaryotic strain derived from ASI 2295 and ASI 2703. The optimum temperature of mycelial growth and fruiting body development were 25～30℃ and 19～24℃, respectively. The pileus was golden to brilliant yellow color. Commercial strain "Gold" was not as prolific as the more commonly cultivated Pleurotus ostreatus in the conversion of substrate mass to mushrooms. However, cultivator can save money for mushroom growing on summer in Korea. Since picking individual mushrooms is tedious and often damages the fragile fruiting bodies compared with other species of oyster mushrooms.

Keywords: Pleurotus cornucopiae var. citrinopileatus, New commercial strain, Golden oyster mushroom

F027

Genetic Variability of Proton Beam Irradiated Strains in *Pleurotus ostreatus*

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Proton beam had higher energy than γ-ray and worked with localized strength. Proton-beam radiation could be valuable tool to induce useful strains of edible mushroom for recycling the biowastes. To assess the effects of proton beam irradiation on oyster mushroom (*Pleurotus ostreatus*), we investigated the proton beam radiation sensitivity and germination rate of the basidiospore and the mycelium. Genetic diversity and phylogenetic relationships among selected proton beam irradiated strains based on clustering analysis were also analysed. According to the analysis of AFLP DNA polymorphisms, all accessions were divided into four groups which coincided with dose rates. Applying the proton beam radiation, the dissimilarity among the induced strains was enhanced with the increase of dose rate. Over 400Gy irradiation led to 46%-58% of genetic dissimilarity of the strains. The evaluation among the proton induced strains of *P. ostreatus* by AFLP technique might provide the mutation effect of proton beam. (Supported by the Cheorwon Agricultural Technology Center through Regional Innovation Development & Enforcement Plan)

Keywords: AFLP, Mutation, Pleurotus ostreatus, Proton Beam

F028

Irisolidone, an Isoflavone Metabolite, Represses JC Virus Gene Expression via Inhibition of Sp1 Binding in Human Glial Cells

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Progressive multifocal leukoencephalopathy (PML) is a fatal demyelinating disease that results from an oligodendrocyte infection caused by the JC virus. Therefore, inhibiting the expression of JC virus is important for preventing and/or treating PML. This study found that irisolidone, an isoflavone metabolite, significantly inhibited the JC virus expression in primary cultured human astrocytes and glial cell lines. Studies examining the underlying mechanism revealed that a mutation of the Sp1 binding site downstream of the TATA box (Sp1-II) dramatically diminished the inhibitory activity of irisolidone. In addition, an irisolidone treatment repressed Sp1 binding to Sp1-II site, which is important for the basal JC virus promoter activity. The results suggest that the inhibitory effect of irisolidone against the JC virus may be attributed at least in part to the suppression of Sp1 binding to the JC virus promoter region. Therefore, the inhibition of the JC virus expression by irisolidone might provide therapeutic potential for PML caused by the JC virus.

Keywords: Progressive multifocal leukoencephalopathy, JC virus, Irisolidone, Gene expression, Promoter, Sp1

F029

Identification of Encystation-Induced Genes in *Giardia lamblia* by Comparative Proteomic Analysis

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Giardia lamblia has a life cycle composed of trophozoite and cyst. Previous study on encystations identified several encystation-induced genes including *myb2*, which encodes a putative transcriptional factor. Using a transgenic *G. lamblia* overexpressing MYB2 protein, we screened target gene(s) controlled by MYB2 protein. A plasmid, pmyb2.pac was constructed by replacing the *gfp* gene of pGFP.pac with the *myb2* gene. Using a 2-D gel electrophoresis, proteome of *G. lamblia* carrying pmyb2.pac was compared with that of *G. lamblia*, which was not transfected. In addition, proteome of encysting cells was compared with that of trophozoites. Ten of the increased protein spots in extracts of *G. lamblia* with pmyb2.pac and 18 increased spots in the encysting cells were identified to proteins. Among them, eight genes were cloned and examined for their expression pattern during encystations as well as in transgenic *G. lamblia* overexpressing MYB2 by real-time PCR. As expected, expression of *cwp1* gene was induced 33-fold upon the cells entered to encystation, while *tim* gene expressed in a constitutive mode during encystation. Expression of heat shock protein 70 and 90 increased 5- to 7-fold and two hypothetical proteins were induced 2- to 7-fold during encystation. In MYB2-overexpressing *G. lamblia*, only the genes for heat shock protein 70 and a hypothetical protein demonstrated 3- to 4-fold increase in their transcription. These results suggest them as target genes controlled by MYB2 protein.

Keywords: Giardia lamblia, Myb2, Transcription factor, Encystation

F030

Genome Sequence Comparison and Superinfection Hierarchy between Two Related *Pseudomonas aeruginosa* Siphophages, D3112 and MP22

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A temperate bacteriophage (MP22) was isolated from a Korean clinical isolate of *Pseudomonas aeruginosa*. It has a coliphage l-like morphology and a double-stranded DNA genome. Here, we present the complete nucleotide sequence and the annotation information of the MP22 genome and its characteristics. The MP22 genome is 36,409 bp long with a G+C content of about 64.2%. The genome contains 51 proposed open reading frames (ORFs), where the 46 ORFs (90%) display significant similarity to the cognate ORFs of the closely related phage, D3112. Four of the predicted ORFs are unique proteins, whose functions are yet to be revealed. Although both MP22 and D3112 phages are closely related at the genome level, the MP22 lysogens as well as the PA14 cells containing the MP22 C repressor gene (C_{MP22}) on a multicopy plasmid were susceptible to D3112 but not MP22 superinfection, whereas D3112 lysogens and the PA14 cells expressing C_{D3112} were immune to the superinfecitons by D3112 and MP22. This superinfection hierarchy (D3112 > MP22) is most likely associated with the dissimilarities in the binding capabilities of both C_{MP22} and C_{D3112} repressors to their operators.

Keywords: Pseudomonas aeruginosa, D3112, bacteriophage, c repressor

F031

Identification of Suppressor Genes for *wspF*, an Autoaggregative Mutant of *Pseudomonas aeruginosa* PA14

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Two major colony morphotypes (typical and rugose) were observed from the static biofilm cultures of *P. aeruginosa* PA14, whereas the biofilm cultures of its isogenic *wspF* mutant exclusively produced rugose colony variants. The *wspF* mutant displalys autoaggregative colony morphology and hyperadherence due to the elevated level of cyclic diguanylic acid (c-di-GMP). We identified and characterized 27 mutants from about 18,000 Tn*phoA* mutant clones, which suppresses the autoaggregation and hyperadhereace phenotype of the *wspF* mutant. Semi-random PCR and direct cloning of the Tn*phoA* insertion sites from the mutant chromosomes revealed the more than 10 non-redundant suppressor genes; two are *pel* genes (*pelA* and *pelB*) which are involved in exopolysaccharide production; four are *wsp* genes (*wspA*, *wspB*, *wspC*, and *wspE*); the other five insertions are located at *pvrS*, PA2144 (*glgP*), PA2436, PA3247 and PA4844. Based on these, we propose that further characterizations of these mutants may help to elucidate the complex regulatory network and its target genes involved in biofilm formation and biofilm-induced morphotypic variations as well as virulence and/or survival of *P. aeruginosa*.

Keywords: Pseudomonas aeruginosa, colony morphotypes, biofilm

F032

pel and *alg*, the Two Genetic Loci for Exopolysaccharide Production are Differentially Regulated by the Cyclic Diguanylic Acid (c-di-GMP) in *Pseudomonas aeruginosa* PA14

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Pseudomonas aeruginosa PA14 is an opportunistic pathogen that produces and secretes the extracellucar polymeric substances (EPSs), which is the extracellular matrix in the highly organized strucutural architecture called biofilm. The two gene clusters (*pel* and *alg*) have been known to be the major genetic determinants of PA14 EPSs involved in mucoidy characteristics and pellicle formation, respectively. Here we performed the GeneChip analysis using an autoaggregative mutant, *wspF*, in which WspR containing both REC and GGDEF domains is constitutively activated, resulting in the elevated level of cyclic diguanylic acid (c-di-GMP). We found that the *pel* gene cluster was upregulated in the *wspF* mutant, whereas the *alg* genes were downregulated, as verified by the promoter-*lacZ* fusions. Both upregulation and downregulation of EPS gene clusters were dependent on the c-di-GMP level. These results and the phenotypic characterization of the double mutants (*wspFpelA*, *wspFalgD* and *wspFmucA*) suggest that the coordinated opposite regulation of both EPS gene clusters by c-di-GMP may help to dismantle the roles of both EPS in the formation of biofilm and the maintenance of its integrity.

Keywords: Pseudomonas aeruginosa, EPS, c-di-GMP

F033

RAM Proteins are Involved in Hyphal Morphogenesis and Potential Targets for Antifungal Drug in *Candida albicans*

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The ability of *Candida albicans* to switch from yeast to hyphal morphology in response to serum is implicated in its virulence. Therefore, understanding the mechanisms for the yeast-to-hypha transition can provide new targets for a novel antifungal drug. In this study, we investigated the roles for the RAM signaling proteins, CaCbk1, CaMob2, CaTao3, CaHym1 and CaSog2 in the morphogenesis of *C. albicans*. We deleted the genes in *C. albicans* and found that the null mutants of the genes were defective in hypha formation, which indicates the RAM proteins are essential components for the hyphal growth of *C. albicans*. To search for downstream effector molecules of the RAM signaling, we performed DNA microarray experiments and compared the transcriptional profiles in the homozygous *Camob2* null mutant and wild type strains. The genome-wide transcription profiling revealed that deletion of *CaMOB2* resulted in down-regulation of the genes involved in ergosterol metabolism, which is consistent with the result that the *Camob2* null mutant was highly susceptible to the antifungal azol drug fluconazole. Taken together, our data suggest that the *in vivo* functions of the RAM proteins in hyphal growth of *C. albicans* may be due to changes in ergosterol biosynthesis and that the RAM signaling proteins can be good targets for a new antifungal drug, implicating clinical benefits with azole antifungal drugs.

Keywords: *Candida albicans,* RAM proteins, virulence, drug target, morphogenesis

F034

Cloning, Expression and Characterization of Enantioselective Epoxide Hydrolases from Marine Microorganisms

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Genes possibly encoding EHases were identified by analyzing open reading frames (ORFs) of several marine microorganisms whose genome sequences are available in public. The EHases were cloned and expressed in *E. coli*. The recombinant EHases were purified by metal affinity chromatography and further characterized. Three EHases - sEEH, nEEH, and rEEH – were highly soluble. Optimal activities of sEEH, nEEH and rEEH occurred at 45 °C, 35°C and 40°C, respectively and the enzymes were very active at neutral pHs. The purified rEEH was highly enantioselective toward styrene oxide. The enzyme could preferentially hydrolyze (*R*)-styrene oxide with ee value of 99% and more than 40% yield. K_m and k_{cat} of rEEH toward (*R*)-styrene oxide were calculated as 5.2 ± 0.3 mM and 31.78 s^{-1}, respectively, while K_m and k_{cat} of rEEH toward (*S*)-styrene oxide were 4.1± 0.3mM and 7.94 s^{-1}. sEEH and nEEH also preferentially hydrolyzed (*R*)-styrene oxide with ee value of 99%, however, the yield of enantiopure (*S*)-styrene oxide was around 10%. K_m and k_{cat} of sEEH and nEEH toward (*R*)-styrene oxide was not much different from those toward (*S*)-styrene oxide. sEEH, nEEH and rEEH could hydrolyze racemic epoxide substrates. The application to chiral resolution of racemic epoxide substrate by sEEH, nEEH, and rEEH is under progress.

Keywords: Enantioselective Epoxide Hydrolase

F035

Difference in Gene Content among *Deinococcus* species - *D. radiodurans*, *D. proteolyticus*, *D. radiopugnans*, and *D. radiophilus*

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All species in the genus *Deinococcus* are extremely resistant to a number of agents and conditions that damage DNA, including ionizing radiation. To date, *D. radiodurans* has received more attention than the other deinococci. The genome of *D. radiodurans* strain R1 is composed of two chromosome, a megaplasmid, and a small plasmid, yielding a total genome of 3,284,156 base pairs. To perform genome hybridizations of *Deinococcus* sp., a customizable CombiMatrix oligonucleotide microarray was constructed with sequences from 3,187 ORFs in *D. radiodurans*. Comparative genomic hybridization revealed about 4% of absent gene content in the chromosome of *D. proteolyticus*, *D. radiopugnans*, and *D. radiophilus* compared to *D. radiodurans*. Our findings also reveal high variability (~40%) in the mega and small plasmids. Thus, it is possible that only chromosomes I and II shares a common ancestry with other *Deinococcus* sp. Examined and the both plasmid may have been acquired separately.

Keywords: Deinococci, comparative genomics, *Deinococcus megaplasmid*

F036

Characterization of DNA Damage-Inducible (*dinB*) Promoter Region from *Deinococcus Radiodurans*

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Deinococcus radiodurans is a bacterium best known for its extreme radioresistance to tolerate both acute and chronic exposure to high levels of ionizing radiation. The secret behinds its incredible radioresistance lies in its ability to repair double strand breaks in its DNA. In order to reveal mechanisms of the extreme radioresistance and DNA repair in *Deinococcus radiodurans*, we examined proteome changes in a wild type strain following γ-irradiation (10 kGy) using two-dimensional polyacrylamide gel electrophoresis. The expression levels of 13 protein spots showed significant changes under radiation stress. Among 8 up-regulated proteins, SSB (single-stranded DNA binding protein), PprA (DNA damage repair protein), DdrA (DNA damage response A), DdrD (DNA damage response D), and DinB (damage inducible protein) are presumably involved in the DNA repair process. Of these spots, we characterized *dinB* promoter region using primer extension assay. The binding site for DinR, a central regulator of *din* loci of *B. subtilis*, was not found in the *dinB* promoter region of *D. radiodurans*.

Keywords: γ-irradiation, Deinococcus radiodurans, dinB

F037

Dual Regulation of CymR on the Expression of *ytmI* and *ytlI* Genes That Functions in Organosulfur Metabolism in *Bacillus subtilis*

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Sulfur, required for the synthesis of amino acids and cofactors, can be supplied either from inorganic sources, such as sulfate, or from organic sources, such as sulfonates, cysteine or methionine. Genes such as those of the *ytmI, yxeL*, and *ssu* operons, which encode proteins that function in the uptake and desulfurization of organic sulfur compounds, are repressed when sulfate or cysteine is present as a sole sulfur source. Both the *ytmI* operon and the divergently transcribed *ytlI* gene are repressed by an Spx-dependent mechanism in sulfate-containing medium. A recent report has implicated that the *ytmI* is activated by YtlI, a LysR-type regulator. In addition, CymR is identified as an another negative regulator of the *ytmI* operon. When *cymR* is inactivated, the expression of the *ytmI* operon was derepressed in the presence of either sulfate or cysteine. This is in contrast to an *spx* mutant, which still exhibits Cys-dependent transcriptional repression. It has been shown that CymR is a negative regulator for the expression of *ytlI*, and hence, indirectly causes repression of the *ytmI* operon. By EMS mutagenesis for *ytmI-ytlI* intergenic regulatory region, this report shows that when CymR regulator protein binds to -35 transcriptional regulatory site of *ytlI*, where it also corresponds to a leader sequence of *ytmI*, CymR directly inhibits the expression of *ytmI* as well as it represses the *ytlI*.

Keywords: sulfur, gene regulation, ytmI, cymR, ytlI

F038

The Redox Capacity-Independent Role of Mitochondrial Thioredoxin in Arginine Biosynthesis

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In the fission yeast *Schizosaccharomyces pombe*, the *trx2*$^{+}$ gene encoding a mitochondrial thioredoxin. The *Δtrx2* mutant grew well as the wild type cells in the complex media containing glucose. However, the mutant did not grow in the glycerol media containing glycerol instead of glucose as carbon source because the *trx2* deletion caused reduction in was depleted, the respiration rate. The *Δtrx2* mutant also showed growth defect in the minimal media and exhibited auxotrophy for arginine and cysteine. The arginine auxotrophy was overcome by introduction of a multicopy *arg3*$^{+}$ gene encoding ornithine carbamoyltransferase (OTCase) in arginine biosynthetic pathway. Trx2 was found to interact directly with Arg3 and the depletion of Trx2 caused a decrease in OTCase activity. Not only wild type but also mutant Trx2 in which one or both of the active site cysteine residues were substituted could restore the decreased OTCase activity. Therefore the active-site disulfide of thioredoxin is not required for the activity of OTCase. It can be proposed the novel redox capacity-independent function of mitochondrial thioredoxin in arginine biosynthesis pathway. (Grant from NRL)

Keywords: Schizosaccharomyces pombe, mitochondria, thioredoxin, arginine biosynthesis, ornithine carbamoyltransferase

F039

Comparative Genomics Revealed that Hypoxic Stress Induces Sexual Development-related Genes in a Human Pathogenic Fungus *Aspergillus fumigatus*

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Aspergillus fumigatus is an imperfect filamentous fungus causing aspergillosis which is an opportunistic fungal disease mainly localized in the respiratory system of human. *A. fumigatus* has no known sexual development process, while *Aspergillus nidulans*, which is a very close relative of *A. fumigatus*, undergoes complete sexual development process. In *A. nidulans*, hypoxic condition is an important environmental factor for activating fruiting body formation. Also, the hypoxic condition is important to *A. fumigatus* because of the environment of host cell is usually maintained as hypoxic condition. To study relationship between hypoxic condition and fungal physiology and virulence, comparative DNA microarray experiment was performed using *A. fumigatus* and *A. nidulans* microarray chips. As a result, SREBP pathway which is an important oxygen sensing system found in fission yeast was up-regulated in hypoxic condition. Furthermore, several important sexual development specific genes found in *A. nidulans* were up-regulated by hypoxic stress in both *A. nidulans* and *A. fumigatus*, suggesting that the hypoxic stress may induce sexual development-specific pathway in *A. fumigatus*. Intensive comparison between *A. fumigatus* and *A. nidulans* microarray result will provide various information of shared and distinct pathway of hypoxic stress response and sexual development process. [This work was supported by KOSEF (R1-2006-000- 11204-0) and KRF (KRF-2005-070-C00123).]

Keywords: *Aspergillus fumigatus, Aspergillus nidulans*, hypoxic stress, microarray, sexual development

F040

IscR Acts as an Activator in Response to Oxidative Stress for the *suf* Operon Encoding Fe-S Assembly Proteins

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In *Escherichia coli*, Fe-S clusters are assembled by gene products encoded from the *isc* and *suf* operons. Both the *iscRSUA* and *sufABCDSE* operons are induced highly by oxidants, reflecting an increased need for providing and maintaining Fe-S clusters under oxidative stress conditions. Three *cis*-acting oxidant-responsive elements (ORE-I, II, III) in the upstream of the *sufA* promoter serve as the binding sites for OxyR, IHF and an uncharacterized factor respectively. Using DNA affinity fractionation, we isolated an ORE-III-binding factor that positively regulates the *suf* operon in response to various oxidants. MALDI-TOF mass analysis identified it with IscR, known to serve as a repressor of the *iscRSUA* gene expression under anaerobic condition as a [2Fe-2S]-bound form. The *iscR* null mutation abolished OREIII-binding activity in cell extracts, and caused a significant decrease in the oxidant induction of *sufA in vivo*. OxyR and IscR contributed almost equally to activate the *sufA* operon in response to oxidants. Purified IscR that lacked Fe-S cluster bound to the ORE-III site and activated transcription from the *sufA* promoter *in vitro*. Mutations in Fe-S-binding sites of IscR enabled *sufA* activation *in vivo* and *in vitro*. These results support a model that IscR in its demetallated form directly activates *sufA* transcription, while it de-represses *isc* operon, under oxidative stress condition. (Grant from NRL)

Keywords: IscR, suf, isc, Fe-S, Fur, OxyR

F041

Molecular Cloning of Genes Encoding Glucoamylase and α-amylase from *Tricholoma matsutake* and Glucoamylase Gene's Expression in the *Pichia pastoris*

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The *Tricholoma matsutake glucoamylase gene(GAT) and α-amylase gene(ATM) were cloned and GAT was expressed in Pichia pastoris*. The GAT sequence of the cDNA fragment reveals an open reading frame of 1,719bp encoding a 573 amino acid mature protein with a calculated molecular weight of 64kDa. And the ATM is 1,470bp in length, encoding a protein of 489 amino acid mature protein with a calculated molecular weight of 55kDa. By analysing and comparing cDNA and genomic DNA, it was confirmed that the GAT was interrupted by 8 introns and ATM was interrupted by 21 introns. By chromosome walking, a DNA fragment of 1,262bp of GAT's 5′ upstream region and 3,520bp of 3′downstream region were cloned. A DNA fragment of 378bp of ATM's 5' upstream region and 341bp of 3'downstream region were cloned. The GAT showed 63% of homology to the glucoamylase(gla1) of Lentinula edodes and the ATM showed 49% of homology to the α-amylase of *Cryptococcus neoformans*. Using the vector pPIC9, the cDNA encoding GAT was inserted in the yeast genome downstream of the 5'AOX1 promoter to replace the AOX1 gene. By inducing with 0.5% methanol for 24h, the glucoamylase was overexpressed in *P. pastoris*.
Keywords: glucoamylase, α-amylase, Pichia pastoris

F042

Quorum Sensing Regulates Flagella Biosynthesis of *Burkholderia glumae*

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Burkholderia glumae, the causative agent of rice grain rot, is motile with two to four polar flagella. In a *B. glumae tofI* mutant, swimming motility was significantly reduced while swarming motility was abolished. The mutant failed to produce flagella. Both swimming or swarming activity and flagellation of a *tofI* mutant can be restored by providing C8-HSL exogenously suggesting that the flagella biosynthesis is under the control of quorum sensing. Here, we describe a regulatory gene homologous to IclR, which we named quorum sensing master regulator (QsmR). The swimming or swarming motilities of *qsmR* mutants were similar to *tofI* mutants and electron microscopic analysis revealed *qsmR* mutant cells were aflagellate. Using DNA mobility shift assay and Tn*3-gusA* report fusions, we showed that expression of *qsmR* is controlled by C8-HSL and its cognate transcription factor TofR. QsmR regulates a flagella master regulator FlhDC. All data indicate that quorum sensing is at the top of the hierarchy of flagella gene regulation. Flagella-deficient mutants lost virulence in rice grain indicating that flagella play important roles in virulence of *B. glumae*.
Keywords: Swarming motility, Swimming motility, Burkholderia glumae, Quorum sensing, Bacterial grain rot of rice

F043

Cloning and Analysis of Fatty Acid Biosynthesis Gene Clusters from *Shewanella hanedai* KMG427

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The fatty acid biosynthetic (fab) gene cluster taking part in the synthesis of middle chain fatty acid and a genomic segment were isolated from eicosapnetaenoic acid (EPA)-producing bacterium *Shewanella hanedai* KMG427. *fabH, fabG, ACP, fabD* and *fabF* genes were conneted in, but *fabB* gene was seperated from these genes. These six gene products are known to synthesis of 3-hydroxyacyl-ACP from Acetyl CoA. *fabH* gene encoding 3-oxoacyl-(acyl-carrier-protein) synthase Ⅲ was composed of 960bp(320aa). *fabD* gene encoding malonyl coA-acyl carrier protein transacylase was composed of 906 bp(302aa). *fabG* gene encoding 3-oxoacyl-(acyl-carrier-protein) reductase was composed of 756 bp(249aa). *acp* gene encoding acyl carrier protein was composed of 399 bp(78aa). And *fabF* gene encoding 3-oxoacyl-(acyl-carrier-protein) synthase Ⅱ was composed of 1254 bp.(355aa) Another one, *fabB* gene encoding β-ketoacyl ACP synthase Ⅰ was composed of 1812bp(404aa). *fabH, acp* showed 88 and 98% homology with that of *Shewanell denitrificans* OS217. *fabD, fabG* showed 75, 88% homology with those of *Shewanella oneidensis* MR-1. *fabF* and *fabB* gene showed 85% and 92% homology with those of *Shewanella* sp.PV4. The order of gene cluster were the same to that of *Escherichia coli* which had fatty acid/phoshpolipid synthesis protein(plsX) gene.
Keywords: fatty acid biosynthesis gene cluster, Shewanella hanedai KMG427

F044

Designation of *Moraxella catarrhalis* Specific Primer and Establishment of Optimal Conditions of PCR Detection on Specimens

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Moraxella catarrhalis is a major cause of upper respiratory tract infections in children and, particularly in adults with chronic obstructive pulmonary disease (COPD). In this study, M. catarrhalis 16S rRNA gene was chosen as the target, and we made specific primer of 2-steps PCR for detection of M. catarrhalis. The primer designated that Tm and GC% of the primer were 56.3℃ and 45.5%. To confirmed of optimal conditions in 2-steps PCR, we processed various conditions with template DNA of M. catarrhalis ATCC 25238. For Optimal condition of 2-steps PCR was 40 cycles of denaturation at 95℃ for 5 sec and annealing/extension at 66℃ for 1 min, and low detection limit of template DNA concentration were 100fg/㎕. The specificity of the reaction verified various bacterial species found in the respiratory tract. The performance of the procedure was examined with 40 nasal aspiration specimens, and the results were compared to those obtained by conventional culture methods. By conventional culture methods, 20 (50%) of the specimens showed growth of the study organism. In contrast, 24 (60%) of the specimens tested positive by the 16S rRNA PCR. None of the culture positive specimens were PCR negative, whereas 4 of the PCR-positive specimens were culture negative. Thus, the 16S rRNA PCR method improves the detection rate significantly compared to that of the conventional culture method.
Keywords: Moraxella catarrhalis, 16S rRNA, 2-steps PCR

F045

The *nsdC* Gene Encoding a Novel Positive Regulator of Sexual Development of *Aspergillus nidulans* is Regulated by Complicated Post Transcriptional Control

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The *nsdC* gene which is predicted to encode a putative transcription factor carrying a novel type of zinc finger DNA binding domain consisting of two C_2H_2 and a C_2HC motifs acts as a positive regulator of sexual development in *A. nidulans*. Two distinct transcripts, 3.2 and 2.8 kb, are synthesized and the smaller one differentially accumulates in various stages of growth and development as well as under the varying nutritional conditions. 5' RACE and northern analysis revealed that there were at least two alternative transcription initiation sites which might be responsible for two kinds of transcript in different size. Two alternative transcription termination sites were also found through 3' RACE and northern analysis of *nsdC* deletion mutant. The gene carries two relatively long introns in its 5'UTR, one of which is spliced alternatively. The results suggest that the expression of *nsdC* is controlled by complicated transcriptional and post transcriptional regulations.

Keywords: Aspergillus nidulans, sexual development, transcription factor, gene expression

F046

Identification and Expression of Phospholipase D from *Streptomyces laodlogenes* CS. 684

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Phospholipase D (PLD, EC 3.1.4.4) hydrolyzes phosphatidylcholine to phosphatidic acid and choline by breaking its phosphodiester bond. PLD also acts on other phosphatidyl esters and catalyzes a transphosphatidylation reaction when alcohol is present as a nucleophilic donor such as the transformation of phosphatidylcholine (PC) and serine to phosphatidylserine (PS) and choline (C). An gene responsible for the extracellular phospholipase D from *Streptomyces laodlogenes* CS. 684, producer of lidlomycin, was amplified from the cosmid with primers, PLD-2F, 5' CCS GGS TGY TGG GGS GAC 3' and PLD-2R, 5' GTC GTA YTC SGT SAC GTT SGG 3'. The product was purified and digested with restriction enzyme and cloned into *E. coli* expreesion vecter, pRSET or pET. The resulting plasmid was overexpressed and checked for their enzyme activity.

Keywords: Identification, Expression, Phospholipase D, *Streptomyces laodlogenes* CS. 684.

F047

Targeting Mycobacterium Tuberculosis rpoB mRNA for Antituberculosis Chemotherapy Monitoring

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Polymerase chain reaction (PCR) has become a popular method for detecting *Mycobacterium tuberculosis* in patients. However, PCR targeting DNA may be inappropriate for monitoring the response of patients to antituberculosis therapy, since mycobacterial DNA persist for long periods in sputum even after the specimens are culture negative. For that reason, there has been an increasing need for developing a PCR targeting mRNA molecules that may truly represent live M. tuberculosis. In order to develope such method, we tested reverse-transcriptase PCR (RT-PCR) and real-time PCR for detecting *rpoB* or antigen 85B mRNA of M. tuberculosis to see whether they can distinguish between viable and non-viable organisms. RT-PCR and real-time PCR results were checked with CFU counts of BCG. In the experiment, the levels of *rpoB* and 85B mRNA were compared in parallel using cultures of BCG that were treated with rifampin for different periods of times. In brief, the results from our study showed that exposure of rifampin to rifampin-susceptible BCG for different periods of times affected the levels of *rpoB* and 85B mRNA. That is, by RT-PCR and real-time PCR, it was observed that the levels of *rpoB* and 85B mRNA decreased gradually as the time for rifampin-treatment increased. Between *rpoB* and 85B mRNA, 85B mRNA disappeared faster than *rpoB* mRNA. For front, we will measure level of *Mycobacterium tuberculosis rpoB* and 85B mRNA in patient's sputum.

Keywords: Mycobacterium tuberculosis, mRNA, Reverse transcriptase PCR, chemotherapy

F048

Functional Analysis and Expression Profiling of the *vip* Genes of *Aspergillus nidulans*

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The *veA* gene in homothallic fungus *Aspergillus nidulans* has been known as a positive regulator of sexual development as well as a repressor of asexual development. To dissect detailed function of the *veA* gene, the *vipA*, *B*, and *C* (**V**eA-**i**nteracting **p**rotein) genes were isolated by using the yeast two hybrid system with a truncated VeA as bait. By PCR using chromosome-specific cosmid library, the *vipA, B,* and *C* genes were isolated. The *vipA* gene has an ORF encoding a 334 amino acid polypeptide and has Gln/Pro rich region and FAR1 domain. The *vipA*-null mutants and over-expressors did not show any remarkable phenotypic difference. The *vipB* gene had an ORF encoding 332 amino acid polypeptide and the amino acid sequence of VipB revealed that it has a putative methyltransferase domain. Similar to the *vipA* deletion mutant, *vipB*-null mutant and a *vipB*-overexpressor did not show any phenotypic difference in development. Although *vipC* showed negative regulation of sexual development in light condition, it has no phenotypic change in dark condition. Since no obvious phenotypic changes were observed in these mutants, the microarray analysis was carried out for obtaining information of *vip*-dependent gene set during mycelial growth and developmental processes. For validating microarray, these expression patterns in the three null-mutants were confirmed by Northern hybridization. Analysis of expression profiles are in progress.
[This work was supported by KOSEF.]

Keywords: *Aspergillus nidulans*, Sexual development, veA, vipA, vipB, vipC, microarray

F049

Comprehensive Analysis of Microbial Histidine Kinases and Screening of Soluble Proteins

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Histidine kinases (HKs) are the most widely used of two-component signal transduction enzymes in bacteria. They mediate multiple biological key functions including chemotaxis, quorum sensing, oxidant stress adaptation and cell wall synthesis. The key functions make HKs very attractive targets for anti-bacterial therapeutic development because of their importance in pathological virulence in some bacteria.Recently, we have initiated building a pool of HK resources. Two hundred and thirty-six genes encoding putative HKs were identified from 28 sequencing-finished genomes of the Comprehensive Microbial Resource. Based upon the genomic data, structural biological data, classical dendrograms, and selectivity data available in public database, we analyzed domain organization and functional modules of the compiled HK genes to identify their signaling domain distribution. The HK genes were then cloned into expression vector possessing hexahistidine-hemoglobin tag and expressed in *E. coli* Rosetta-gami™ (DE3). Soluble fraction of the expressed HKs were accessed by using colony filtration blotIn this study, we present a fundamental data resource of bacterial HK pool, thereby providing potential opportunities for a biochemical study of prokaryotic signaling system and for a chemogenomical study applicable to drug discovery. This work is supported by a grant from the 21C Frontier Microbial Genomics and Applications Center.
Keywords: Histidine kinase, Domain analysis, colony filtration blot

F050

Regulation of Oxidative Stress Response by Quorum Sensing in *Burkholderia glumae*

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Burkholderia glumae produces a broad-host range phytotoxin, called toxoflavin, which is a key pathogenicity factor in rice grain rot and wilt in many crops. Toxoflavin biosynthesis and transport are regulated by quorum sensing. *tofI* (biosynthesis of N-octanoyl homoserine lactone) and *qsmR* (quorum sensing master regulator) mutants did not survive at the stationary phase. The phenomena are similar to the phenotypes that bacterial cells are exposed to oxidative stresses. Using catalase activity staining and H_2O_2 treatment, we found that *tofI*::Ω and *qsmR*::Ω mutants have less catalase activity. We analyzed genes involved in oxidative stress in *B. glumae* and found seven catalase genes (*katB, katE, katG, katM1, katM2, katS*, and *katQ*) and two alkyl hydroperoxide reductase genes (*ahpC* and *ahpF*). Gel shift analysis demonstrated that TofR/C8-HSL binds regulatory region of *ahpCF* and QsmR binds regulatory region of *katB*. Using Tn3-*gusA* transcriptional fusions, we showed that TofR/C8-HSL regulates expression of *ahpCF*. Insertional mutation in *ahpF* conferred slow growth at the early stationary phase. Single mutation in the genes encoding catalase and alkyl hydroperoxide reductase had little effect at stationary phase survival. This is a first report that quorum sensing and transcriptional activator QsmR regulate expression of catalase genes responding to oxidative stress in *B. glumae*.
Keywords: oxidative stress, Burkholderia glumae, Quorum sensing, catalase, alkyl hydroperoxide reductase

F051

Cloning of the cDNA Encoding a Laccase from *Ganoderma lucidum* and Expression of Soluble Laccase in *Escherichia coli*

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Laccases are multicopper-containing enzymes which catalyse the oxidation of phenolic and nonphenolic compounds with the concomitant reduction of molecular oxygen. In this study, a full-length cDNA coding for laccase (GLlac1) was isolated from *Ganoderma lucidum* by RT-PCR. The corresponding open reading frame is 1560 nucleotides long and encodes a protein of 520 amino acids. The cDNA encodes a precursor protein containing a 21 amino-acid signal sequence. The deduced N-terminal amino-acid sequence was similar but not identical to those of other laccase proteins.GLlac1 was released out of cells when expressed in *E. coli* and has a functional enzyme activity. In addition, we found that GLlac1 has potential anti-oxidative effects, and thus GLlac1 could be considered in the pharmacological application.
Keywords: Ganoderma lucidum, laccase, cDNA cloning, expression, enzyme activity

F052

Proteome Analysis of Total Proteins Regulated by HrpB from the Plant Pathogenic Bacterium *Burkholderia glumae*

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Plant pathogenic bacteria deliver variable numbers of effector proteins into plant cells through the Hrp (hypersensitive response and pathogenicity) type III protein secretion system (Hrp T3SS) during infection. Genes encoding the Hrp T3SS are expressed under plant apoplastic conditions in an AraC-type transcriptional activator HrpB dependent manner. To identify proteins controlled by HrpB in *Burkholderia glumae*, we constitutively expressed *hrpB* under the control of *trc* promoter and analyzed HrpB-dependent extracellular and cellular proteins by the two-dimensional gel electrophoresis (2DGE) and mass spectrometry techniques. All HrpB-dependent proteins had no significant homology to known T3SS-dependent proteins except a HrpK of *Pseudomonas syringae* pv. *syringae* and two T3SS-associated cytoplasmic proteins of *Ralstonia solanacearum*. Secretion of all identified extracellular proteins was independent of the Hrp T3SS. Secretion of sixteen proteins was dependent upon the type II protein secretion system (T2SS) and eight of them had predicted signal peptide sequences. Mutants lacking the T2SS or Hrp T3SS were less virulent to rice panicles indicative of important roles in pathogenicity.
Keywords: Burkholderia glumae, HrpB, Secretion, Two-dimensional electrophoresis

F053

Analysis of Gene Cluster for Trehalose Biosynthesis from *Streptomyces albus*

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Trehalose is produced in bacteria and yeast cells, fungi, algae, and a few higher plants, and plays very important roles to protect macromolecules in the cell. A putative biosynthetic gene cluster for salbostatin was cloned from the *Streptomyces albus* ATCC21838 and analyzed. Interestingly, many genes next to the salbostatin biosynthetic genes were assumed to be involved in trehalose biosynthesis. The sequence data revealed that 8 genes were clustered in a 10-Kb DNA. Among them sugar kinase/glucokinase, trehalose-phosphate synthase, transporter, trehalose-6-phosphate phosphatase, and ADP-glucose synthase were expected to biosynthesize trehalose from glucose by conversion into glucose 6-phosphate and ADP-glucose, condensation to trehalose 6-phosphate, and subsequently trehalose. To elucidate the biosynthetic function of these genes, the DNA containing above 5 genes were cloned into *S. lividans* and the ability to produce trehalose was examined. The productivity of trehalose in the transformant has been studied by thin layer chromatography and high performance liquid chromatography in various conditions. [Supported by the 21C Frontier Microbial Genomics and Application Center Program, Ministry of Science & Technology, Republic of Korea.]
Keywords: Trehalose, S. albus, salbostatin

F054

The Pre-Membrane of JEV Dose not Affect the Viral Production and Infectivity in Murine Leukemia Virus Pseudotyped Expressing *Japanese encephalitis* Virus

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The pre-Membrane (prM) and envelope (E) protein of *Japanese encephalitis* virus (JEV) are related in virus infectivity, cell-association, and elicitation of neutralizing antibody (NAb). We produced JEV pseudotyped viruses with JEV (strain: Nakayama-NIH; NK and Beijing-1; BJ) *prM-env* and *env* gene and tested their infectivity, titer of JEV pseudotyped viruses. We also analyzed neutralization capacities with anti-JEV sera from JEV-immunized mouse. Whether contain a prM or not, JEV-pseudoviruses had a same size of envelope protein and similar infectivity in several cell lines. We have measured the titer of NAb in immunized mouse sera (derived NK and BJ) using JEV-pseudotyped expressing prM-env and env proteins. The 50% reduction neutralizing antibody titers of NK and BJ were about 1:10,000 at each strain immunized sera. In result, there was no difference between the JEV *prM-env* pseudotyped virus and the JEV *env* pseudotyped virus in infectivity, titer, and neutralization activity.
Keywords: Japanese encephalitis virus, pre-membrane, Pseudotyped virus

F055

Construction of the Recombinant Baculovirus Vector System for Bladder Cancer Therapy

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It was recently reported that HIV-1 Tat protein transduction domain (PTD) is responsible for the highly efficient attachment and cross of the plasma membrane. Also M gene expressing the anti-tumor activity and TERT or survivin (SV) promoters posessing specific induction in cancer cell are pretty effective molecules for bladder cancer therapy. we constructed a novel recombinant baculovirus vector expressing M gene (pBacG-SV/TERT-M) and a M-Tat fusion protein (pBacG-SV/TERT-M-Tat). Enhanced delivery of M via Tat-mediated spread on negative human cancer was investigated. In our result, M-Tat showed more efficient and safty than other system in view of translocation into tumor cells, inhibition of tumor cell proliferation and induction of apoptosis. Therefore, we suggest that recombinant baculovirus vector containing Tat fused M was effectively and saftly delivered between infected cells and uninfected cells.
Keywords: baculovirus, cancer, M, PTD

G001

Molecular Characterization of *bla*$_{IMP-1}$ in Multi-Resistant *Acinetobacter baumannii* Clinical Isolates

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Among 46 *Acinetobacter baumannii* isolated in 2004, two imipenem-resistant isolates were obtained from clinical specimens taken from patients hospitalized in Busan, Republic of Korea. Two carbapenemase-producing isolates were investigated further to determine the mechanism of resistance. Two cases of infection by *A. baumannii* producing the IMP-1 β-lactamase were detected. The isolates were characterized by a modified cloverleaf synergy test and EDTA-disk synergy test. Isoelectric focusing of crude bacterial extracts detected nitrocefin-positive bands with the pI value of 9.0. PCR amplification and characterization of the amplicons by direct sequencing indicated that the isolates carried *bla*$_{IMP-1}$ determinant. The isolates were characterized by a multidrug resistance phenotype, including penicillins, extended-spectrum cephalosporins, carbapenems, and aminoglycosides. These results appear to represent that the observed imipenem resistance among two Korean *A. baumannii* isolates is due to the spread of an IMP-1-producing clone. This study shows that the *bla*$_{IMP-1}$ resistance determinant, which is emerging in Korea, may become an emerging therapeutic problem, since clinicians are advised against the use of extended-spectrum cephalosporins, imipenem, and aminoglycosides. [Supported by grants from the NIH of KCDC and BioGreen 21 Program (20050301034479) of Rural Development Administration in Republic of Korea].

Keywords: *Acinetobacter baumannii*, carbapenemase, IMP-1, EDTA-disk synergy test, ERIC-PCR

G002

Hepatitis C Virus Nonstructural Protein 5 Activates Beta-Catenin-Dependent Transcription Activity

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HCV infection often leads to liver cirrhosis and hepatocellular carcinoma. The majority of HCV infection progress to chronic hepatitis. HCV nonstructural protein 5 (NS5) consists of 5A and 5B. The NS5A protein is a multifunctional protein and promotes tumor growth and a number of cell cycle regulatory genes. The NS5B is an RNA-dependent RNA polymerase (RdRp) that catalyzes the replication of HCV. To elucidate the molecular mechanism of HCV-induced hepatocellualr carcinoma, we examined the effects of HCV NS5 proteins on wnt/beta-catenin signal transduction cascades. We show that beta-catenin-mediated transcription activity was elevated in replicon cells as determined by luciferase assay. We confirmed this result using a transient transfection experiment in hepatoma cell line. To further investigate the role of NS5A and NS5B in beta-catenin-mediated transcription activation, we examined the protein-protein interaction between NS5A/5B and beta-catenin. Both *in vitro* and *in vivo* binding data show that both NS5A and NS5B protein directly interact with beta-catenin in replicon and hepatoma cell lines. We also found that NS5A and NS5B proteins were colocalized with beta-catenin in the cytoplasm. These results indicate that both NS5A and NS5B proteins activate beta-catenin-dependent transcriptional activity through interaction with beta-catenin and NS5A/5B may be involved in wnt/beta-catenin mediated neoplasmic transformation.]

Keywords: HCV, Wnt/beta catenin, NS5A, NS5B, Hepatocellular carcinoma

G003

Dramatic Decrease of Erythromycin Resistance in Group A *Streptococci* by Change of *emm* Distribution Isolated from Schoolchildren in Jinju

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Serotyping is essential for epidemiologic study of group A streptococcus (GAS) infections. The antibiotic resistance and its mechanism of GAS varies according to nations or study period. Throat cultures were taken from 2,351 elementary school children to isolate GAS. Antibiotic susceptibility test to erythromycin (EM), clindamycin (CC), and tetracycline (TC) was performed by agar dilution method. T typing and *emm* genotyping as well as macrolide resistance phenotype and genotype were studied. Isolation rate of GAS were 14.0% (328/2351). T5/27/44 were the most prevalent, accounting for 29.6%, T12 and T6 were 13.4% and 10.7%, respectively. *emm*44/61 was the most prevalent, it accounted for 29.3% and *emm*6 and *emm*1 were 11.6% and 9.8%, respectively. Resistance rates of GAS to EM, CC and TC were 9.8%, 8.8% and 18.3%, respectively. Constitutive resistance (CR) was observed in 87.5%, M phenotype in 9.4% and inducible resistance (IR) only in 3.1%. The *erm*B and *mef*A genes were present in 90.6% and 9.4% of the strains, respectively. T12, *emm*12, which showed highly resistant to macrolides, were significantly decreased compared to the past. Resistance rate of EM was dramatically decreased compared to 51.0% in 2002. There has been a gradual increase of macrolide consumption during last few years. Dramatic decrease of EM resistance during short period might be related to the change of distribution of T and *emm* types, rather than a decrease of consumption of macrolides.

Keywords: Streptococcus pyogenes, epidemiology, antibiotic resistance, emm

G004

Analysis of Syncytium Formation Mechanism Induced by Ecotropic Murine Retrovirus

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To study the mechanism of syncytium formation, novel syncytia -inducing ecotropic murine retrovirus was used. Our previous result showed that amino acid substitutions at the RBD(receptor binding domain) of envelope glycoprotein contribute to syncytium formation. In this study, we have investigated if this fusion phenomenon could occur with retroviral vectors pseudotyped with the novel syncytia-inducing ecotropic murine leukemia virus Env. We have found that these vectors were not able to mediate virus-to-cell fusion in *M.dunni* murine cell lines. These findings indicate that syncytia-inducing ecotropic murine leukemia virus is capable of generating syncytia during its replication. There was also no correlation between the level of ecotropic murine leukemia virus receptor(mCAT-1) and the fusogenic effect.

Keywords: ecotropic mouse retrovirus, envelope, mCAT receptor, M.dunni, syncytium

G005

Characterization of a Third Extracellular Domains of Ecotropic Receptor in Moloney Murine Leukemia Virus Infection

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The murine ecotropic retroviral receptor has been demonstrated to function as a mouse cationic amino acid transporter 1(mCAT1) composed of multiple membrane-spanning domains. The feral mouse *Mus dunni* cells are not susceptible to infection by the ecotropic Moloney murine leukemia virus (MoMLV), although they can be infected by other ecotropic murine leukemia virus such as Friend MLV and Rauscher MLV. The relative inability of MoMLV to replicate on *M.dunni* cells has been attributed to two amino acid(V214 and G236) within the third extracellular loop of the *M.dunni* CAT1 receptor(dCAT1). By exchanging the third extracellular loop of the mCAT1 cDNA encoding receptor from the permissive mouse and corresponding portion of cDNA encoding nonpermissive *M.dunni* receptor, we have identified the most critical amino acid residue glycine at position 236 in the third extracellular loop of the dCAT1. We also examined the role of third extracellular loop of the *M.dunni* CAT1 receptor in syncytium formation. To investigate the relationship between dCAT1 and virus-induced syncytia, we infected 293-dCAT1 or chimeric dCAT1 cells with S82F pseudotype virus. The S82F pseudotype virus did not cause syncytia, but showed increased susceptibility to 293 cell expressing dCAT1. Our studies suggest that S82F induced syncytium formation is thought to arise by cell-cell fusion but not by virus-cell fusion.

Keywords: retrovirus receptor, pseudotype virus, syncytia, virus-cell fusion

G006

In vitro Antifungal Activity of Xanthorrhizol Isolated from *Curcuma xanthorrhiza* Roxb. Against Opportunistic Filamentous Fungi

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Xanthorrhizol was isolated from the rhizome of *Curcuma xanthorrhiza*. (Zingiberaceae) and its *in vitro* activity against opportunistic filamentous fungi was evaluated by using the NCCLS (M38-A) standard method. Xanthorrhizol was found to be active against all the species tested, namely *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium oxysporum, Rhizopus oryzae,* and *Trichophyton mentagrophytes*: MICs were 2.0-, 2.0-, 2.0-, 4.0-, 1.0-, and 1.0 μg/mL, while MFCs were 4.0-, 4.0-, 4.0-, 8.0-, 2.0-, and 2.0 μg/mL, respectively. Susceptibility of xanthorrhizol against 6 species of filamentous fungi was comparable to that of the commercial antifungal, amphotericin B. Xanthorrhizol also has activity to inhibit conidial germination of all tested species. The results strongly suggest that xanthorrhizol can be developed as a natural antifungal agent.

Keywords: antifungal, in vitro, opportunistic fungi, xanthorrhizol

G007

Inhibition of Bacterial Swarming by Macelignan Isolated from *Myristica fragrans* Houtt

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Inhibition of growth and swarming motility of *Bacillus subtilis* was investigated using macelignan isolated from nutmeg (*Myristica fragrans* Houtt.). Macelignan was found to inhibit both the growth of *B. subtilis* and its swarming motility. Macelignan strongly affected the growth of *B. subtilis* on LA plates (no swarming) in a concentration-dependent manner and 64 mg/cm^2 of macelignan completely inhibited the colony growth in 48 h. The growth of *B. subtilis* was also completely inhibited at 40 μg/ml of macelignan in liquid medium. Colonies of *B. subtilis* without macelignan began to swarm at a rate of 0.28 μm/s 12 h after inoculation. In contrast the swarming speed of colonies at 16-, 32-, and 64 mg/cm^2 of macelignan was 0.19-, 0.10-, and 0 μm/s, respectively. Macelignan at 64 μg/cm^2 completely inhibited swarming of *B. subtilis*, but its growth was still present. Thus, macelignan has potential to control the multicellular behaviour of Gram-positive bacteria.

Keywords: *Bacillus subtilis*, inhibition, macelignan, swarming bacteria

G008

Emergence of G Un-typeable Strains of Human Rotavirus in a University Hospital

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Introduction: The second generation rotavirus vaccine has been introduced since 2005, but the vaccine strains are different from company to company. To evaluate the success of candidate vaccines, it is essential to know the serotypes or genotypes of prevailing rotavirus strains in each country or region. The objective of this study was to characterize rotavirus G and P genotypes circulating among infants and young children hospitalized with severe diarrhea in a hospital in Gyeonggi province, Korea, in 2005. **Method**: Stool specimens were tested for rotavirus by enzyme immunoassay. A total of 128 rotavirus antigen-positives stool specimens were genotyped for G and P genes by multiplex PCR. **Results**: From January to December 2005, a total of 1,297 fecal specimens were tested for rotavirus antigen at Hanyang University Guri hospital, of these, 173 (13.3%) were rotavirus-antigen positive. Among the rotavirus antigen-positives, 128 isolates were genotyped. The globally common strains constituted 18.3% only (G3P[8] 10.3%, G2P[4] 4.0%, G1P[8] 2.4%, G4P[8] 1.6%,), and the uncommon strain, G4P[6] constituted 46.0% and G3P[4] 1.6%. Two strains were PCR negative. The new and most interesting feature of our study was the high incidence of G untypeable strains, which constituted 34.1%. **Conclusion**: The high incidence of G untypeable strain in our study could imply the emergence of new strains of human rotavirus or the appearance of genetic variation in G gene of rotavirus in Korea.

Keywords: Rotavirus, Genotype

G009

A Novel Strategy to Enhance Anti-viral Potency: Effects of Co-treatment of a Small-Interfering RNA and RNA* in Coxsackievirus B3

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Coxsackievirus B3 (CVB3) is the major pathogen of a wide spectrum of human diseases, including aseptic meningitis, encephalitis, and myocarditis. The promising potential of RNA interference (RNAi)-based antiviral therapies has been well established. We and other research groups previously demonstrated that CVB3 specific siRNA induces specific antiviral effects via direct downregulation of virus replication. However, the anti-viral potency by a single treatment of a siRNA is not satisfactory.Here, we provide the first evidence demonstrating the enhanced anti-CVB3 effects by the combined treatment of a siRNA with a novel form of RNA called RNA*. RNA* is made from *in vitro* transcription of T7 RNA polymerase that utilizes single-stranded DNA. Our recent studies have shown that RNA* leads anti-viral activity through distinct pathway from that of siRNA. When MRC cells were infected with CVB3, the cells suffered from morphological abnormality and subsequent loss of cell viability. However, in the presence of 10-20 nM of siRNA or 50pg/ml of RNA*, the cells were partially protected from virus infection. Moreover, the co-treatment of siRNA and RNA* at the identical concentration, the detrimental effects by CVB3 was drastically demolished. We believe that the similar strategy can be successfully applied to other distinct virus groups.
Keywords: siRNA, RNA*, coxsackievirus B3, antiviral effect, co-treatment

G010

A Novel Small-Interfering RNA Design Software CAPSID: To Effectively Select siRNAs Targeting Viruses with High Genetic Variation and Instability

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The promising potential of RNA interference (RNAi)-based antiviral therapies has been well established. However, the antiviral efficacy is largely limited in various viruses with genomic diversity and genetic instability. Human enterovirus B (HEB), a major causative agent of numerous human diseases, is one of them. To overcome these obstacles, we developed new siRNA design software CAPSID **(Convenient Application Program for siRNA Design) (**http://compbio.sookmyung.ac.kr/~capsid/). CAPSID can rapidly search conserved sequence patterns and these extracted conserved patterns are then applied for siRNA design. The program allows us to select siRNAs capable of targeting the conserved regions in viral genomes. Its potency as a universal anti-viral siRNA design tool was confirmed against various serotypes of HEB strains. Thus, CAPSID can be readily utilized to design effective siRNA for viruses with many different serotype viruses, along with genetic instability.
Keywords: RNA interference, small interfering RNA, anti-viral agent, CAPSID, genetic instability

G011

Role of UL112-113 Self-Interaction in Human Cyto megalovirus Replication

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Four phosphoproteins of 34, 43, 50, and 84-kDa with common amino termini are synthesized via alternative splicing from the UL112-113 region of human cytomegalovirus (HCMV) genome. We previously showed evidence that the four UL112-113 proteins both self-interact and interact with each other, and that the N-terminal region encompassing amino acids from 1 to 125 is required for both self-interaction and nuclear localization as foci. We also found that the ability of the UL112-113 proteins to relocate UL44 (the viral polymerase processivity factor) to the pre-replication sites relied on self-interaction, and reached maximal levels when the four proteins were coexpressed. To study the role of UL112-113 self-interaction in the virus context, we generated a mutant HCMV (Towne)-BAC clone encoding the N-terminal 25 amino acid-deleted UL112-113 proteins. These proteins were defective in self-interaction. When human fibroblast cells were transfected with BAC clones, the growth of mutant virus was significantly impaired compared to the wild-type and revertant viruses. We also found that the UL112-113 proteins interact with UL44 in virus-infected cells and in vitro GST pull-down assays. Our data suggest that interactions occurring among UL112-113 proteins via their shared N-terminal region are required for their intranuclear targeting and the recruitment of UL44 to pre-replication sites, and that these activities of UL112-113 are important for efficient viral DNA replication.
Keywords: HCMV, replication, UL112-113, self-interaction

G012

Investigation of Pseudorabies Virus Latency in Nervous Tissues of Seropositive Pigs by Nested and Real-Time PCR

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The prevalence and quantity of latent pseudorabies virus (PrV) in nervous tissues of pigs exposed to field strain in Korea was investigated by nested and real-time PCR. Nervous tissues including trigeminal ganglion (TG), olfactory bulb (OB), and brain stem (BS) were collected from 94 seropositive pigs. PrV latent infection in nervous tissues was initially investigated by nested PCR targeting three glycoprotein genes (gB, gE, and gG.). Based on the obtained result, latent infection was detected in 95.7% of screened animals. Furthermore, it was revealed that the examined tissues harbored different copy numbers of latent PrV genome ranging from $<10^{2.0}$ to $10^{7.1}$ copies per microgram of genomic DNA in real-time PCR analysis. These results show that under normal conditions, levels of latent PrV in the nervous tissues of pigs can vary across a wide range. Therefore, the data presented here provides information regarding control of the endemic state of PrV in Korea.
Keywords: Latent infection, porcine herpes virus, nested PCR, real-time PCR

G013

Altered Expression of Type 1 Inositol Triphosphate Receptor in the Ngsk *Prnp* Null Mice

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Some lines of PrP knockout mice showed ataxia and Purkinje cell degeneration in the cerebellum caused by the ectopic expression of the prion-like doppel protein (Dpl), which prevented by expression of the cellular prion protein (PrP^C). But physiological functions of Dpl and PrP^C and underlying mechanisms are unknown. In this study, to clarify the possible implication of both proteins on the intracellular calcium homeostasis, we have investigated the expression levels of sarco/endoplasmic reticulum Ca^{2+} ATPase type 2b (SERCA 2b) and IP3 receptor type 1 (IP3R1), the major calcium-release channel in the cerebellum, in cerebella Ngsk *Prnp* null mice and wild type mice. Both mRNA and protein levels of IP3 R1 gene were reduced in the cerebella of Ngsk with different time point compared with wild mice, but that of SERCA 2b gene was not changed. Interestingly the level of IP3 R1 mRNA expression was down regulated at early time in parallel with protein levels. The results of this study suggest that Dpl may effect on the expression of IP3 R1 in Purkinje cell so that may cause Purkinje cell degeneration, and as a result, the onset of ataxia in Ngsk Prnp deficient mice

Keywords: Prion protein, Doppel, Type 1 inositol 1,4,5 triphosphate receptor, Ataxia, Purkinje cell

G014

A Novel Spherical ssRNA Virus in *Agaricus bisporus*

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A novel mycovirus was isolated from cultivated *Agaricus bisporus* with a severe epidemic of white button mushroom malformation. This novel virus was a 35-37nm spherical virus encapsidating two single-stranded RNA(ssRNA) of about 8 and 1.7 kb with a coat protein of approximately 23kDa. This virus was wide spread in Kyungbuk and Chungnam provinces.

Keywords: *Agaricus bisporus*, Mycovirus, spherical ssRNA virus

G015

Biomarker Database: a Web-Based Biological Information of Infectious Disease Biomarkers

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Biomarker covers the use and development of tools and technologies, monitoring of drug discovery and development and understanding of prediction, causes, progression, regression, outcome, diagnosis and treatment of disease. Infectious diseases in humans have been increased in the past decades. The global burden of currently classified emerging or re-emerging infectious diseases has also increased dramatically and threatens in the near future without any national boundaries. To apply a resource for developing and maintaining biomarkers for the diagnosis, detection and pathogenic modeling research of infectious diseases, we have constructed Biomarker Database (http://biomarker.cdc.go.kr), which consisted of approximately 4000 biomarkers information related to 40 of gastrointestinal, respiratory and high-risk pathogen infectious diseases. The website provides several omics information and global news of biomarkers as well as the description of pathogens and disease. We hope it can effectively utilize this website as new information entry for researchers in the biotechnology field.This study was collaborated with Korea University and supported by a research grant of the Korea Centers for Disease Control and Prevention Project, Ministry of Health & Welfare, Republic of Korea.

Keywords: biomarker infectious disease

G016

Evidence of Ciprofloxacin Resistance Mechanism Assocoated with Efflux Pump System of *Neisseria gonorrhoeae* Isolates in Korea

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Neisseria gonorrhoeae is a gram-negative, diplococcal, pathogen, causing sexually transmitted disease, gonorrhea. The percentage of fluoroquinolone-family antibiotics, ciprofloxacin-resistant *N. gonorrhoeae* has dramatically increased every year, recently. In case of ciprofloxacin-resistant mechanism, it has been known that the mutations of DNA gyrase A, drug importing porin protein in OMP or efflux pump system endow the gram negative bacteria with drug resistance. To verify the resistance mechanism of *N. gonorrhoeae* against ciprofloxacin, we examined the strains showing high resistant levels (MIC≧16㎍/㎖) and low resistant levels (1≦MIC<4㎍/㎖), which were clinically isolated from 2001 to 2005 in Korea. To see the biological effect of the efflux pump system, various concentrations (10,30,60㎍/㎖) of efflux pump inhibitor, PAβN(phe-arg B-naphthylamide dihydrochloride) were treated with ciprofloxacin. In conclusion, the activity of PAβN(60㎍/㎖) inhibitable efflux pump demonstrated decrease the maximum of 4 fold ciprofloxacin MIC value in most of N.gonorrhoeae isolates. It is probably suggest that efflux pump system is responsible for acquiring resistance against ciprofloxacin.

Keywords: *Neisseria gonorrhoeae*, Ciprofloxacin, Efflux Pump, PAβN

G017

Construction of a Cholera Vaccine Candidate in Which Cholera Toxin B Subunit is Overexpressed

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Vibrio cholerae is a gram-negative bacterium that causes a severe diarrheal disease cholera. For the protection against cholera, the antibacterial immunity is thought to play the dominant role. In addition to antibacterial immunity, cholera toxin B subunit (CTXB) has also been shown to afford protection against cholera. To obtain a CTXB-overexpressing vaccine candidate strain, we employed the delivery transposon system by Chiang and Mekalanos. The engineered transposon, pSCGR1, which contains coding region of *ctxb* with ribosome binding site was randomly transposed into the chromosome of *V. cholerae* M7922 to place the *ctxb* gene under the control of a strong promoter. Several CTXB-expressing *V. cholerae* strains were isolated. Among them, the highest CTXB-expressing isolate produced 2.21±0.72 ug/ml of CTXB and was found to harbor an independent insertion in VC0972 gene which encodes a putative porin protein. Since the presence of the antibiotic resistance gene is not desirable in a vaccine strain, the chloramphenicol resistance gene of the transposon was removed by the expression of FLP recombinase of pCP20. The removal of the cholramphenicol marker did not affect the expression of CTXB. Although the expression level of CTXB expression in this mutant was not relatively high, this study indicates the possibility of application of the delivery system for the construction of cholera as well as other pathogenic vaccine candidate strains.

Keywords: cholera vaccine, cholera toxin B subunit, vibrio cholerae

G018

Production of Rabbit-Derived Peptide Antibodies against Coxsackievirus Capsid Proteins: Application for Detection of Enteroviral Proteins

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Coxsackieviruses B (CVB1–6) in enterovirus genus is the nonenveloped virus containing a single-stranded positive sense RNA as a genome. Coxsackievirus of icosahedral symmetry has four capsid proteins called VP1, VP2, VP3 and VP4. Although VP1 is a major antigenic determinant, VP2 and VP3 may be also important proteins for viral physiology, such as viral maturation and entry. For maturation and functional study of viral proteins, availability of specific antibodies to viral proteins is crucial. However, only commercialy available antibody against CVB is monoclonal antibody (MAb) originated from CVB5 VP1. In this study, we generated and characterized six different peptide-based polyclonal antibodies (NO1-5 and B3 targeting CVB3 VP1 and VP2, respectively). The detection potency of the antibodies was characterized by Westernblotting analysis and immunofluorescent assay. We found that NO1 is specific to CVB3, while NO3 and NO5 can detect many different coxsackievirus serotypes. Their sensitivity and availability seemed to be mostly better than commercial VP1 MAb. Moreover, these rabbit-derived antibodies found to be useful for double staining. Thus, these antibodies would allow us to study virion assembly, the kinetic expression of viral proteins and the role of viral proteins in depth.

Keywords: Coxsackievirus, Peptide antibody

G019

Development of Recombinant Lentivirus Containing siRNA Sequence Targeting Coxsackievirus 2C Region

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Coxsackieviruses B (CVB) 1 to 6 types are one of the important human pathogens which induce myocarditis, hepatitis, meningitis, pancreatitis and other diseases. However, there are no vaccines or therapeutic reagents in clinical uses. Recently, small interfering RNA (siRNA) approaches to prevent viral production have been developed. Although some reports showed effective reduction to CVB production in cell culture system, the delivery of siRNA *in vivo* still has been hampered. To overcome this limitation, we constructed a recombinant lentivirus containing siRNA sequence to Coxsackievirus 2C region, called Met-2C. Recently, we demonstrated that Met-2C siRNA functions as a universal and persistent anti-enteroviral agent in *in vitro* cell system. Recombinant lentivirus containing Met-2C (Met-2C lentivirus), clearly reduced viral production in CVB3-infected cells. In addition, Met-2C lentivirus-infected mice showed the reduction of CVB3 production in heart and liver of mice challenged with CVB3 compared with that in the mice infected with recombinant lentivirus containing GFP gene. In conclusion, we developed the Met-2C lentivirus as the therapeutic candidate for CVB infection. Moreover, as far as we know, Met-2C lentivirus is the first available reagent for *in vivo* approach in CVB3 infection.

Keywords: Coxsackievirus, small interfering RNA, Lentivirus

G020

Increased Expression of VEGF-A in the Brains of Scrapie Infected-Mice

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Prion diseases are one of fatal neurodegenerative diseases in human and animals, which are characterized by neuronal cell death, astrogliosis, spongiosis and accumulation PrP^{Sc} in the brains. In addition to its major roles in angiogenesis, vascular endothelial growth factor (VEGF) has neurotrophic and neuroprotective effects. VEGF is an ischemia-inducible factor and increased expression of VEGF has been described in other neurodegenerative disease including Alzheimers disease. In order to understand the possible roles of the VEGF-A in prion disease, we investigated the expression levels of VEGF-A in ME7 scrapie infected mice by using Western blot analysis, and immunohistochemistry. The expression level of VEGF-A had tendency to increase relative to the stage of scrapie development, and were significantly higher at end stage in brains of scrapie-infected mice compared with control mice at same time. In addition, the immunoreactivity of VEGF-A was colocalized within glial fibrillary acidic protein (GFAP) positive reactive astrocytes of hippocampus in scrapie-infected mice. In conclusion, we found that VEGF-A was upregulated and secreted in reactive astrocyte in hippocampus in scrapie infected-mice. For future study, to study relationship between neuron and VEGF secreted in reactive astrocytes, we will investigate the expression levels of VEGF-A receptor 2 in neuron of scrapie infected-brain.

Keywords: Prion diseases, VEGF-A, Reactive astrocytes, scrapie

G021

Neuronal Differentiation Upregulated during Postnatal Development of Prion Protein (PrP) Knock Out Mice Brains

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Normal cellular prion protein (PrP^C) is highly conserved in mammals and expressed predominantly in the brain. PrP^C has putative roles in many cellular processes including signaling, survival, adhesion, and differentiation. Nevertheless, the exact function of the PrP^C in the CNS has not been fully elucidated. To study the function of PrP^C in relation to cell differentiation, we investigated the expression levels of various cell marker in the brains of both Zurich I PrP(−/−), and ICR PrP(+/+) mice during postnatal development stage by Western blot and immunohistochemical analysis. Our results show that the protein level of nestin, neural stem cell marker, was increased significantly in Zurich mice during postnatal day 7-10 (P7-10). The expression levels of Neuronal nuclei (NeuN), and microtubule-associated protein (MAP2) used as a neuronal markers were also elevated robustly during P0-10 in Zurich mice compared with ICR mice. In contrast, glial fibrillary acidic protein (GFAP) used as an astrocytic marker and F4/80 used as a microglial marker were expressed at same level both in Zurich and in ICR mice. Imunohistochemically, NeuN was strongly immunostained throughout the forebrain at P7 in zurich mice compared with ICR mice. It was observed that GFAP was expressed strongly during P4-10 in brains of both ICR and Zurich mice. These results suggest that cellular PrP^C may influence in the neuronal differentiation during postnatal development stage.

Keywords: Prion protein (PrP) knock out mice, PrPC, neuronal differentiation

G022

Isolation and Molecular Characterization of G9 Human Rotavirus Strains in Korea

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Group A rotavirus is the most common cause of gastroenteritis among infants and young children. The outer layer of the viral capsid is composed of two structural proteins, VP4 and VP7 and they play important roles in protection by eliciting neutralization antibodies. Group A rotaviruses are subdivided into distinct G and P serotypes according to the antigenic differences of the VP7 and VP4, respectively. Rotavirus G9 serotype was thought to be the fifth most common serotype circulating among the population worldwide. In this study, G9 human rotaviruses were isolated from fecal samples using MA104 cells and characterized. Characteristic cytopathic effects of rotavirus were observed and rotaviral antigens were detected by ELISA and indirect immunofluorescence in MA104 cells inoculated with isolated HRV strains. Rotavirus antigen was detected in fecal samples using ELISA and G type was performed by RT-PCR and sequencing of the complete VP7 gene. The nucleotide sequences of G9 HRV isolated in this study were compared with those of other recent and prototype G9 strains from other parts of the world. Also, the nucleotide sequences of VP4 and NSP4 gene of HRV isolated in Korea were compared with those of other HRV strains from other countries. The results suggest that HRV strains isolated in Korea belong to a G9, P1A[8], NSP4[A] type.

Keywords: Human rotavirus, G9 serotype, VP4, VP7, NSP4

G023

The Effect of Simian Virus 40 (SV40) Large T Antigen in Glioblastoma Multiforme

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During the initial manufacture of the salk polio vaccine from 1954 to 1960, the vaccine was grown on rhesus monkey kidney cells, which were then treated with formalin to inactivate virus infectivity while preserving antigenicity. In 1960, there was fairly rapid recognition of SV40 contamination of these lots because SV40 is found frequently in rhesus monkey but is more resistant to formalin inactivation than polio.We gained the brain tumor tissues from surgery which were diagnosed as glioblastoma multiforme (GM). Tissues were cultured as primary condition with culture media, consequently obtained three naturally immortalized cell clones from one patient and analyzed the cells using molecular biological. Immortalized cells were characterized as oligodendroglia cells, where viruses usually anchor and proliferate. Immunofluorescence results clarified the location of SV40 large T antigen as nuclear. SV40 large T antigen was detected in brain tissue, blood sample, cultured cells, and cell cultured media by PCR method, however we failed to find virus particles by electronic microscope (EM). Instead, using cytogenic research we found the tumor cell's chromosomes were enormously changed when compared with normal cells.SV40 large T antigen is postulated to be effect on chromosomal changes in oligodendroglial cells of brain tissue. Since the patient was 60-years-old, thus we suspect that this GM patient was vaccinated with SV40 contaminated polio vaccine and induced the tumorigenesis.

Keywords: Glioblastoma Multiforme (GM), Simian virus 40 (SV40), Oligodendroglial cells, tumorigenesis

G024

Roles of Global Regulatory Proteins under Oxidative Stressed Condition in Bacteria

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Salmonella is intracellular pathogen that can survive and replicate in macrophages even though these cells are equipped with a plethora of anti-microbial mechanisms. The survival of *Salmonella* under this harsh conditions is very important to maintain infection ability in the intestinal tract. Among various survival mechanisms of *Salmonella* in stressed conditions, we have focused on *Salmonella* survival mechanism in iron-induced toxic condition. Multiple proteins were expressed in stationary *S. typhimurium* in high-iron condition. An approximately 20 kDa protein was detected as a stationary-phase specific protein in cytosolic fraction. It was identified as a Dps (DNA binding protein in stationary phase) by analysis of MALDI-TOF assay. It has been known that Dps allows DNA to form chromatin by binding to DNA nonspecifically and protects DNA from ROS (reactive oxidative species). Fur, a major regulator in iron metabolism, was revealed as a new positive regulator of *dps* expression irrespective of iron condition and functioned separately from RpoS well known as positive regulator of *dps*. Fur box in *dps* promoter region didn't concern the regulation of *dps* expression by Fur. To investigate the role of Dps, we constructed a *dps* defined deletion mutant by allelic exchange. A *dps* mutants was more sensitive to oxidative stress in iron-depleted condition, indicating that Dps play an essential role in the iron metabolism.

Keywords: Dps, Fur, iron, oxidative stress

G025

Application of Virus-Induced Gene Silencing on Study of Non-host Resistance Against *Ralstonia solanacearum* on *Nicotiana benthamian* and *Phytophthora capsici* on Pepper

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Phytophthora capsici and *Ralstonia solanacearum* are economically important pathogens in the field crops particularly Solanaceae family. To date, among commercial plant cultivars, no resistance ones have been reported. While control means of the pathogens have been appropriated, no solid solution except crop rotation was practically exploited. Recent studies revealed that a wild pepper cultivar CM334 and *Nicotiana benthamiana* showed strong resistance against *P. capsici* and *R. solanacearum* respectively indicating that the CM334 and *N benthamiana* are nonhost plants. To understand mechanism(s) related to the nonhost resistance, we employed *Tobacco rattle virus* (TRV) as a virus-induced gene silence (VIGS) vector that have utilized on functional genomics of plant science. 27 Solanaceae genes which are involved on signaling components of its defense mechanism were silenced with leaf infiltration on pepper and *N. benthamiana*. Two weeks after infiltration of TRV, sporangia ($>10^3$) of *P. capsici* and bacterial suspension ($>10^8$) of *R. solanacearum* were drenched on the crown part of each plant. MEK1, MEK2, EDS1, WRKY2, and TGA1.2 silenced pepper and NPR1, ICS, MYB1, and WRKY2 silenced *N. benthamiana* were appeared typical symptoms on the leaves and stems two weeks after challenge of each pathogen. Our results suggest that VIGS can be used a useful tool to study nonhost resistance against irresistible plant pathogenic bacteria or fungi on crops.

Keywords: Phytophthora capsici, Ralstonia solanacearum, Nicotiana benthamiana, nonhost resistance, pepper, VIGS

G026

Establishment of AAV5-Specific Monoclonal Antibodies Using the Expressed AAV5 Capsid Protein

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Adeno-associated virus (AAV) is a member of the *Parvoviridae* family. AAV is one of the most ideal delivery vectors for gene therapy, owing to non-pathogenic property, very limited host immune response and long-term persistent expression of trans-genes. However, there are concerns that presences of pre-existing immunity to delivery vectors in human might decrease its therapeutic efficacy. To overcome this limitation and to select promising vectors, we consider investigations for immune status of human against delivery vectors. For this purpose, AAV5 cap gene was cloned, expressed and monoclonal antibodies (MAbs) against AAV5 were produced. The AAV5 *cap* gene was subcloned into pBlueBac4.5/V5-His-TOPO^R vector and expressed in baculovirus expression system. The expression of AAV5 capsid in baculovirus expression system was verified by immunofluorescence assay (IFA) and Western blot analysis. For production of AAV-specific MAbs, the immune cells from mouse were fused with SP2/O myeloma cells. The recombinant baculovirus expressing AAV5 protein reacted with His_6-specific MAb by IFA. As expected, three major spliced protein bands of 78, 92 and 100 kDa were detected with an anti- His_6 MAb or anti-AAV2 polyclonal antibody. Produced MAbs reacted with recombinant baculovirus expressing AAV5 capsid protein on inoculated Sf9 cells. Our results suggest that expressed AAV capsid protein and AAV-specific MAbs would be usefully applicable for diagnosis of AAV5 infection in human.

Keywords: AAV, cap gene, expression, monoclonal antibodies

G027

Molecular Epidemiology of an Outbreak of Hepatitis A in Ansan, Korea in 2006

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Background: Hepatitis A virus (HAV) is a causative agent of acute viral hepatitis, which represents a significant public health problem. HAV is usually circulated in the environment and transmitted by oral-fecal route. **Methods:** From the end of May to mid-June 2006 a HAV outbreak occurred among employees in OO company in Ansan, Korea. Blood and fecal samples were collected from 7 HAV IgM(+) patients, who manifested an acute viral hepatitis. To detect and characterize HAV strains, RT-PCR and sequence analyses were carried out. A nested RT-PCR was performed to detect a 185 bp fragment of HAV VP3-VP1 junction region. The sequences were compared with those of corresponding reference strains of genotype I-III for phylogenetic analysis. **Results:** HAV RNA was detected in six stool samples by RT-PCR. Phylogenetic analysis of the VP3-VP1 region of detected HAV strains revealed that all 12 clones belonged to genotype III and showed nucleotide identities ranging from 90.9% to 95.2% when compared to foreign strains such as 005ROTT, 038ROTT, 068ADAM and BRAB15, which were reported in Netherlands (importation from Pakistan). **Conclusion:** The present study provides valuable information on this outbreak indicating that HAV genotype III strain, which is rarely detected in Korea, caused this viral hepatitis outbreak and that this outbreak might be associated with importation of HAV strains from foreign countries such as Pakistan and India.

Keywords: HAV, Outbreak, Genotype 3

G028

The *in vitro* Biological Control of *Fusarium solani* by Chitinases DY-2, DY-16, DY-59 and VS-9

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Four chitinolytic fungal strains, *Auarthron reticulatum* DY-2, *Trichoderma harzianum* DY-16, *Trichoderma aureoviride* DY-59 and *Rhizopus microsporus* VS-9, were isolated from soil samples. Among that three were two new chitinolytic species, *A. reticulatum* and *R. microsporus,* identified by morphology and phylogenetic analysis. Chitinases, DY-2, DY-16, DY-59 and VS-9 had some basic characteristics such as optimal temperature at 50°C, 50°C, 40°C and 60°C and pH at 5, 4, 4 and 4, respectively, and were inhibited by metal ions Ag^{++} and Hg^{++}. Soluble chitin (DD 50%) was the best suitable substrate for four chitinases and enzymatic hydrolysis productions from swollen chitin were chitin monomer and chitin dimmer. Four chitinases DY-2, DY-16, DY-59 and VS-9 inhibited germination of *Fusarium solani* microconidia at protein concentration of 0.18, 0.02, 0.004 and 0.02mg/ml, respectively, during 20 h treatment. Those chitinases digested cell wall of hypha *F. solani* to produce *N*-acetyl-*β*-D-glucosamine (GlcNAc), *N,N'* -diacetylchitobiose $(GlcNAc)_2$, and *N', N'', N''', N'''', N'''''* -acetylchitopentaose $(GlcNAc)_5$.

Keywords: Chitinase, *Aurarhron reticulatum, Trichoderma harzianum, Rhizopus microsporus, Fusarium solani*, N-acetyl-β-D-glucosamine, Biological control

G029

The Efficacy of Recombinant Protective Antigen (rPA) Anthrax Vaccine Produced from *Bacillus brevis*

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Because Anthrax Vaccine Adsorbed-Biotrax(AVA) has been reported to have several drawbacks such as partial side-effects and inconvenience of multiple-vaccination, current researches are mainly focused on the development of recombinant protective antigen(rPA) vaccine that are known to have protective effects on anthrax toxin. For the development of rPA vaccine, establishing marker to verify the efficacy of newly developed vaccine is considered to be an important task. To achieve this goal, we examined the efficacy of vaccine in guinea-pig model by applying various amounts of rPA. The rPA vaccine was prepared by adsorption of 2-fold diluted rPA from the concentration of 100ug/ml to 6.25ug/ml in 0.85% saline(pH 6.0), contained 0.5mg/ml of rehydrogel HPA. The vaccine was intramuscularly injected to animals and leaved them for 14 or 28 days as their immunization period. Then, we challenged them with virulent anthrax spores to compare their survival data to the serum levels of anti-PA IgG ELISA and toxin neutralization assay(TNA) titers. The results clearly showed that the immunization efficacy of rPA tend to increase with the addition of adjuvant than rPA alone. In addition, we could conclude that 28 days of immunization period would be suitable for obtaining decent level of protection titer in TNA, which might be used as the standard of predicting the survival rate of animals in challenge rather than anti-PA IgG ELISA.
Keywords: Anthrax vaccine, recombinant protective antigen(rPA), Efficacy test

G030

Development of a Novel Bacterial Protein Secretion System

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Protein overexpression systems have been adapted in the *Salmonella* vaccinology to facilitate foreign antigen expression. It has been shown that secreted protein antigens elicit stronger immune response than cytosolic antigen does. In order to induce high secretion, we constructed a new delivery system for foreign antigens using key components of type-II Sec dependent system, which the key components are LepB (signal peptidase), SecA (ATPase), SecB (chaperone). LepB activity in the delivery system was analyzed by *araC::*P_{araBAD}*::lepB* system, named CK76. Since CK76 harboring pBP158 and pBP244 was possible of survival on MacConkey agar plate without arabinose, we concluded that LepB in the delivery system play a normal role. SecA and SecB in the delivery system showed high production. When *Salmonella* host harboring pBP438, a derivative of pBP244 containing a *pspA* (*Streptococcus pneumoniae* surface protein A) gene, was cultured to stationary phase, it exhibited twice secretion of PspA compared that of pYA3494, a derivative of pYA3493 containing the *pspA* gene. Through western blotting with Lon, cytosolic protein, and OmpA, outermembrane protein, PspA was not secreted due to cell lysis, but showed to transfer extracellular matrix by a normal secretion. This system in *Salmonella* host maintains very stable more than 60 generations. [This work was supported by grant No. RT105-03-02 from MOCIE].
Keywords: LepB, SecA, SecB, PspA, delivery system

G031

A Tegument Protein of Human Cytomegalovirus Contains Ubiquitin-Specific Protease Activity

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Human cytomegalovirus (HCMV) UL48 encodes a high molecular weight tegument protein of 253-kDa. Despite of genetic evidence that UL48 is essential for viral growth, its function remains unknown. Recently, its homolog of herpes simplex virus type-1 (HSV-1) has been shown to exhibit a novel deubiquitinating protease (DUB) activity in its N-terminal region. UL48 was also shown to contain ubiquitin (Ub)-binding activity. In this study, we characterized protease activity of UL48. We purified the N-terminal 359 amino acid protease domain of UL48 in *E.coli* and performed in vitro assays for hydrolase activity. The result showed that UL48 (1-359) efficiently released Ub from Ub-HA fusion but not from other Ub-like protein-HA fusions such as SUMO-1-HA, ISG15-HA, or Ufm1-HA. Mutation of the conserved cysteine at 24 to serine completely abolished this activity, demonstrating that UL48 has a specific DUB activity. To investigate a role of the UL48 DUB activity in viral replication, we constructed a Towne strain-based bacterial artificial chromosome (BAC) clone, which encodes a mutant UL48 protein containing a cysteine at 24 to serine substitution. When permissive human fibroblast cells were transfected with the mutant BAC clone, reconstitution of the mutant virus was comparable to those of the wild-type and revertant viruses. Our data suggest that DUB activity of UL48 is not essential for viral growth at least in cultured human fibroblast cells.
Keywords: HCMV UL48, ubiquitin-specific protease

G032

Recombination Analysis of Porcine Endogenous Retrovirus Envelope Gene

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Human tropic Porcine Endogenous Retrovirus (PERV) is major concern in zoonosis for xenotransplantation. PERV could not be eliminated by specific pathogen free breeding. Recently recombinant PERV A/C was isolated and showed 500-fold more infectious than PERV-A to human cell. Also presence of Human endogenous retrovirus (HERV) suggests another risk for xenotransplantation. An often-proclaimed risk of PERV infection is the recombination possibility of PERV with HERV. However, potential for recombination of PERV with other viruses or recombination among the PERVs can not be passed over. Here. PERV envelope genes were cloned in domestic pigs and miniature pigs from Korea and natural recombinant were screened. Among the 169 clones, we found five recombinants. Four of five isolates showed PERV-B and A recombinants. Another one was PERV-A and B recombinant. Natural recombination rate was estimated at least three percents. Thus, our data suggest that recombination event among human tropic PERVs have the potential risk during and after xenotransplantation.
Keywords: PERV, Xenotransplantation, recombination, envelope, pigs

G033

Interference Against Heterologous and Heterotypic Lethal Challenge in Mice by Cold-Adapted Live Influenza Vaccine

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A live attenuated influenza vaccine has been evaluated as fast-acting prophylactic measures against acute influenza infection. In mouse infection model, the cold-adapted (ca) influenza virus derived from the X-31 virus (H3N2) provided efficient protection of mice against heterologous (H1N1) and heterotypic (influenza B virus) lethal challenge 1-4 days after vaccination. Even simultaneous vaccination upon virulent challenge resulted in notable reduction of pathogenic symptoms of acute infection. The vaccination resulted in immediate release of pro-inflammatory cytokines including IL-6, TNF-alpha and IL-1 representing stimulation of innate immune response. The vaccination immediate before challenge also led to generation of significant pool of reassortant viruses between the vaccine strain and the virulent viruses, suggesting that the acquirement of gene(s) of live vaccine further contributes toward attenuation of the virulence. Our results suggest that innate immune responses and genetic reassortment may play a role independently and synergistically in early protection by live influenza vaccination. The immediate protection by live influenza vaccination could be further extended to various influenza strains and may offer new options for the control of potential influenza pandemic.

Keywords: influenza, vaccine, cold-adapted, interference, innate immunity

G034

Rapid Selection of Reassortant Live Attenuated Influenza B Vaccine Strain

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Live attenuated influenza vaccine can be generated by reassortment between attenuated donor strain and virulent wild type viruses. The reassortant viruses inherit the two RNA segments encoding HA and NA surface glycoproteins from the epidemic wild type virus and the six internal RNA segments (PB2, PB1, PA, NP, M and NS) from an attenuated donor strain. The annual production schedule for influenza vaccine necessitates rapid generation and characterization of vaccine strains within narrow window of time frame. Most importantly, the identification of reassortant with desired 6:2 RNA constellation is time –consuming and labor-intensive, and therefore, efficient screening system for reassortant is essential. In this work, a rapid and simple multiplex RT-PCR system was developed to identify the origin of the genome segments. The PCR primers were designed for specific amplification of RNAs from the cold-adapted donor strain, B/Lee/40 ca virus, by multiple sequence alignment of variable region. The system was adapted and optimized with 8 independent wild type influenza B viruses. The one-step RT-PCR method is sensitive and efficient for routine identification of reassortant vaccine viruses of the desired 6:2 RNA constellation carrying the attenuation property of donor virus and immunogenicity of wild type virus. This method could be practically implemented as preliminary genotyping of reassortant live influenza vaccine strains.

Keywords: influenza, vaccine, cold-adapted, RT-PCR, genotyping

G035

Characterization of Live Influenza Vaccine Donor Strain Derived from Cold-Adaptation of B/Lee/40 Virus

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Influenza B virus is distinguished from influenza A virus in genetic structure and its host range. Vaccination against influenza B virus is important especially considering occasional severe cases especially in infants and elderly person. In this study, we developed new live attenuated influenza B virus by multiple passage of B/Lee/40 influenza virus at progressively low sub-optimal temperatures (30℃, 27℃ and 24℃). The cold-passaged mutant exhibited both temperature-sensitive (ts) and cold-adapted (ca) phenotypes in embryonated chicken eggs and MDCK cells. The pathogenicity and immunogenicity were determined in BALB/c mice following intranasal inoculation. The mice inoculated with ca virus did not show any clinical signs even at high titer(10^7 p.f.u.) infection. Replication of ca virus in mice were also monitored. The shedding of ca virus in the lung and upper respiratory tract was highly limited, and yet, the mice elicited significant level of protective antibodies. Therefore, this attenuated B/Lee/40 ca virus have potential as live vaccine for protection from influenza B virus infection.

Keywords: influenza, vaccine, cold-adaptation, characterization, B/Lee/40

G036

Distribution of Gastroenteritis Virus in Korea, 2005-2006

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From Nov. 2005 to Jan. 2006, total of 91 stool specimens were collected from children with and without acute gastroenteritis. All stool samples were tested for the presence of rotavirus A, rotavirus C, norovirus GI, norovirus GII, sapovirus, astrovirus, and adenovirus by RT-multiplex PCR method. Among these viruses, rotavirus was most prevalent with 82.4% (75/91), followed by norovirus GII (39.6%, 36/91), norovirus GI (9.9%, 9/91), and astrovirus (1.1%, 1/91). Sapovirus, rotavirus C and adenovirus were not detected in this period. We were isolated one of G1, G2 and G3, and G9 strains of rotavirus. The VP7 sequences of these strains were analyzed with previous isolated Korea strains and other countries strains. All norovirus genogruop I and II isolates associated with the outbreaks of acute gastroenteritis were further characterized for their genotypes and genetic relationship with the reference strains base on the recent norovirus capsid region classification sheme of Kageyame, 2004. The pholygenetic tree of nucleotide sequences reveal that isolates of novirus GI formed a cluster with Japan reference strain (Saitama KU19a GI) known as norovirus genogoup GI/12. In case of GII, GII-4 group was presented major group and followed GII-2 and GII-12.One astrovirus strain was isolated in this study and the strain was adapted to Caco-2 cells. It was found that astrovirus detected in the present study were serotypes 1, and showed high relationship with Newcastle strain from UK.

Keywords: Rotavirus, Norovirus, Astrovirus, Gastroenteritis

G037

Purkinje Cell Loss Induced by Autophagic Cell Death in the Ngsk *Prnp* Deficient Mice

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The functional loss of PrP^C and the ectopic PrPLP/Dpl expression induce the neurodegeneration in ataxic *Prnp* deficient mice. Ngsk *Prnp* deficient mice, one of the *Prnp* deficient mice models, show late-onset ataxia and Purkinje cell degeneration and as a result have a deficit in motor coordination. However, the mechanism of Purkinje cells degeneration was not sufficient in Ngsk *Prnp* deficient mice. Here, to elucidate the cellular mechanism involved in Purkinje cell degeneration, we observed cerebellar Purkinje cell degeneration of Ngsk *Prnp* deficient mice by using electron microscope at the ultrastructural level with different age. Degenerated Purkinje cells were more increased in cerebellum of Ngsk *Prnp* deficient mice compared with aged matched control mice. Interestingly, degenerating Purkinje cell was detected at the 3 months of age in Ngsk *Prnp* deficient mice, which contained autophagic vacuoles with various size and stages. Autophagic vacuoles contained cytoplasmic components, intracellular organelles and residues. However, chromatic condensation and nuclear fragmentation, the characteristics of apoptosis, were not observed. These results suggest that Purkinje cell degeneration may be induced by autophagic cell death in the Ngsk *Prnp* deficient mice.

Keywords: Ngsk Prnp deficient mice, ataxia, Purkinje cell, autophagic vacuole, autophagic cell death

G038

In vitro and *in vivo* Infections of Murine Gammaherpesvirus 68 in the Central Nervous System

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Gammaherpesviruses such as Epstein-Barr virus and Kaposi`s sarcoma-associated herpesvirus are important human pathogens. Recent reports argue that gammaherpesviruses may be associated with diverse neurological diseases. We used recombinant virus expressing either enhanced green fluorescence protein (MHV-68/EGFP) or Lac Z gene (MHV-68/Lac Z) to monitor MHV-68 infection in neuronal system in vitro or in vivo, respectively. Upon the infection of MHV-68/EGFP in the neuronal cell lines, expressions of EGFP and lytic viral proteins and increased number of viral genome copies were detected in a dose-dependent manner, although the efficiency to support lytic replication varied depending on the origin of cell lines. Upon intracerebroventricular injection of MHV-68/LacZ into either young or adult mice, virus replication was examined using beta-gal staining and immunohistochemistry with an antibody against a capsid protein. The results showed that virus infection was found in neuronal cells, ependymal cells, and endothelial cells. The infection was preceded more aggressively in young mice than in old ones. Consistent with this result, the mortality rate was significantly higher in young mice than in old mice, suggesting age-dependent host defense system is involved in protection against MHV-68 infection in the brain. Currently, viral genes required for this neurovirulence is under investigation. [This work is supported by KOSEF R04-2004-000-10048-0.]

Keywords: gammaherpesvirus, murine gammaherpesvirus

G039

The Detection of Human Endogenous Retroviruses in the Cerebrospinal Fluid of Individuals with Sporadic Creutzfeldt-Jacob Disease

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Human endogenous retroviruses (HERVs) constitute approximately 8% of the human genome. The possible involvement of retroviruses in prion disease has been suggested by many studies. In this study, we investigated the expression of 10 HERV families from the cell-free cerebrospinal fluids (CSFs) of individuals with 87 sporadic Creutzfeldt-Jakob disease (CJD) by RT-PCR analysis. The retroviral RNAs of HERV-W, HERV-K, HERV-FRD, ERV-9, HERV-T, HERV-L, HERV-H, HERV-F, HERV-I, and HERV-E were found in 86 (98%), 76 (87.4%), 62 (71.3%), 53 (60.9%), 33 (37%, 21 (24.1%), 12 (13.8%), 11 (12.6%), 7 (8.0%), and 2 (2.3%) CSF samples of individuals with sporadic CJD, respectively. Interestingly, the detection rate of HERV-T and HERV-L in CSFs of sporadic CJD patients was significantly increased than in those of 14-3-3 negative samples. Our results suggest that the expression of HERVs may be associated with the development of prion diseases, and it might lead to improve methods for the diagnosis of sporadic CJD.

Keywords: Prion, Creutzfeldt Jacob diease (CJD), Human endogenous retrovirus (HERV), Cerebrospinal fluid (CSF)

G040

Search and Discovery of Novel Genes on *Bacillus anthracis* Genome by Suppression Subtractive Hybridization Analysis

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Bacillus anthracis, the etiological agent of anthrax has been taxonomically classified into the *B. cereus* group with *B. cereus, B. thuringiensis, B. mycoides* and *B. weihenstephanensis*. Although the pathogenesis and ecological manifestation may be different, *B. anthracis* shares a strong degree of DNA sequence similarity with the closely related species. Therefore, the discrimination of *B. anthracis* from other members of the *B. cereus* group is still complicated. Suppression subtractive hybridization was performed to search for genomic differences between *B. anthracis* and the most closely related *B. cereus*. About 230 subtracted *B. anthracis*-specific libraries were obtained by Southern hybridization and comparative sequences were analyzed using NCBI Blastin. Subsequently, 8 primer sets specific to *B. anthracis* were designed from the sequences of subtracted clones and the specificities were evaluated from 8 *B. anthracis*, 32 *B. cereus,* 10 *B. thuringiensis,* 6 *B. mycoides* and 20 other bacillus strains. Of the primer sets, primers based on glycosyltransferase gene did not amplify PCR products from any of the bacillus strains which examined except *B. anthracis*. The data obtained can be useful for future development of efficient diagnostic tools allowing rapid identification of *B. anthracis* from other members of the *B. cereus* group.

Keywords: *Bacillus anthracis, B. cereus,* Suppression subtractive hybridization

G041

Molecular Epidemiology of Enteric Adenovirus Infections in Korea during 2001-2005

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Aim: The enteric adenoviruses type 40 (Ad40) and 41 (Ad41) are important causes of acute gastroenteritis. We analysised a partial hexon gene of adenovirus strains from patients during 2004-2005 and compared with our previous adenovirus data of 2001-2003.
Materials and Method : We collected 201 adenovirus EIA [Bioincell; Viro-capture™] positive samples from acute gastroenteritis patient in Korea during 2004-2005 in colleaboration with provincial laboratories. We performed PCR using primers targeting a partial hexon region and a 482 bp of hexon gene were sequenced (ABI Prism Dye terminator Kit, USA) and compared with adenovirus strains using DNAstar Lasergene program.
Result : Among 201 adenovirus EIA positive stool samples, we detected 132 PCR positives and sequences of 127 Ad strains were determined. The proportion of Ad 41 among adenovirus infection changed considerably during 2001-2005 period. A Ad 41 strains were detected in 64.9% during 2001-2003 and in 96% (n=127) in 2004-2005 among adenovirus PCR positive samples. Our results also coincides with global patterns of Adenovirus infections.
Conclusion : Our study showed that the proportion of Ad 40 and 41 among adenovirus infection changed in recent years propotion of Ad41 increased whereas incidence of Ad 40 decreased among adenovirus infections.
Keywords: enteric adenovirus, genotyping

G042

Development of Antigen Delivery System using *Salmonella typhi* Cytolysin A

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The cytolysin A (ClyA) is a 34-kDa pore-forming cytotoxic protein and expressed by some enteric bacteria including Salmonella typhi. This toxin is transported on the bacterial surface and secreted without posttranslational modification. Using the surface display of ClyA, the expression vectors for 193-aa immunogenic antigen of spike protein (termed S1E) from severe acute respiratory syndrome coronavirus (SARS-CoV) were constructed. The vectors carried a gene encoding *S. typhi* ClyA conjugated to S1E at the C terminus (termed ClyA-S1E) and asd gene in pGEM-T and pBR322, named pGApLCS1E and pBApLCS1E. ClyA-S1E recombinant proteins from these vectors were expressed on the surface of the attenuated *S. typhimurium* deficient of global virulence gene regulator, ppGpp. However, they did not possess the hemolytic activity on the blood agar plate and cytotoxicity against HeLa cells. To examine whether bacteria expressing ClyA-S1E induced the immune response against S1E, *S. typhimurium* deficient of ppGpp and Asd was transformed with these vectors and orally immunized in mice. In the western blotting against GST-conjugated S1E using the immunized mouse sera, it was shown that the significant band was detected in the mouse serum by the bacteria transformed with pGApLCS1E but not pBApLCS1E. It indicated that the immune response producing antibody was dependent on the expression level of ClyA-S1E. Therefore, ClyA delivery system can be used for SARS vaccine development.
Keywords: Cytolysin A, SARS-S1E, vaccine, Salmonella typhimurium

G043

Discrimination of *Streptococcus pneumoniae* from Viridans Group Streptococci by Suppression Subtractive Hybridization Analysis

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Streptococcus pneumoniae is a major cause of bacterial disease in humans, including pneumonia, otitis media, septicemia, and meningitis. Although the pathological manifestation may be different, S. pneumoniae shares over 99% of 16S rDNA homology with the closely related species, *S. mitis* and *S. oralis*. Therefore, classification of these organisms has long been considered difficult. Suppression subtractive hybridization was performed to search for genomic differences between *S. pneumoniae* and the most closely related *S. mitis*. About 115 S. pneumoniae-specific clonal libraries were evaluated with Southern hybridization and completely sequenced. Subsequently, *S. pneumoniae*-specific primers were designed and specificities were examined by gradient PCR using genomic DNAs extracted from 40 oral streptococcal species. Of those primer sets, primers based on alpha 1-6-glucosidase amplified only the genomic DNAs from *S. pneumoniae* strains. These new oligonucleotide primers may be very useful for the rapid identification and diagnosis to discriminate from other viridans group Streptococci.
Keywords: *Streptococcus pneumoniae, Streptococcus mitis*, Suppression subtractive hybridization

G044

Distribution of Human Group A Rotavirus VP7 and VP4 Types Circulating in Seoul, Korea Between 2004 and 2006

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Five hundred and four fecal specimens collected from young children with acute diarrhea between 2004 and 2006 were determined for G and P types by RT-PCR method. Of these, only 393 (77.98%) of 504 stool samples were confirmed positive-rotavirus and genotyped for outer capsid proteins VP7 and VP4. In serotyping of VP7, G1 (35.6%) were as dominant circulating serotype, following by G3 (26.5%), G4 (14.8%), and G2 (12.0). The G9 (1.0%) and G12 (0.3%) have also been found in this study. In genotyping of VP4, P[8] were as the most circulating genotype (53.2%), following by P[4] (15.3%), P[6] (15.3%), and P[9] (2.3%). The mixed genotypes of P[4]/[8] and P[6]/[8] were 5.6% and 0.8%, respectively. Determination of G- and P-type combinations revealed that G1P[8] were the most prevalent strains (25.5%), following by G3P[8] (16.8%), G2P[4] (6.4%), and G4P[6] (6.1%). Of interest of this study is that the G9 was very rare in comparison to recently reports from rural regions of Korea. This study was the continuous study of Song *et al.* (2003) in our laboratory.
Keywords: Human Group A Rotavirus, G-type, P-type, RT-PCR

G045

Characterization of Human Rotavirus Strains with G12 and P[6] Detected in Korea

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G12 strains were first detected from diarrheic children in the Philippines in 1990. After more than 10 years, human G12 strains were found in Thailand, the United States, India and Japan. A G12 human rotavirus strains, CAU195 was isolated from the previous genotyped diarrheic stools of an infant in Korea. The full length VP7, VP4 and NSP4 genes of strain CAU195 were sequenced and compared with those of G12 rotavirus available on GeneBank. VP7 gene sequences of strain CAU195 showed high identity with that of the G12 prototype strain L26, and with those of G12 strains reported recently from other countries. VP4 gene sequences of strains CAU195 showed the highest identity with those of P[6] rotaviruses. This is a first report of isolation and characterization of human rotaviruses with G12 serotype in Korea
Keywords: Human group A rotavirus, G12 serotype, VP7, VP4, NSP4

G046

Comparison of Proteome Components of *Helicobacter pylori* in Different O_2 Concentration and Knockout Mutants

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Helicobacter pylori is a Gram-negative microaerophilic bacterium that selectively colonizes the human stomach. To address the influence of oxidative stress and its underlying mechanisms, we have compared protein expression profile of *H. pylori* incubated various oxygen pressures under 5%, 2% and 18% O_2 conditions by a global proteomic analysis, which includes high-resolution 2-DE followed by Q-TOF-MS and bioinformatic databases search/peptide-mass comparison. The results revealed that more than 39 proteins were differentially expressed under oxidative stress. Most notably, the protein expression levels of 14 proteins including Pfr are up-regulated in 2% O_2 condition. Under 18% O_2 condition, the protein expression levels of 4 proteins including NapA are up-regulated. In addition, under 5% O_2 condition, the protein expression levels of 12 proteins containing AtpD are up-regulated. Within a large family of peroxidases, one member that catalyzes the reduction of organic peroxides to alcohols is known as AhpC. We have obtained *ahpC* deletion mutant of *H. pylori*. The a*hpC* deletion mutant differed in their protein expression level, compared with wild type strain. We have also performed an extensive proteome analysis of the *ahpC* deficient mutant of *H. pylori* utilizing Q-TOF-MS.
Keywords: *Helicobacter pylori*, Q-TOF, Oxidative stress

G047

Development of a Capture Enzyme-Linked Immunosorbent Assay for the Detection of *Clostridium botulinum* Neurotoxin Serotype A

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A capture enzyme-linked immunosorbent assay (ELISA) was developed to detect *Clostridium botulinum* neurotoxin serotype A (BoNT/A). The assay is based upon affinity-purified mouse and rabbit polyclonal antibodies directed against the BoNT/A complex, which was purified from culture filtrate of *C. botulinum* ATCC19397. For the capture ELISA, the optimized amount (0.2 ug/well) of rabbit polyclonal antibody was immobilized on ELISA plates to detect BoNT/A (ranging from 0.1 ug to 97.66 pg/well), which was recognized by 0.2 ug/well of mouse polyclonal antibody. From five independent repeated experiments, standard curves were linear over the range of 0~12.5 ng/well and the coefficients (r^2) ranged from 0.9962~0.9994 for all assays. The inter-variations were typically 5-12% and the specificity was confirmed by showing no cross reactivity against BoNT/B and /E. The detection limit of capture ELISA was 0.78 ng/well, which was a 4-fold improvement compared with that of indirect ELISA method using mouse or rabbit polyclonal antibody (3.125 ng/well). These results support that the capture ELISA could serve as a rapid and sensitive screening tools for detecting BoNT/A.
Keywords: capture ELISA, BoNT/A

G048

The Polymorphisms of the Prion-like Protein Gene (PRND) in Korean Normal Population and Sporadic Creutzfeldt-Jakob Disease

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To investigate whether the PRND polymorphisms are associated with an increased risk for developing sporadic CJD in the Korean population, we compared the genotype and allele frequencies of PRND polymorphisms in 95 sporadic CJD patients with those in 102 healthy Koreans. Polymorphisms of PRND gene in Koreans were found at codons 56, 174 and the 3' untranslated region (UTR) +28 site. One heterozygote at codon 56 was observed in normal controls, not in sporadic CJD patients. A strong significant difference of PRND genotype frequency at codon 174 was found between normal Korean population and various European populations. In sharp contrast to results in the German population, our study does not show a significant difference in PRND genotype or allele frequency at codon 174 between sporadic CJD and normal controls. However, a significant difference was found between sporadic CJD and normal controls in genotype distribution and in allele frequency at 3' UTR +28. These results suggest that a specific genotype (T/T) or allele (T) at 3' UTR +28 of the PRND gene is a risk factor for sporadic CJD.
Keywords: prion-like protein gene, Creutzfeldt-Jakob disease, polymorphism

G049

Salmonella typhimurium and its LPS Induce Ornithine Decarboxylase via TLR-4 Signal Pathway

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Salmonella typhimurium can infect many different hosts by its protein and nonprotein structures which act as pathogen-associated molecular patterns (PAMPs). Among the PAMPs, one of the prime candidates are Lipopolysaccharide (LPS) that binds to eukaryotic cell's PAMP receptor proteins like Toll-Like Receptor (TLR) family, especially TLR-4. A p38 mitogen-activated protein kinase (MAPK) which is a conserved subfamily of MAPKs involved in the response to stress found in eukaryotic cells is activated through the TLR-4. When cells are treated with p38 inhibitor named SB202190, p38 phosphorylation and expression of Ornithine decarboxylase (ODC) induced by S. Typhimurium and LPS were down-regulated. S. typhimurium and LPS induced a significant increase in ODC western blotting at 4 hours after stimulation. These suggest that p38 MAPK signaling can affect the expression of ornithine decarboxylase which plays essential roles in various biological functions, including cell proliferation, differentiation and cell death.

Keywords: *Salmonella*, TLR-4, p38, ODC

G050

Genotyping of the JC Virus in Urine Samples of Healthy Korean Individuals and Herpes Zoster Patients

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Herpes zoster is a neurocutaneous disease caused by the varicella zoster virus (VZV) as a consequence of declining cell-mediated immunity or by immune suppression and conditions with immunodeficiency. Reactivation of JC virus (JCV) may be linked with immunodeficiency or immunosuppressive therapy. To evaluate the relationship between the herpes zoster and JCV reactivation, the prevalence of the JCV was investigated in 102 Korean herpes zoster patients and 100 healthy Korean individuals. There are no significant differences in the incidence of JCV between the Korean herpes zoster patients and healthy controls (P = 0.5391). In order to investigate genotypes of JCV, we analyzed 45 JCV isolates amplified from Koreans by DNA sequencing and nucleotide sequence analysis. Six distinctive JCV strains were identified in the VT-intergenic region. Based on phylogenetic analysis, JCV types 1 (11%), 2A (22%), and 7B (67%) were found in these Korean patients. Interestingly, JCV type 1 found in Koreans was sub-classified into Type 1C subtype, which is different from type 1A and B substrains prevalent in European. These results indicate that herpes zoster may not play an important role in JCV reactivation and are not associated with JCV types.

Keywords: JC virus, Herpes zoster, genotype

G051

Identification of Genes Differentially Expressed in RAW264.7 Cells Infected by *S. typhimurium*

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Salmonella typhimurium causing mouse typhoid infects the host such as macrophage cells and proliferated in the intracellular vacuoles. The infected cells trigger many response genes against the infection. In this study, we tried to identify such genes of RAW264.7 cells by PCR screening method using degenerate primers. 14 genes were found to be differentially expressed after 4hr infection in which 8 genes were increased but the others decreased. Most of genes were related to proinflammatory responses such as cytokines and cell death. The mutation in *msbB* gene encoding the myristoyl transferase in lipid A of lipopolysaccharide (LPS) causes much less toxicity to the inoculated animals. We compared the expression of the identified genes in wild-type and *msbB*-mutated *S. typhimurium* infections and found that *Lyzs* encoding lysozyme type M was differentially expressed. This gene is probably related to bacterial survival in the host cells.

Keywords: *Salmonella typhimurium*, macrophage, infection, gene expression, lysozyme

G052

Investigation of *Helicobacter pylori* Alkyl Hydroperoxide Reductase (AhpC) Kinetics Using AhpC Cys-residue Mutant Series

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Helicobacter pylori is a human pathogen that causes chronic gastritis and peptic ulceration and is directly linked to gastric carcinogenesis. A long-term exposure to ROS, such as O_2^-, H_2O_2, OH^-, it's damage to *H. pylori*. But *H. pylori* has defence mechanisms from ROS. *H. pylori* alkylhydroperoxidase C (AhpC) belongs to the antioxidants, and it contains two cysteine residues(C49S, C169S) in its active site. We made mutant series C49S, C169S and double mutant(C49S/C169S mutant) and checked enzyme activity by DTT oxidation. Reduced AhpC and three type mutants were reveal 26kDa in SDS-PAGE. Non-reduced AhpC and single mutants revealed 52kDa-protein, but double mutant didn't. Active-site Cys mutants show that all two cysteine residues are important for activity. Cys-49 plays a more important role in activity than Cys-169. Interestingly, the C49S mutant is inactive, but double mutant C49/169S shows significant revertant activity. Kinetic parameters indicate that the C169S mutant is active, although much less efficient. Suprercoiled DNA can form nicks in the presence of oxidative radicals, the wild AhpC, C169S and double mutant were protection pBK DNA forming nicks but the C49S mutant didn't. AhpC is capable of acting as a general antioxidant by protecting a range of substrates including supercolied DNA. We suggest that *H. pylori* AhpC, therefore, belongs to peroxiredoxin family and might follow disulphide-relay reaction mechanism.

Keywords: alkylhydroperoxidase C, revertant activity, peroxiredoxin

G053

Polymorphisms of the Prion Protein Gene (PRNP) in Hanwoo (*Bos Taurus Coreane*) and Holstein Cattle

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Three polymorphisms were found in ORF region, the third position of codon 78, the third position of codon 192, and the deletion of a single octa-repeat. An analysis of codon 78 revealed no difference in the genotype or allele frequencies between Hanwoo and Holstein animals. However, there were significant differences in the genotype ($P < 0.0001$) and allele ($P < 0.0001$) frequencies at PRNP codon 192 between Hanwoo and Holstein animals. The rate of Holstein animals with deletion of a single octa-repeat was 91.5% undeleted homozygotes, 8.5% heterozygotes (with R3 deletion), and 0% deleted homozygotes. However, none of the 120 Hanwoo animals had any octa-repeat deletions. In the promoter region of PRNP, a significant difference ($P = 0.0249$) in allele frequency of 23 bp indel polymorphism were observed between Hanwoo and bovine spongiform encephalopathy (BSE)-affected German cattle previously described. Interestingly, the genotype and allele frequencies of 23 bp indel polymorphism in Korean Holstein was greatly similar to those previously reported for BSE-affected German cattle and healthy U.S. cattle sires. These results suggest that Korean Holstein may be more sensitive to BSE than Hanwoo.

Keywords: bovine spongiform encephalopathy, prion protein gene, Hanwoo

G054

Providing *in vitro* Models to Study Endogenous Retrovirus, Murine Leukemia Virus (MuLV), Derived from Senescence-Accelerated Mice (SAMP8) and Senescence-Resistant Mice (SAMR1)

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Senescence-accelerated mouse (SAM), senescence-prone (SAMP) and senescence-resistant (SAMR), strains were originated from ancestral AKR/J mice strains. SAMP8 strain was provided as a murine aging model because SAMP8 has shown marked impairment of learning and memory while SAMR1 has shown normal aging phenotype. Accelerated aging phenotype of SAMP8 was postulated to be resulted from endogenous retroviruses, murine leukemia viruses (MuLVs), however the reason is not clarified yet.To investigate the effect of MuLVs in SAMP8 mice, we have established hippocampal and cortex neuronal cell lines from SAMP8 and SMAR1 mice. We have used Western blot analysis, immunocytochemistry, RT-PCR, and UV plaque assay to characterize the cell lines. The morphology of the cell lines and the cell growth rate/doubling time were determined by inverted microscope and hemacytometer, respectively. So far, we have confirmed the immortalization of SAMR1 cell lines which did not express MuLV gene by RT-PCR using SV40 primers. Although we have not fully characterized SAMR1 cell lines and we need to establish SAMP8 cell lines, the establishment and characterization of these cell lines will be a very useful *in vitro* model for the study of endogenous retrovirus.

Keywords: senescence-prone mice (SAMP8), senescence-resistant mice (SAMR1), Murine leukemia virues (MuLV)

G055

The Cellular Prion Protein Prevents Autophagic Cell Death in Neuronal Cell-Line

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Recently, we reported the cellular prion protein (PrP^C), a glycosylphosphatidylinositol (GPI) -anchored membrane protein, is involved in anti-apoptotic roles against neuronal cell death induced by serum deprivation in prion protein gene (Prnp)-deficient neuronal cell line. However, the exact cellular mechanisms involved are still controversial. Autophagy is an intracellular bulk degradation system, which delivers cytoplasmic components to the lysosome/vacuole. To elucidate the mechanisms in which PrP^C is involved in autophagic cell death pathway, we compared expression patterns of microtubule-associated protein 1 light chain-3 (LC3), an autophagy marker, in Prnp-deficient ($Prnp^{-/-}$) neuronal cell line to those with wild-type (WT) neuronal cell line. The expression level of LC3-II was increased in the $Prnp^{-/-}$ neuronal cell line compare to controls under serum deprivation conditions, but not in normal growth medium cultured cells. Interestingly, co-localization of LC3 and lysosomal membrane glycoprotein-2 (LAMP-2), indicating the presence of autophagolysosomes, were present in $Prnp^{-/-}$ neuronal cell line under serum deprivation conditions but not in WT neuronal cell line. Our data suggest that PrP^C is implicated in autophagic cell death pathway in neuronal cell line.

Keywords: prion, autophagy, microtubule-associated protein 1 light chain-3 (LC3)

G056

Expression and Application of Hydrophilic Extra-viral Domain (HEVD) of A75/17-CDV H Gene in *E. coli*

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Canine distemper virus is a highly contagious lethal pathogen causing multi-systemic disease in dogs. The modified live vaccines have greatly reduced the risk of this pathogen. However, vaccine failure occurs in the presence of maternally-derived antibody. Therefore, evaluating the level of anti-CDV antibody is prerequisite for the vaccination of CDV. For the purpose of helping determine anti-CDV antibody titers, we expressed hydrophilic extra-viral domain (HEVD) of A75/17-CDV H gene in *E.coli*. The expression of HEVD was confirmed by dot hybridization as well as western blot. However, density shown by dot hybridization was darker than by western blot, indicating that *E. coli*-expressed H protein may be conformationally dependent. The *E. coli* expressed-H protein was purified and its antigen was used for ELISA. Sera of 12 dogs grouped by anti-CDV antibody titers were tested and there was a reasonable correlation between ELISA titers and antibody titers by the conventional CDV antibody detection kit. This suggests that ELISA using HEVD could be useful for the serological estimation to avoid CDV vaccine-break

Keywords: Canine distemper virus, H gene, hydrophilic extra-viral domain, E.coli, ELISA

G057

Characterization of the Locus of Enterocyte Effacement (LEE) in Different Attaching and Effacing *Escherichia coli* (AEEC) Serotypes

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Attaching and effacing *Escherichia coli* (AEEC) cause enteric infections in humans and animals. Attaching indicates the intimate attachment of bacteria to the enterocyte, effacing relates to the localized effacement of brush border microvilli. AEEC groups together enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC). The genes responsible for the development of this lesion are clustered on the chromosome forming a pathogenicity island called LEE for locus of enterocyte effacement. This 35-kb long DNA fragment includes genes encoding a type III secretion system, genes encoding type III secreted proteins (espA, espB, espD and tir) and the *eae* gene encoding intimin, an outer membrane protein involved in the intimate attachment. These genes have been sequenced from several AEEC strains and the sequences alignments revealed the presence of constant and variable regions. Development of multiplex PCR allowed us to type five of the most important genes implicated in the formation of the AE lesion. Associating the results of *eae, tir, espA, espB* and *espD* gene typing in the 42 strains studied, three pathotypes are observed: eae_{γ}-tir_{γ}-$espA_{\gamma}$-$espB_{\gamma}$-$espD_{\gamma}$(O157), eae_{β}–tir_{β}–$espA_{\beta}$–$espB_{\beta}$–$espD_{\beta}$(O26) and eae_{γ}-tir_{α}-$espA_{\alpha}$-$espB_{\alpha}$-$espD^{-}$(O111).

Keywords: AEEC, LEE, MultiPlex PCR, Pathotype

G058

The Genome-Scale Metabolic Network of *Vibrio vulnificus* CMCP6 for *in silico* Drug Targeting

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Vibrio vulnificus is a highly human-pathogenic bacterium infected through contaminated seafood or wounds. Drug development is crucial as this pathogen shows very high mortality rate when infected. However, drug development process is very costly in terms of time, money, and manpower. Therefore, we took advantage of the computational biology. In study, we reconstructed a genome-scale metabolic network of *V. vulnificus* in order to perform various *in silico* experiments for drug targeting. Subsequently, we employed constraints-based flux analysis to assess the metabolic capability of the organism. We particularly performed single gene deletion simulations, in which the whole set of reaction was deleted one by one so as to identify reactions, and thus genes, that lead to the zero biomass formation. This newly identified set was considered as first drug target candidates. This candidate set should further be narrowed down using bioinformatics techniques and verified.
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Keywords: *Vibrio vulnificus,* genome-scale metabolic network, constraints-based flux analysis, drug targeting

G059

Molecular Characterization and Pathogenicity Evaluation of a Novel Type-2a Strain of Canine Parvovirus Isolate from a Dog in Korea

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A CPV isolate from the diarrheic fecal sample of a domestic dog suffering from enteritis underwent molecular characterization and pathogenicity evaluation. Based on the result of nucleotide and amino acid sequence analysis, the isolate was classified as novel type-2a CPV with Asn in the 426th position and Val in the 555th position in VP2. Infection studies on young beagle dogs confirmed strong pathogenicity of the isolate. Two dogs inoculated with the isolate died from severe enteritis. Histologic examination of the duodenum and jejunum of the dead dogs revealed destructive lesions of villi and mucosal layers typical in CPV infection. On the contrary, three other dogs inoculated with the isolate passaged four times on A72 cell line did not develop any clinical symptoms, with their antibody titers against CPV rising significantly on 7~11dpi. This presumably was caused by the rapid attenuation of the isolate during the cellular adaptation process.
Keywords: canine parvovirus, novel type-2a CPV, molecular characteristics, pathogenicity

G060

Generation of Prion Protein (PrP) Transgenic *Drosophila*

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Although many prion protein (PrP) transgenic or knockout animal models have been generated for studying the biology and etiology of prion diseases, the neurodegenerative mechanisms of these diseases and the function(s) of prion protein remain unclear. It is suggested that a possible role of PrP^{C} may be masked by functional redundancy of other proteins. Since *Drosophila* has several advantages for investigating in the etiology and pathogenesis of many neurological disorders, we evaluated transgenic (Tg) *Drosophila* expressing wild or mutant type of human (Hu-) or mouse (Mo-) prion protein. When various forms of PrP were expressed in Drosophila neurons, the Mo-PrP was localized in pre-synaptic terminals as well as axon tracts, and the expression of mutant Mo-PrP (P101L) was found to be highly localized to presynaptic terminals compared to that of normal PrP. It is of interest that only mutant type flies exhibit bang sensitivity in early stages. These results suggest that PrP Tg *Drosophila* models may be a good model for investigation of predisposing defects in early stages as well as neurodegenerative mechanisms of prion diseases.
Keywords: Prion Protein, Neurodegeneration, Synapse, Transgenic *Drosophila*

G061

Mapping the Immunodominant Region in OmpA Using Various Recombinant OmpA

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Similar to other membrane proteins, OmpA protein is a major transmembrane outermembrane protein with multiple biological functions. However, limited information is available about its antigenic properties. We previously identified the immunodominant antigen in *Salmonella,* which was OmpA protein. In this study, it was further questioned to identifying actual epitope on OmpA antigen. To prove the role of OmpA in pathogenesis and location of precise epitope the antigenic determinants on OmpA protein, several *ompA* clones responsible for the antigenisity of OmpA, which might be absent or modified of OmpA proteins. The antigenicities of purified OmpA variants was examined by Westernblot analyses. We found the epitope of OmpA was distributed over several regions, not limited in the C-terminal domain of *Salmonella typhimurium* OmpA. Moreover, we observed a variant of OmpA protein, deleted 23 amino acids, disappeared the antigenicity on westernblot analyses. *S. typhimurium* defined *ompA* 69 bp deletion mutant, CK52(*ompA*D69bp) and *S. typhimurium ompA* 23 amino acid replacement mutant, CK75(*ompA* 23 amino acid replacement) constructed by gene replacement with recombinant plasmid pBP243 and pBP402. The immunodominant surface antigen seen in wild-type *Salmonella* diminished in proteins from *S. typhimurium* CK52 and CK75. In addition, the regions of OmpA responsible for the antigenicity were defined to prove possible mechanism involved in the subunit vaccine.

Keywords: Salmonella typhimurium, OmpA, Antigenicity

G062

Identification and Characterization of Abnormal Citrullinated Proteins by Peptidylarginine Deiminase 2 (PAD2) in Scrapie-Infected Mice

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Prion diseases are transmissible neurodegenerative disorders caused by the deposition of an abnormal isoform of the prion protein (PrP^{Sc}) in the brain. It has been reported the possible role of Ca^{2+} and neuronal cell death in prion diseases. Peptidylarginine deiminase 2 (PAD2) is a Ca^{2+}-dependent enzyme that catalyze the conversion of peptidylarginine to peptidylcitrulline. PAD2 is a unique expressed type in brain and its cellular localization was found in glial cells, especially astrocytes and microglia. It has been reported that the increased citrullinated proteins were observed in various degenerative diseases. To identify and characterize the citrullinated proteins in scrapie-infected mouse model, we first investigate protein citrullination in the brains of control and scrapie-infected mice. The expression level of PAD2 and the content of citrulline were significantly increased in the brains of scrapie-infected mice. Interestingly, we have found that the citrullinated proteins of varied molecular weights (15~100 kDa) were more intensively detected in scrapie-infected brains. We are currently identifying and characterizing these various proteins that were abnormally accumulated after scrapie infection. This study will suggest a useful biomarker for the pathogenesis of prion diseases. [This work was supported by the grants of Ministry of Health and Welfare (A020007) and the Korea Research Foundation (KRF-2006-003-200287)].

Keywords: Citrullination, Prion, PAD2, Calcium, Scrapie, Astrocyte

G063

Nuclear Transport of Hepatitis B Virus Core Protein is Mediated by Mutiple Importins

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Hepatitis B virus (HBV) is a main cause of liver disease including liver cirrhosis and hepatocellular carcinoma. Among Hepatitis B viral proteins, core protein, 21kDa, is a major capsid protein. This protein has been found both in the nuclei and the cytoplasm of infected hepatocytes. Although a nuclear localization signal (NLS) has previously been identified in the core protein sequence, details of the mechanism by which it enters the nucleus remain to be clarified. A variety of viral proteins which are translocated into nucleus have been identified and their import was facilitated by the interaction with importin α/β via their NLS

Here, we investigated the nuclear localization of HBV core protein by the expression of various types of flag-tagged importin α family and importin β in Huh 7 cell line. We show that HBV core protein interacts with multiple member of importin α family, mainly α1, α5, α7. This study suggests that the nuclear import of HBV core protein is mediated by the importin α/β dependent pathway and multiple importin a function as a mediator for the nuclear entry of HBV core protein.

The specific interactions between core and importins are under investigation using deletion mutant of core protein and knock-down of importin α proteins.

Keywords: Hepatitis B Virus, core protein, importin

G064

Upregulation of Transactivity of HBx is Correlated with Over-Expression of Ribosomal Proteins in HBV-Associated Hepatocellular Carcinoma

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Hepatitis B virus (HBV) infection remains a major public health problem including chronic hepatitis and hepatocellular carcinoma (HCC). Hepatitis B virus X protein (HBx) was estimated to play a key role for the development of hepatitis and HCC during HBV infection. Although lots of studies on the function of HBx have been performed, the relationship between HBx and HCC development was poorly understood. Recently, cellular proteins which are over-expressed in HBV-associated HCC patients have been reported. Interestingly, among 29 over-expressed genes, 13 genes were ribosomal proteins. Therefore, we focused on the roles of ribosomal proteins on HBx activity. After cloning and transfection of 18 over-expressed genes, mainly ribosomal subunits, we checked the HBx activity using NF-kb luciferase reporter system. Here, we report that most of the over-expressed ribosomal proteins upregulate the transactivity of HBx. Furthermore, we found that the enhanced activity of HBx was correlated with the solubility of HBx, judged by the reduction of punctate expression of HBx under fluorescent microscopic examination. The activity of the HBx was maximized when co-transfected with ribosomal protein S3a (RPS3a). The solubility of HBx was increased by RPS3a in a dose-dependent manner. In conclusion, we show that the over-expressed ribosomal proteins in HBV-associated HCC patients are associated with the HBx activity, suggesting the possible role of HBx on the development of HCC.

Keywords: Hepatitis B virus, Hepatitis B virus X protein, Ribosomal protein S3a, Solubility

G065

HCMV Induces Mitochondria-Targeted Apoptosis in HFF Cells

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Mitochondria often play a central role in an apoptotic pathway. Disruption of mitochondrial transmembrane potential (ΔΨm) has been observed in various cells undergoing apoptosis. HCMV infection induces apoptosis in permissive cells, however, little has been known about the mitochondria-targeted apoptosis in HCMV infected human foreskin fibroblast (HFF) cells. Here, we studied the mitochondrial pathway of apoptosis in HCMV infected HFF cells. Flow cytometry analysis using JC-1 revealed that HCMV infection induced a disruption of ΔΨm in HFF cells which started at 24 hours post infection (h.p.i) and maximized at 48h.p.i. Cytochrome C (Cyt-C), an inner membrane protein of mitochondria, was detected by western blot analysis in cytoplasmic extracts of the HCMV infected cells after 24 h.p.i., but not of mock-infected cells, suggesting that ΔΨm was disrupted and Cyt-C was released from mitochondria to cytoplasm. Caspase-3 activity assay based on fluorescence spectrophotometery using fluorogenic substrate (Ac-DEVD-AMC) revealed that caspase-3 activity was increased after 48 h.p.i. in HCMV infected cells. Therefore, HCMV infection induced mitochondria-targeted apoptosis in HFF cells.

Keywords: HCMV, mitochondria, apotosis, cytochrome C

G066

Universal Primer for Hepatitis B Virus Detection Using MATCH-UP Program

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Traditionally primers for PCR detection of viruses have been selected from genomic sequence of single or representative viral strain. However, high mutation rate of viral genomes often results in failure in detecting viruses in clinical and environmental samples. Thus, it seems necessary to consider primers designed from multiple sequences in order to improve detection of viral variants. MATCH-UP is a program intended to select universal primers (UP) from multiple sequences. We designed using MATCH-UP program UP sets for HBV detection from 12 whole genomic sequences of HBV isolated from Korean patients and 691 worldwide HBV sequences. Thousands of primer candidates were initially extracted and these were sequentially filtered down to 11 UP sets (6 Korean sets and 5 worldwide sets). These UP sets were tested by PCR using 5 HBV Korean HBsAg(+) patient sera, and eventually 6 UP sets were selected and named MUK-1,2,3 and MUW-1,2,3. These UP sets and 3 HBV reference primer sets reported by others were compared using 37 HBV HBsAg(+) sera from Korean patients. The detection rates were between 35 to 57% for MUK-2,3, MUW-1,2, and –3. MUK-1 was exceptionally efficient in detecting HBV from Korean sera, since 94.5% (35/37) of the samples were positively detected. Reference primer sets detected between 16% and 24% of the Korean samples. These results suggest that the UPs designed by the MATCH-UP program from multiple sequences could be useful in detecting viruses from clinical samples.

Keywords: PCR, universal primer, MATCH-UP

G067

Glycosylation of Japanese Encephalitis Virus prM is Important for Virus Release and Pathogenesis in Mice

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The prM protein of Japanese encephalitis virus (JEV) contains a unique potential N-linked glycosylation site at amino acid positions 15-17. To investigate its roles in JEV biology, the genome was engineered to generate three mutants (N15A, T17A, and N15A/T17A) bearing Ala substitutions at either one or both residues. Analysis of these mutants in the presence or absence of endoglycosidases revealed that the glycosylation site was indeed utilized for the addition of glycans. Three mutants lacking the glycosylation site resulted in about 20-fold reduction in the release of infectious virions without changes in intracellular accumulation levels of viral RNA and proteins; this reduction was correlated with small plaque morphology, delayed virus growth, and reduced virus yield. Significantly, three mutants lacking the glycosylation site showed a drastic reduction in mouse neuroinvasiveness after IP and IM inoculations, but no difference was observed in mouse neurovirulence after IC inoculations. Our findings suggest that glycosylation of JEV prM is required not only for its proper intracellular cleavage and virus assembly/release in cell culture but also for neuroinvasiveness in mice.

Keywords: Japanese encephalitis virus, Glycosylation, Virus release, Pathogenesis

G068

Ssk2 MAPKKK Governs the Unique Regulation of the Stress Activated Hog1 Pathway in Human Fungal Pathogen *Cryptococcus neoformans*

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The Hog1/p38 stress-activated MAPK pathway is structurally conserved in many organisms as diverse as yeast and mammals and modulates a myriad of cellular functions. The Hog1 pathway is uniquely specialized in a majority of clinical *Cryptococcus neoformans* serotype A and D strains to control differentiation and virulence factor regulation. Here we identified and characterized the Ssk2 MAPKKK upstream of the MAPKK Pbs2 and the MAPK Hog1 in *C. neoformans*. The *SSK2* gene was identified as a potential component responsible for differential Hog1 regulation between the serotype D sibling f1 strains B3501 and B3502 through comparative analysis of their meiotic map with the meiotic segregation of Hog1-dependent sensitivity to the fungicide fludioxonil. Ssk2 is the only polymorphic component in the Hog1 MAPK module, including two coding sequence changes between the *SSK2* alleles in B3501 and B3502 strains. To further support this finding, the *SSK2* allele exchange completely swapped Hog1-related phenotypes between B3501 and B3502 strains. In the serotype A strain H99, disruption of the *SSK2* gene dramatically enhanced capsule biosynthesis and mating efficiency, similar to *pbs2* and *hog1* mutations. Furthermore, *ssk2*, *pbs2*, and *hog1* mutants are all hypersensitive to a variety of stresses and completely resistant to fludioxonil. Taken together, these findings indicate that Ssk2 is the critical interface protein connecting the two-component system and the Pbs2-Hog1 pathway in *C. neoformans*.

Keywords: Ssk2 MAPKKK, *Cryptococcus neoformans*, Hog1, The stress-activated MAPK pathway

G069

In vitro Activity of Kaempferol Isolated from the *Impatiens balsamina* alone and in Combination with Antibiotics Against *Propionibacterium acnes*

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We investigated the *in vitro* antibacterial activity of kaempferol alone and in combination with antibiotics against antibiotic-resistant *Propionibacterium acnes*. We performed doubling dilutions of the antimicrobial agents from 1024 mg/L to 0.0625 mg/L in the GAM broth. Minimum inhibitory concentrations (MICs) of kaempferol, quercetin, and five antimicrobial agents were determined by microbroth dilution method for antibiotic-sensitive and resistant *P. acnes*. The antibiotic combination effect against resistant *P. acnes* was studied by chequerboard method. Kaempferol and quercetin demonstrated antibacterial activities and MICs for both compounds were ≤16 mg/L and ≤64 mg/L for clindamycin sensitive and resistant *P. acnes*, respectively. The combination formulations (kaempferol and erythromycin or clindamycin, quercetin and erythromycin or clindamycin) showed synergic effects on the inhibition of the growth of *P. acnes*. The combination of clindamycin with kaempferol or quercetin showed higher synergic effect than that of erythromycin with kaempferol or quercetin. Thus, it is predictable that the combination of antibiotics with flavonols is helpful to treat acne caused by antibiotic resistant *P. acnes*.

Keywords: Kaempferol, *P. acnes*, Chequerboard test

G070

Deferiprone can Inhibit the *in vitro* Growth of *Staphylococci* by Reducing Iron-Availability

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Deferoxamine has been widely used as the standard iron-chelating therapeutic agent for the improvement of iron-overload. Coagulase-negative staphylococci (CoNS) are known to be major pathogens in patients with iron overload. However, we found that deferoxamine can stimulate the growth of staphylococci, especially coagulase-positive staphylococci (CoPS). Accordingly, we tested whether deferiprone, a new oral iron-chelating agent, can effectively inhibit the growths of CoPS as well as CoNS by reducing iron-availability. In contrast with deferoxamine, low doses of deferiprone did not stimulate the growths of CoPS (n=26) as well as CoNS (n=27). Moreover, high doses of deferiprone could inhibit the growths of CoPS including methicillin-resistant strains (n=14) as well as the growths of CoNS on non-transferrin-bound-iron. At the same doses, deferiprone reduced the iron-saturation level of transferrin and thus could inhibit the growths of CoNS on transferrin-bound iron, but did not affect the growths of CoPS on transferrin-bound-iron. These findings indicate that deferiprone, but not deferoxamine, can be utilized to prevent human staphylococcal infections by lowering iron-availability in patients with iron-overload [supported by R13-2003-009].

Keywords: Staphylococci, Deferiprone, Deferoxamine, Iron, Transferrin

G071

Ubiquitous Desferal®-Specific Receptor in *Vibrio vulnificus*

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Deferoxamine (Desferal®) has been used as the standard iron chelator for the treatment of iron overload. However, some bacteria including *Vibrio vulnificus* have been known to be able to use the drug for iron-uptake. The *desA* gene encoding DesA which can bind to Desferal was recently cloned and sequenced in a *V. vulnificus* strain. In this study, we determined whether the *desA* gene ubiquitously exists in *V. vulnificus* strains and DesA is the real Desferal-specific receptor. In PCR, the *desA* gene existed in 10 of 10 clinical strains and in 9 of 10 environmental strains. The growths of all the *desA*-positive strains were stimulated by Desferal, but the growth of one *desA*-negative strain was not. In RT-PCR, the *desA* transcription was observed only in the presence of Desferal under iron-deficient conditions, but not in the absence of Desferal under iron-deficient conditions. A *desA*-deletion mutant did not grow despite the presence of Desferal under iron-deficient conditions, but its suppressed growth was completely recovered by the complementation of wild type *desA* gene. All these findings were also observed in cirrhotic ascites, a human *ex vivo* background. These results indicate that DesA ubiquitously exists in *V. vulnificus* strains and is the real Desferal receptor expressed only in the presence of Desferal under iron-deficient conditions, and suggest that Desferal therapy may predispose iron overloaded patients to *V. vulnificus* infections [supported by R13-2003-009].

Keywords: Vibrio vulnificus, Deferoxamine, Iron, Iron chelation

G072

Vibrio vulnificus Metalloprotease VvpE Production via the Type II General Secretion System during the Early Growth Phase

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Vibrio vulnificus is a gram-negative estuarine bacterium that opportunistically causes fatal septicemia in susceptible patients with underlying diseases. It had been believed for long time that *V. vulnificus* metalloprotease (VvpE), one of the putative virulence factors, began to be produced during the late growth phase. However, recent delicate studies revealed that *vvpE* transcription was initiated from the early growth phase albeit a low level without the verification of extracellular VvpE production. In this study, by using a simple zymography as well as the Western blot method using rabbit polyclonal anti-VvpE-body, we could verify extracellular VvpE production during the early growth phase which was consistent with *vvpE* transcription. In addition, we found that extracellular VvpE production was delayed by mutation of the *pilD* gene encoding the type IV prepilin peptidase/N-methyltransferase, and the delayed VvpE production was recovered by *in trans pilD*-complementation, which indicates that extracellular VvpE production during the early growth phase is mediated by the type II general secretion system [supported by R13-2003-009].

Keywords: *Vibrio vulnificus*, Metalloprotease, Zymography

G073

Ferrophilic Characteristics of *Vibrio vulnificus* and Potential Usefulness of Iron Chelating Therapy

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We determined the ferrophilic characteristics of *Vibrio vulnificus* to evaluate the potential usefulness of iron chelation therapy for the prevention of *V. vulnificus* infections. Readily available non-transferrin-bound iron (NTBI) is required for the growth initiation of *V. vulnificus* under *in vitro* iron-limited conditions and a human *ex vivo* condition. NTBI aided efficient transferrin-bound iron (TBI) utilization by *V. vulnificus* and vulnibactin-mediated iron-uptake system was expressed after bacterial growth had started by NTBI. *V. vulnificus* required higher NTBI levels for growth initiation, produced siderophores at lower levels and utilized TBI less efficiently than other bacteria. In addition, the growth of *V. vulnificus* was inhibited by deferiprone, a clinically available iron chelator. These results show that *V. vulnificus* is a ferrophilic bacterium which requires higher NTBI levels than other pathogens and that iron-chelation therapy may be an effective means of preventing the *in vivo* growth of *V. vulnificus* in susceptible patients [supported by R13-2003-009].

Keywords: Vibrio vulnificus, Ferrophilic bacterium, Iron, Deferiprone

G074

Subcellular Targeting of a Major Outer Membrane Protein Omp38 of *Acinetobacter baumannii*

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Acinetobacter baumannii is an important opportunistic pathogen that is responsible for hospital acquired-infections, but the pathogenic mechanism of *A. baumannii* remains unclear. A major outer membrane protein Omp38 of *A. baumannii* ATCC19606^T consisted of 356 amino acids and showed β-barrel porin. Omp38 targeted to the cell membrane of epithelial cells and played a significant role in cell invasion by *A. baumannii*. Omp38 targeted to the mitochondria and induced apoptosis of host cells by caspase- and apoptosis-inducing factor dependent pathways. Furthermore, Omp38 targeted to the nucleus of the eukaryotic cells and degraded eukaryotic DNAs by endonucleolytic and exonucleolytic attack. With regard to the nuclear targeting of Omp38, we identified a novel monopartite nuclear localization signal (NLS), KTKEGRAMNRR, between residues 320 and 330 in C-terminal region. NLS is predicted to form α-helix structure and is exposed to the bacterial surface. NLS region is conserved in the major Omps of pathogenic *A. baumannii, Acinetobacter* 3 and 13TU species, but not in *A. radioresistens* and *Acinetobacter* ADP1 strain. We propose a new pathogenic mechanism of *A. baumannii* in aspects of subcellular targeting of a major outer membrane protein Omp38.

Keywords: Acinetobacter, Nuclear localization signal, Pathogenesis

G075

Multiplex PCR for Screening of Intimin Alleles in Enterohemorrhagic *Escherichia coli* and Enteropathogenic *Escherichia coli*

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Enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) are diarrheagenic human pathogens belonging to distinct family of enteric bacteria that form unique structures called attaching and effacing(A/E) lesions on the surfaces of intestinal epithelial cells. The objective of this study was to devise a multiplex PCR for rapid detection of *eae* gene and identification of the specific three intimin alleles(α, β, γ) in *E. coli* strains. To test the allele-specific PCR assay, we examined 623 strains of diarrheagenic *E. coli* isolates during the past 9 years (1997-2005) in Korea. The results provides a specific way to classify suspected pathogens into the major clonal group of EPEC and EHEC. All 623 clinical isolated strains of diarrheagenic *E. coli* in this study were successfully categorized and easily analyzed for the presence of virulence adhering factor; intimin types.

Keywords: EPEC, EHEC, Mutiplex PCR, intimin alleles

G076

Staphylococcus aureus Skin Colonization in Children with Atopic Dermatitis

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Atopic dermatitis is a chronic inflammatory skin disease associated with colonization of the skin with *Staphylococcus aureus* known to produce staphylococcal enterotoxins (SEs) with superantigen activity. *S. aureus* and SEs have important roles in the exacerbation and prolongation of atopic dermatitis. The aim of this study was to determine the colonization rates of *S. aureus* and methicillin-resistant *S. aureus* (MRSA) in the skin of children with atopic dermatitis. We examine the colonization rates of *S. aureus* and MRSA from lesional skin of seventy-six atopic dermatitis children. *S. aureus* was isolated from 65 atopic dermatitis children (86%) and 21% of atopic dermatitis children had MRSA. This results may help to determine whether anti-*S. aureus* treatments should be considered in the treatment of atopic dermatitis.

Keywords: *Staphylococcus aureus*, atopic dermatitis, MRSA

G077

Classification of CagA Type in *Helicobacter pylori* Strains Isolated from Gastrointestinal Tract using Polymerase Chain Reaction

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H. pylori CagA protein uses type Ⅳ secretion system to be injected into gastric epitherial cells to undergo tyrosine phosphorylation, and is combined with SHP-2(Src homology 2 domain-containing tyrosine phosphatase) to disturb signal transmission system, affecting cell's proliferation, differentiation, apoptosis, and formation of hummingbird phenotype. This reaction manifests higher bonding capacity in East Asian isolates, than in Western geographic isolates, which leads to the presumption that it plays an important role in diverse sets of gastrointestinal diseases. In this experiment a total of 73 *H. pylori* strains isolated from Korean gastrointestinal tract were examined the distribution of Western *cagA* type and East Asian *cagA* type using PCR(Polymerase Chain Reaction). Experiment was conduced at the Yong-In Severance Hospital attached to Yonsei University College of Medicine, from August to November 2005. With multiplex PCR, designed in this experiment, it was possible to identify the frequency of repetition for C domain that includes WSS(Western specific sequence). Distribution ratio of Western *cagA* type is low with 4.1%, and that of East Asia *cagA* type comprises the most with 94.5%. The relationship of CagA type of *H. pylori* and the gastrointestinal disease progression should be further more studied.

Keywords: *H. pylori*, CagA, SHP-2, Western, East Asian, subtype, multiplex PCR

G078

Yolk Immunoglobulin (IgY) against Bacterial Flagellin

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Colonic inflammation by pathogenic bacteria are increasing. Recently, the pathological role of Toll-like receptor 5 (TLR-5) engagement by bacterial flagellin in colonic inflammation was reported. The aim of this study was to develop the specific yolk immunoglobulin (IgY) against bacterial flagellin. Bacterial flagellins were produced and immunized to laying hens for specific anti-flagellin IgY production. IgY titer against flagellin was determined by ELISA. Titer of anti-flagellin IgY was over 1:300,000 after 3rd immunization. Anti-flagellin IgY was significantly inhibit the bacterial flagellin in vitro and in vivo. This results are expected that specific anti-flagellin IgY can used to prevent and treat the bacterial colonic inflammation by inhibition of flagellin-TLR5 interaction.

Keywords: flagellin, IgY, TLR5

G079

Rapid Detection of the Pathogenic Agent of Bacterial White Enteritis of Larval and Juvenile Stages in Olive Flounder

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Two bacterial isolates obtained from rotifer and diseased olive flounder larvae, *Paralichthys olivaceus*, were identified as *Vibrio ichthyoenteri* based on the results of phenotypic characterization. In an attempt to develop rapid PCR method the detection of *V. ichthyoenteri*, we examined the 16S-23S rRNA intergenic spacer region(ISR) of *V. ichthyoenteri* and developed species-specific primer for *V. ichthyoenteri*. Analysis of the ISR sequences showed that *V. ichthyoenteri* contains one types of polymorphic ISRs. The size of ISRs ranged 348 bp length and not contains tRNA genes. Mutiple alignment of representative sequences from different Vibrio species revealed several domains of high sequence variability, and allowed to design species-specific primer for detection of *V. ichthyoenteri*. The specific of the primer was examined using genomic DNA prepared from 19 different Vibrio species, isolated 18 group Vibrio species and most similar sequence of other known Vibrio species.

The results showed that the PCR reaction using species-specific primer designed in this study can be used to detect *V. ichthyoenteri*.

Keywords: *V. ichthyoenteri*, 16S-23S rRNA intergenic spacer region, species-specific primer

G080

Characterization of *Streptococcus parauberis* from Cultured Flounder (*Paralichthys olivaceus*)

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This study performed for the purpose of identification of *Streptococcus parauberis* from culture flounder(Paralichthys olivaceus) with Streptococcosis in the Jeju island. The result of BIOLOG^{TM} test were Streptococcus uberis that simility of 0.5 and 98% identified in MicroLog^{TM} system(Release 4.05). Carbohydrate utility pattern were Dextrin, N-Acetyl-DGlucosamine, Arbutin, Maltose, Maltotriose, D-Cellobiose, D-Fructose, D-Mannose, α-D-Glucose, D-Mannitol, β -Methyl D-Glucoside, Salicin, Sucrose, D-Trehalose, Pruvatic Acid Methyl Ester, Mono-methyl Succinate, Glycerol. In addition hemolysis test for S. parauberis were hemolysis and hemolysis test for S. iniae hemolysis in BAP(Blood agar plate). Antibiotic test for S. parauberis were Ampicillin, Amoxicillin and Fluoroquinolone sensitivity. Mutiplex PCR assay were detected *S. pauberis*(718bp), *S. iniae*(870bp) *L. garviae* (1,100bp). Dectected *S. parauberis*(718bp) were result of 16S rRNA sequence identified with *S. parauberis*(Gene bank accession number X89967). All isolated *S. parauberis* that with bouned by one group. The result were *S. pauberis* that ɣ-hemolytic chain form cocci and negative reaction of catalase, mPCR assay were 718bp amplicon size.

Keywords: *Paralichthys olivaceus*, *Streptococcus parauberis*, Mutiplex PCR

G081

Analysis of Low Molecular Weight Proteins of *Helicobacter pylori* Strain 26695

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Investigations on low molecular weight (LMW) proteins and peptides are increasingly exploiting developments of technologies and methodologies for proteomics with a hope of discovering better indicators of the onset or progression of diseases. About fifty percents of proteins of which molecular size are lesser than 15,000 dalton were reported as hypothetical proteins in the genome sequence data of *Helicobacter pylori* strain 26695. Preparation of LMW proteins were carried out by the elution of factions from SDS-PAGE gel. The eluted proteins were cleaned and precipitaed and then the precipitated proteins were applied to two-dimensional electrophoresis, HPLC and protein identification with MALDI-TOF-MS and Q-TOF MS. As a result, we newly identified low abundant LMW proteins such as ribosomal protein L23, membrane- associated lipoprotein, hypothetical protein HP1083, hypothetical protein HP0369, which had been not visible from whole cell lysate. In addition, several hypothetical proteins were conformed to real proteins expressed from authentic genes. Analysis of LMW proteins may provide diagnostic candidates for H. pylori infection and its relative diseases.

Keywords: *Helicobacter pylori*, Proteome analysis, Low molecular weight protein

G082

The Analysis of Autoinducer Synthesis Gene Expression Involved in Quorum Sensing Mechanism with Catheter Associated Urinary Tract Infection (CA-UTI)

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Catheter-associated urinary tract infection (CA-UTI), which is frequently occurring in the patients with indwelling foley catheter, can cause higher mortality in immune deficient patients. Formation of biofilm is involved quorum sensing mechanism between infected bacteria and it has resistant to immune system of host and antibiotics. The purpose of this study is conduct to discover the mechanism of quorum sensing that forms biofilm in catheters and it's related bacteria. Biofilm forming bacteria, *E. coli, S. aureus* and *P. aeruginosa,* were isolated from infected foley catheters. Bacteria were grown in LB medium at 37℃ with shaking for 30days as single cultures and mixture culture. The cultures were collected at regular intervals (every 2 day to 30days). We observed no significant differences in the expression of four genes (*ygaG*, *luxS*, *rhlI*, and *lasI*) from samples (single and mixture culture) in each time by RT-PCR. Seems to, each autoinducer synthesis genes were expressed minimum cell density and then expressed continuously for 30 days. However, real-time PCR assays we used for quantification of the transcript levels of four genes different results. Interestingly, each genes mRNA expression was increased in mixture cultures than single cultures. And for 30 days expressed mRNA levels were increased to 9 days (or 17 days) and then decreased.

Keywords: Quorum sensing, Catheter-associated urinary tract infection (CA-UTI), Autoinducer

G083

Expression of Outer Membrane Protein H (ompH) in Transgenic Tobacco Containing *ompH (A:3)* of *Pasteurella multocida* serogroup A:3

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It has been known that outer membrane protein H (OmpH) from *Pasteurella multocida* serogroup A:3, induces fowl cholera. We cloned a gene, *ompH*, from *P. multocida* (A:3). The gene was transfected to tobacco by *Agrobacterium* –based plant gene transfer system. A tobacco harboring the *ompH(A:3)* was confirmed by DNA sequencing. And it's expression was also examined by Western-blot. Offsprings of the first and second generations are growing in pots under the controlled growth chamber.

Keywords: outer membrane protein H, Pasteurella multocida, transgenic tobacco

G084

Molecular Characterization of Human Rotavirus with G9 and P[8] Specificity Detected in Korea

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Rotaviruses are the most common etiologic agent of severe diarrhea in infants and young children throughout the world. Previous epidemiological studies reported that G1 to G4 serotypes were common, particularly G1 as the predominant strain during the past years. Recently, increasing numbers of studies have documented that G9 strain may represent a fifth epidemiologically important G serotype. In this study, a G9 human rotavirus strain, CAU202 was isolated from the previous genotyped diarrheic stools of an infant in Korea. The full length VP7, VP4 and NSP4 genes of CAU202 were sequenced and compared with those of human rotaviruses available on public databases. Phylogenetic analysis demonstrated substantial sequence of VP7 gene of CAU202 was related very closely to the G9 strains that were isolated in the Belgium (B897-00, B6590-01, B629-02) and Sweden (R46). With respect to the VP4 gene, CAU202 fell into the major genetic lineages of genotype P[8] with isolates of Hungary (Hun9) and Thailand (CU52P8). These results suggest that a Korean CAU202 strain detected in this study is belong to G9P[8] genogroup.

Keywords: Human group A rotavirus, G9 serotype, VP7, VP4, NSP4

G085

Studies on the Thymidine Kinase of Varicella-Zoster Virus

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Varicella-zoster virus (VZV), one of human herpesviruses is a member the Alphaherpesvirinae subfamily. It causes two distinct clinical diseases - varicella (chickenpox) and herpes zoster (shingles). Chickenpox, a seasonal childhood disease. is a manifestation of primary VZV infection. During primary infection, VZV establishes a latent infection in the dorsal root ganglia. Shingles results from the reactivation of the latent infection. It is a sporadic disease, reactivation appears dependent on a balance between virus and host factors, specially the host immune system, The incidence of shingles increases age-dependently, especially among elderly people. The thymidine (Thd) kinase (TK) encoded VZV is a pyrimidine salvage enzyme responsible for the synthesis of thymidine monophosphate (TMP) from Thd and ATP. It catalyzes the initial activation (phosphorylation) of antiviral nucleoside analogues such as acyclovir (ACV), ganciclovir (GCV), (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVdU), and many 5-substituted deoxyuridine compounds. Like the HSV-1 TK it can further phosphorylate TMP and BVdU-monophosphate to diphosphate-forms. It has been less studied compared to the HSV-1 TK. So we report results of the cloning, expression and DNA sequencing of the VZV TK gene and the enzyme activity of the recombinant TK protein and the localization of the TK protein in virus-infected cells.
Keywords: Varicella-zoster virus, Thymidine kinase, DNA sequence, Intracellular localization, Expression, Enzyme activity

G086

Optimal Growth Condition for *Helicobacter pylori* in the Thin Layer Liquid Culture

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In General, shaking incubation jar or plastic pack that maintain a microaerobic condition have been used for liquid culture of *Helicobacter pylori*. Recently, it was reported that *H. pylori* can grow equally well *in vitro* under microaerobic or aerobic conditions at high bacterial concentrations by shaking shallow-layered liquid culture and behaved like oxygen-sensitive microaerophiles at low cell densities. However, study on the growth of *H. pylori* by standing thin layer liquid culture under various atmospheric conditions has not been reported yet. Here, we compared the growth of *H. pylori in vitro* under different oxygen and carbon dioxide partial pressures with variations of media, volumes and additives such as serum or Isovitalex. This study showed that *H. pylori* grew best in brucella broth with a thin-layered liquid volume of 3 ml which contained 10% horse serum under 10% CO_2 and 18% O_2 atmospheric condition. Viability of cells was also better in liquid media at the atmosphere with 10% CO_2 and 18% O_2 than that with 10% CO_2 and 5% O_2, which has been known as the best atmospheric condition for *H. pylori* growth. Addition of Isovitalex or DMCD instead of horse serum exhibited nothing to be favorable to growth of *H. pylori*. The growth curve entered an exponential phase at 12 hours and a stationary phase at 28 hours. In this study, we found an optimal growth condition for *H. pylori* in the thin layer liquid culture.
Keywords: *Helicobacter pylori*, thin layer liquid culture, atmospheric condition

G087

Galectin-3 Expression Correlates with Prion Protein Accumulation in Murine Scrapie and Human Creutzfeldt-Jakob disease

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To investigate the involvement of galectin-3, a novel substrate for matrix metalloproteinases (MMP)-2 and -9, in the process of neurodegeneration in prion diseases, the expression and cellular localization of galectin-3 were studied in the brains of a mouse model of prion disease (scrapie) and human Creutzfeldt-Jakob Disease (CJD). Both messenger RNA and protein of galectin-3 were significantly increased in scrapie-affected brains, particularly at the time when the abnormal prion protein, PrP^{Sc}, began to accumulate in the brains. In addition, we found that galectin-3 was co-immunoprecipiated with MMP-2, but not with MMP-9 and that galectin-3 binds to normal prion protein, PrP^{C}. Immunohistochemically, immunostaining for galectin-3 was found mainly in B4 isolectin-positive cells (presumably activated microglia/macrophage), but not in astrocytes; the staining was in areas of PrP^{Sc} accumulation and neuronal death in scrapie infected brains as well as in human brains with CJD. These findings show that induction of galectin-3 is associated with MMP-2 activation in brain phagocytes in human CJD and in its mouse model; these results suggest that increased expression of galectin-3 plays an important role in the neurodegenerative processes in prion diseases.
Keywords: Prion disease, Galectin-3, Matrix metalloproteinase-2, Microglia, Macrophage

G088

The Study about Functional Domain Essential to Surface Localization of SipB in *Salmonella Typhimurium*

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It has been known that SipB, one of invasion proteins encoded in *Salmonella* pathogenisity island 1 (SPI-1), is secreted outside cell and localized on outermembrane. However, a functional domain essential to surface localization of SipB is not investigated yet. This study was undertaken to investigate the functional domain essential to surface localization of SipB. We show that only the first ~ 160 amino acid of SipB N-terminal sequence was directed on outermembrane and could mediate the recombinant reporter proteins to outermembrane localization. The 100 ~ 140 amino acids fragment of SipB was indispensable to the localization to outer membrane. Proteinase K susceptibility and immunofluorescence assay indicated that SipB was not incorporated into outermembrane but displayed bacterial surface. We also showed that when the fragment (31 ~ 160 amino acids of SipB) devoid of secretion signal of SipB was expressed in bacterial cytoplasm, the fragment could be neither secreted into culture supernatant nor displayed on cell surface.
Keywords: Salmonella, SipB, outermembrane localization

G089

Cellular Intoxication Mechanism of Cleaved MAPKK in Murine Macrophages RAW264.7 Treated with Anthrax LeTx

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Anthrax, a disease of mammals (including humans) is caused by *Bacillus anthracis*, a gram-positive, spore-forming, rod-shaped, toxin-produced bacterium. Anthrax lethal toxin (LeTx) consists of protective antigen and lethal factor. Intoxication of murine macrophages (RAW 264.7) with the anthrax lethal toxin (LeTx 100 ng/ml) results in profound alterations in the host cell gene expression. The role of LeTx in mediating these effects is unknown, largely due to the difficulty in identifying and assigning function to individual proteins. In this study, we have used two-dimensional polyacrylamide gel electrophoresis /MALDI-TOF mass spectrometry and GeneChip (Affymetrix) to analyze earlier responses of murine macrophages treated with the LeTx, In 2DE/MALDI-TOF analysis, cleaved mitogen-activated protein kinase kinase (MAPKK1) were increased in the LeTx treated macrophage . In GeneChip analysis, dual-specificity phosphatase 6 (Dusp6), Jun oncogene (Jun) were decreased in LeTx treated macrophage. Our results suggest that these approaches are a useful tool to study earlier response in intoxicated macrophages and contribute to the identification of a putative substrate for LeTx.

Keywords: Anthrax, *Bacillus anthracis*, Lethal toxin, two-dimensional polyacrylamide gel electrophoresis, GeneChip

G090

Natural Iminosugar Derivatives of 1-deoxynojirimycin Inhibit Glycosylation of Viral Hepatitis Proteins

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A silkworm extract known to contain naturally occurring iminosugars including 1-deoxynojirimycin derived from the mulberry was evaluated in surrogate HCV and HBV in vitro assays. The antiviral activity of the silkworm extract and one of its purified constituents, 1-DNJ, was demonstrated against BVDV and GB virus-B both members of Flaviviridae, and against WHV and HBV both members of Hepadnaviridae. The silkworm extract exhibited a 1300 fold greater antiviral effect against BVDV in comparison to the purified 1-DNJ. Glycoprotein processing of BVDV envelope proteins was disrupted upon treatment with the naturally derived components. The glycosylation of WHV envelope proteins was affected to a greater extent upon treatment with silkworm extract than the purified 1-DNJ as well. The mechanism of action for this therapy may lie in the generation of defective particles which are unable to initiate the next cycle of infection as demonstrated by inhibition of GBV-B in vitro. We postulate that the 5 constituent iminosugars present in the silkworm extract contribute, in a synergistic manner, toward the antiviral effects observed and may complement conventional therapies. These results indicate that pre-clinical testing of the natural silkworm extract regarding the efficacy of treatment against viral hepatitis infections can be evaluated in the respective animal models, in preparation for clinical trials in humans.

Keywords: iminosugar, silkworm, HCV, HBV, anti-viral

G091

Lithium Aluminum Hydride ($LiAlH_4$) and Sodium Borohydride ($NaBH_4$) Cause Reductive Cleavage of Oxidized Molecules Leading to a Reduction of Prion Infectivity and Levels of PrP^{Sc}

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Previous evidence that RNA is required for PrP^{res} amplification *in vitro* suggested that RNA molecules can function as cellular co-factors for PrP^{Sc}. An approach to this issue would employ chemicals that can cleave phosphodiester bonds of RNA and then assess the effects on the infectious agent. Lithium aluminum hydride ($LiAlH_4$) is a reducing agent that can induce reductive cleavage of oxidized molecules such as carbonyls, carboxyl acids, esters, and phosphodiester bonds. Starting with scrapie brain homogenates, we found that $LiAlH_4$ destroyed PrP^{Sc}, extended the scrapie incubation period and markedly reduced total RNA concentration. These findings prompted us to investigate whether RNA molecules are co-factors for PrP^{Sc} propagation. RNase A treatment of partially purified PrP^{Sc} and of 263K brain homogenates was sufficient to increase sensitivity of PrP^{Sc} to PK, providing the first evidence that RNA molecules are a component of PrP^{Sc}. However, RNase A alone and PrP^{Sc} degradation by RNase A plus PK *in vitro*, did not show profound loss of scrapie infectivity compared with $LiAlH_4$, suggesting oxidized molecules can be important in the scrapie agent replication process. Our data suggest that RNA molecules can be important in maintaining the structure of PrP^{Sc}. Chemicals that cleave highly oxidized molecules, such as $LiAlH_4$, have a profound effect on infectivity.

Keywords: PrPSc, Oxidized molecules, lithium aluminum hydride, RNA

G092

Arabidopsis Tonoplast Protein TIP1 and TIP2 Interact with the *Cucumber mosaic virus* 1a Replication Protein

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Cucumber mosaic virus (CMV) replication complex is associated with cellular membranes. However, it is unknown whether any host factors participate in this process. In this study, five groups of *Arabidopsis tonoplast intrinsic protein* (*AtTIP*) genes were isolated and their interaction with CMV proteins was tested. TIP1 and TIP2 proteins interacted more strongly with CMV 1a protein in the yeast two-hybrid system than proteins from the other three subgroups. The interaction of CMV 1a with TIP1 and TIP2 proteins was confirmed in the Sos-recruitment system and co-immunoprecipitation assays, whereas interactions with the other three subgroups were not observed in these two assays. CMV 1a was co-localized with TIP1 and TIP2 in transfected *Arabidopsis* protoplasts. In addition, the accumulation of CMV CP was slightly delayed in *tip* mutant plants and the viral RNA accumulation was delayed compared to wild type Col-0. These results suggest that the two TIP groups could affect the formation of replication complex in the tonoplast by interacting with CMV 1a.

Keywords: Cucumber mosaic virus, tonoplast intrinsic protein, protein-protein interaction

G093

AIDS Pathogenesis in Association with p53 and HIV-1 Tat

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Few papers have reported the HIV-1 inhibition in line with p53 of infected cells. However, the detail mechanism for the p53-medicated HIV-1 suppression has not yet been clearly revealed. In the consecutive experiments, we found that the Tat was phosphorylated by transfection of p53 and the phosphorylation intensity was increased in proportional to the amounts of transfected p53. Through the kinase experiment we found that PKR is likely to be involved in the p53-mediated Tat suppression. Recombinant PKR was co-immunoprecipitated with recombinant Tat. The expression of PKR was markedly enhanced by the expression of p53. It means that PKR-Tat interaction depends on the p53 and the interaction seems to result in PKR-mediated Tat-phosphorylation. PKR-knock out Jurkat cells didn't show the p53-mediated Tat suppression. In a series of mutation studies, we found that p53-mediated Tat suppression is strongly associated with Tat phosphorylation at a specific sites via activated PKR. It was also newly found that the PKR-mediated Tat-phosphorylation blocks the Tat/TAR binding, probably followed by inhibition of the Tat-mediated transcription. It means that the HIV-1 latency in the early stage of infection is due to the p53-triggered Tat-phosphorylation through activated PKR. Localization of phospho-Tat and HIV-1 replication in the PKR-knock-out Jurkat cells are under investigation.AIDS therapeutic drugs and vaccine development will be discussed when the time is allowed.
Keywords: HIV-1, PKR, tat

G094

PKR Regulates Tat-Mediated Transactivation and HIV-1 Replicaiton by Disrupting the HIV-1 Tat and Humen-CycT1 Interaction

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Human immunodeficiency virus type 1(HIV-1) Tat activates the HIV-1 long terminal repeat (LTR), mainly by recruiting the P-TEFb (hCycT1 & Cdk9 complex) to the viral TAR RNA element. It was reported that the p53 is induced and activated by HIV-1 infection during the integration of proviral cDNA into the host chromosome. It was found recently in our Lab that the activated p53 induces PKR expression. These results are consistent with the report that PKR is induced and activated in the presence of HIV-1 infection. Furthermore, we also found that PKR directly interacts with HIV-1 Tat and phosphorylates the Tat protein at a highly conserved serine and threonine residues around the activation domain. Here, we show that Tat is phosphorylated by PKR at least three serine/threonine residues in the CycT1 binding domain (or activation domain). In addition, a Tat-TAR-CyclinT1 ternary complex is completely inhibited in the presence of PKR. We propose that PKR is a negative regulator of Tat-mediated transactivaiton, indicating that the viral transcription and replication is suppressed by Tat phosphorylation.
Keywords: HIV-1, tat, cyclin T1, phosphorylation

G095

Newly Designed Six-Membered Azasugar Ncleotide-Containing Phosphorothioate Oligonucleotide (AZPSON) as a Potent HIV-1 Drug

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A series of modified oligonucleotides (ONs) containing a phosphorothioate (P=S) backbone and a six-membered azasugar (6-AZS) as a sugar substitute in a nucleotide were newly synthesized and tested for their ability to inhibit the human immunodeficiency virus type 1(HIV-1) by simple treatment of HIV-1 infected cultures without any transfection process. While unmodified P=S ONs had little anti-HIV-1 activity, the six-membered azasugar nucleotide (6-AZN)-containing P=S oligonucleotides (AZPSONs) showed remarkable inhibition of HIV-1/SHIV replication and syncytium formation (EC_{50} = 0.02~0.2 mM) without any cytotoxicity up to 100 mM. DBM-2198, one of the most effective AZPSONs, showed antiviral activity against a broad spectrum of HIV-1, including T-cell tropic, monotropic and even drug-resistant variants of HIV-1. These anti-HIV-1 activities of DBM-2198 were similarly maintained in peripheral blood mononuclear cells. When severely infected cultures were treated with DBM-2198, syncytia disappeared completely within 2 days. Taken together, our results suggest that DBM-2198 can be considered for further development as a safe and effective AIDS-therapeutic drug against a broad spectrum of HIV-1 variants.
Keywords: AZPSOIN, oligonucleotide, HIV-1, antiviral drug

G096

Azasugar-Containing Phosphorothioate Oligonucleotide DBM-2198 Inhibits Human Immunodeficiency Virus Type 1 (HIV-1) Replication by Blocking HIV-1 gp120 without Affecting V3 Region

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A series of modified oligonucleotides (ONs) containing a phosphorothioate (P=S) backbone and a six-membered azasugar (6-AZS) as a sugar substitute in a nucleotide were newly synthesized and tested for their ability to inhibit the human immunodeficiency virus type 1(HIV-1) by simple treatment of HIV-1 infected cultures without any transfection process. While unmodified P=S ONs had little anti-HIV-1 activity, the six-membered azasugar nucleotide (6-AZN)-containing P=S oligonucleotides (AZPSONs) showed remarkable inhibition of HIV-1/SHIV replication and syncytium formation (EC_{50} = 0.02~0.2 mM) without any cytotoxicity up to 100 mM. DBM-2198, one of the most effective AZPSONs, showed antiviral activity against a broad spectrum of HIV-1, including T-cell tropic, monotropic and even drug-resistant variants of HIV-1. These anti-HIV-1 activities of DBM-2198 were similarly maintained in peripheral blood mononuclear cells. When severely infected cultures were treated with DBM-2198, syncytia disappeared completely within 2 days. Taken together, our results suggest that DBM-2198 can be considered for further development as a safe and effective AIDS-therapeutic drug against a broad spectrum of HIV-1 variants.
Keywords: AZPSON, HIV-1 antiviral mechanism

G097

Identification and Analysis of Human Papillomavirus Genotypes in Korean Women

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Human papillomavirus genotypes isolated from 99 Korean women with cervical lesions were identified and analyzed.The high-risk type HPV is the main epidermiological factor of cervical cancer, which is one of the leading causes of cancer deaths in women worldwide, and also in Korea. Therefore, the identification of HPV types is very important and necessary for the diagnosis and remedy of cervical cancer. In this paper, we detected and identified types of human papillomavirus purified from cervical lesions obtained from 99 Korean women using 4 primer sets and DNA chip by "PCR/direct sequencing" and the BLAST program analysis. We performed PCR analysis with 4 different primer sets (PGMY, G4, G6, GP1) and the detection rates per primer set for 99 patient-samples were 22 %(22/99), 36 % (36/99), 59 % (59/99), 74 % (74/99), respectively. Among 4 different primers, the sensitivity of the GP1 primer was the highest. In total 99 cases, 71 cases were identified as HPV-16 (21%), HPV-58 (6%) and HPV-26, 31, 52, 66, and 68 (4% each), HPV-6, 11, 18, 33, 35, 39, 40, 42, 44, 51, 53, 56, 59, 61 and 70 (each 3%), HPV-30, 32, 34, 45, 54 and 55 (each 1.4%). The frequency of high-risk type was the highest (72%) in 30-40 years old.
Keywords: HPV, diagnosis

G098

Human Metapneumovirus Infection in Seoul from 2004 to 2006

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The human metapneumovirus (hMPV) is a recently identified Paramyxovirus associated with acute respiratory tract infections (ARIs) in humans. The clinical features were similar to those caused by influenza-like illnesses (ILI) to severe bronchiolitis and pneumonia. This study was conducted the isolation and characteristics of hMPV from patients with ARIs as part of ILI surveillance during 2004-2006. A total of 757 specimens collected by nasopharyngeal aspirate or swab from ARI patients. Of these, 27 were detected the L and N gene of hMPV by reverse transcription-PCR. The age range of the patients with hMPV infection was 1 months to 67 years with similar numbers of females (59.3%) and males (40.7%). The main symptoms were fever (80%), nasal mucus (66.7%), vomit (26.7%), diarrhoea (20%), and difficult breathing (20%) and the main diagnosis were pneumonia, bronchiolitis, and nasoharyngitits rhinitis. Virus activity peaked in March and May. Phylogenetic analysis of the L and N gene confirmed the existence and simultaneous circulation within one epidemic season of hMPV isolates belonging to two genetic lineages.
Keywords: hMPV, ARI

G099

Comparison of Appearance between Origin and Korean Outbreak on Experimentally Transmit Chronic Wasting Disease Agent into elk^{prp}TG Mice

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Chronic Wasting Disease (CWD), a transmissible neurodegenerative disease caused by unusual pathogens called prions, has been raged in North America's cervidae population recently. Also, in Korea, it has been reported that outbreak from imported Canadian elk and deer. The aim of this study was to document which entity of CWD agent have entered Korean and to study distinction of CWD agent in TGelk^{prp} mice by lesion profiling. The homozygous TGelk^{prp} mice were infected with three CWD agents (originated from one Korea's and two North America's cevidae) and carried out an experiment on incubation time, neuropathological lesion profile, pattern of PrP^{sc} deposition and western blot profile. Interestingly, We found that the TGelk^{prp} mice infected with Korea CWD case's brain homogenate showed more short incubation period and severe vacuolar degeneration than control, as well, amount of PrP^{sc} deposit as a hallmark in prion diseases. These results suggested that homozygous TGelk^{prp} mice efficiently transmit in short incubation periods are valuable not only as research tool of cervid prions but also as reliable diagnostic tools. Furthermore, CWD agent that outbreak in Korea could be more virulent than North American's.
Keywords: Chronic Wasting Disease, Incubation time, Lesion profile, Cervidized homozygous mice

G100

Emergence of Multidrug-Resistant *Salmonella enterica* Serovar Typhi Containing Class 1 Integron in Nepal

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A total of 121 *Salmonella enterica* serovars Typhi and Paratyphi A isolated from the enteric fever patients at a University Hospital in Nepal between February 2004 and January 2006 were tested for their antimicrobial susceptibility. The occurrence and cassette content of integrons as well as molecular mechanisms of resistance among the multidrug resistant (MDR) *S. typhi* were evaluated. Thirty nine percent of the isolates were susceptible to all the antimicrobial agents tested. Seven of the *S. typhi* strains were MDR. All MDR *S. typhi* isolates contained class 1 integron with a single cassette, *dfr*A7, conferring resistance to trimethoprim. Analysis of molecular mechanism of resistance in the MDR isolates identified six different resistant genes. Resistance to sulfamethoxazole, streptomycin, ampicillin, tetracycline and chloramphenicol were mediated by *sul*1, *str*A-*str*B, bla_{TEM} like, *tet*B and *cat*A genes respectively. To our knowledge, this is the first report of the occurrence of integron and molecular characterization of mechanism of resistance of *S.typhi* isolated from Nepal. Thus, this study suggests the need for all round surveillance of the integron and antibiotic resistance genes because *S. typhi* could become resistant to broad spectrum cephalosporins by integrating cassettes that encode resistant to them , such as veb-1, a common cassette in Asia.
Keywords: multidrug-resistance, *Salmonella typhi*, mechanism of resistance, class 1 integron, Nepal

G101

Amikacin-Resistant Isolates of *Enterobacteriaceae* Collected from a University Hospital in Korea for 11 Years

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We investigated antimicrobial susceptibility and resistance mechanism of 96 isolates of amickacin-resistant *Enterobacteriaceae* collected from a University hospital in Korea for 11 years. The amikacin-resistant strains isolated in 1995 and 1996 showed low-level resistance to aminoglycosides such as kanamycin, tobramycin, gentamicin, and amikacin. However, the amikacin-resistant strains isolated after 1997 revealed high-level resistance to those aminoglycosides (MIC, $\geq$ 512 μg/㎖). The *armA* and *rmtB*, 16S rRNA methylase genes confer high-level resistance to aminoglycosides, were found in 64 strains and 17 strains, respectively. In addition, most of the strains carrying *armA* or *rmtB* revealed resistance to extended-spectrum beta-lactam, double-disk synergy test (DDST) was performed to test whether these isolates produce extended-spectrum β-lactamase (ESBL) as well as 16S rRNA methylase. Sixteen (64%) of 25 strains of *E. coli* carrying *armA* or *rmtB*, and 39 (93%) of 42 strains of *K. pneumoniae* carrying *armA* or *rmtB*, showed positive result in DDST. The result strongly suggests that the 16S rRNA methylase gene and ESBL gene may locate on the same plasmid and thereby enable the strains carrying this plasmid to confer resistance to both aminoglycosides and extended-spectrum β-lactam.

Keywords: amikacin-resistance, armA, rmtB, ESBL

G102

Conjugative R-plasmids Found in Amikacin-Resistant Isolates of *Escherichia coli* and *Klebsiella pneumonia* Carry both Extended-Spectrum Beta-Lactamase Gene and 16S rRNA Methylase Gene

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In a previous study, we found that most of the clinical isolates of *Enterobacteriaceae* carrying 16S rRNA methylase gene were presumptive extended-spectrum beta-lactamase (ESBL)-producers. To determine whether both 16S rRNA methylase gene and ESBL gene locate the same conjugative plasmid, we performed the conjugation experiment with 12 isolates of *E. coli* and 35 isolates of *K. pneumoniae* which revealed a positive result in double-disk synergy test and found to carry 16S rRNA methylase gene, such as *armA* and *rmtB*. From the conjugation experiment, 47 transconjugants were obtained which grown on selective agar plates containing amikacin and extended-spectrum beta-lactam and they were further characterized for the incompatibility group of the conjugative plasmid as well as for the presence of 16S rRNA methylase and ESBL genes. Among 47 strains, *armA* and *rmtB* were detected from 30 strains and 17 strains, respectively. Among 30 strains carrying *armA* gene, bla_{SHV}, bla_{SHV}/bla_{DHA-1}, $bla_{CTX-M-3}$, and bla_{CMY-1} were detected from 12, 11, 6, and 1 strain, respectively. Among 17 strains of carrying *rmtB* gene, $bla_{CTX-M-14}$, bla_{SHV}/$bla_{CTX-M-14}$, and bla_{TEM} were detected from 9, 7, and 1 strain, respectively. Interestingly, RepL/M replicon was detected in all six strains, carrying both *armA* and $bla_{CTX-M-3}$, and RepA/C replicon was detected in all 16 strains, carrying both *rmtB* and $bla_{CTX-M-14}$.

Keywords: amikacin-resistance, 16S rRNA methylase gene, ESBL

G103

Impairment of p38 MAPK-Mediated Cytosolic Phospholipase A_2 Activation in the Kidneys is Associated with Pathogenicity of *Candida albicans*

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In studying the mechanisms underlying kidney susceptibility to candida infection, we previously reported that the reduced production of cytokines (i.e. Tumor necrosis factor (TNF)-α) via platelet-activating factor (PAF)-induced activation of Nuclear factor-κB (NF-κB) renders the organ susceptible to the fungal burden. In this study, we investigated the possibility that pathogenic *Candida albicans* may evade clearance and perhaps even multiply by inhibiting elements in the signaling pathway that leads to the production of TNF-α. The fungal burden of pathogenic *C. albicans* in the kidneys was 10^4-10^5-fold higher than that of a nonpathogenic strain. PAF-induced early activation of NF-κB and the expression of TNF-α mRNA were observed in the kidneys of mice infected with nonpathogenic, but not pathogenic, strains of *C. albicans*. Impairment of PAF-mediated early NF-κB activation following infection with pathogenic *C. albicans* was associated with prevents activation of the enzyme cytosolic phospholipase A_2 ($cPLA_2$) as well as the upstream pathway of $cPLA_2$, p38 mitogen-activated protein kinase. Collectively, these findings indicate that *C. albicans* appears to exert its pathogenicity through impairing production of anti-candidal cytokines by preventing $cPLA_2$ activity. This novel mechanism provides insight into understanding and perhaps a target for the treatment of pathogenic *C. albicans*.

Keywords: *Candida albicans*, PLA_2, TNF, NF-κB, MAPK

G104

DNA and Recombinant Protein Vaccines for Foot-and-Mouth Disease Virus Induce Antigen-specific Antibody Immune Responses

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Foot-and-mouth disease virus (FMDV) is a small single stranded RNA virus which belongs to the family *Picornaviridae*, genus Apthovirus. It is a principal cause of FMD that highly contagious disease in livestock. Among other serotypes of FMDV, type O has been extremely hard on farmers and global market economically last five years. Structural protein VP1 is involved in the neutralization of viral infectivity, and it has both B and T cell epitopes. RNA-dependent RNA polymerase 3D is highly conserved among other serotypes and immunogenic. Therefore, we selected VP1 and 3D as vaccine targets. VP1 and 3D genes were cloned into pcDNA3.1 vector for DNA vaccine and into pET for recombinant protein production. Expressed VP1, 3D proteins were confirmed by Coomassie staining and Western blot analysis using Abs generated with B cell peptides. Ten μg of each VP1 and 3D DNA or proteins were co-immunized into 5 weeks old BALB/C mice intradermally or intramuscularly, respectively. Ab titers in protein vaccine group were higher than those in DNA vaccine group as determined by ELISA. IgG isotyping analysis showed that Th2 type immune responses were induced following immunization with recombinant protein of VP1 and 3D. Whether theses Abs have neutralizing activities need to be determined further. Our results suggest that both intradermal DNA and intramuscular recombinant protein vaccine administration efficiently induce Ag-specific Ab immune responses.

Keywords: FMDV, VP1, 3D, DNV vaccine, Recombinant protein vaccine

H001

The Enhancement of Th2-Type Humoral and Th1-Type Cellular Immunity against Pseudorabies Virus DNA Vaccine by GM-CSF Genetic Adjuvant

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Ganulocyte/macrophage colony-stimulatory factor (GM-CSF) is an attractive adjuvant for a DNA vaccine on account of its ability to recruit antigen-presenting cells (APCs) to the site of antigen synthesis as well as its ability to stimulate the maturation of dendritic cells (DCs). This study evaluated the utility of GM-CSF cDNA as a DNA vaccine adjuvant for glycoprotein B (gB) of pseudorabies virus (PrV) in a murine model. The co-injection of GM-CSF DNA enhanced the levels of serum PrV-specific IgG with a 1.5- to 2- fold increase. Moreover, GM-CSF co-injection inhibited the production of IgG2a isotype. However, it enhanced production of IgG1 isotype resulting in humoral responses biased to the Th2-type against PrV antigen. In contrast, the co-administration of GM-CSF DNA enhanced the T cell-mediated immunity biased to the Th1-type, as judged by the significantly higher level of cytokine IL-2 and IFN-g production but not IL-4. When challenged with a lethal dose of PrV, the GM-CSF co-injection enhanced the resistance against a PrV infection. This suggests that co-inoculation with a vector expressing GM-CSF enhanced the protective immunity against a PrV infection. This immunity was caused by the induction of increased humoral and cellular immunity in response to PrV antigen.
Keywords: DNA vaccine, Pseudorabies virus, GM-CSF, Th1/Th2 type, Protective immunity

H002

PMA Induces c-FLIP Expression via PKC and ROS

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The molecular mechanisms regulating c-FLIP has not been studied in detail. In the present study, we studied mechanisms regulating c-FLIP expression. PMA significantly increased expression of c-FLIP. Actinomycin D inhibited PMA-induced c-FLIP expression, suggesting that regulation of c-FLIP expression occurs at the transcriptional level. Induction of c-FLIP by PMA was inhibited by GF, an inhibitor of PKC, suggesting that PKC plays important role in regulation of c-FLIP. PMA increased phospohrylation of ERK, p38 MAPK, and Akt. The activation of these signaling molecules was also inhibited by GF. Addition of PMA significantly increased ROS. The induction of ROS by PMA was inhibited by GF. Therefore, effect of ROS on expression of c–FLIP was determined. H_2O_2 increased expression of c-FLIP. H_2O_2 increased phosphorylation of p38 MAPK and Akt, but not that of ERK. The inhibition of p38 MAPK by SB and that of Akt by LyYdecreased expression of c-FLIP, suggesting that p38 MAPK and Akt function upstream of c-FLIP. PMA significantly enhanced motility of cancer cells and this required activation of p38 MAPK and Akt. Both PMA and H_2O_2 increased phosphorylation of IκB, suggesting that NF-κB mediate induction of c-FLIP. Sulfasalazine, an inhibitor of NF-κB, suppressed induction of c-FLIP. Since PMA enhanced motility and increased c-FLIP, it was necessary to determine whether c-FLIP affected motility of cancer cells.
Keywords: c-FLIP, motility, reactive oxygen species, PMA

H003

ROS Mediates Hyaluronic Acid-Promoted Motility of Melanoma Cells by Activating Akt, p38 MAPK, and NF-κB

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Hyaluronic acid (HA) is a component of extracellualr matrix and has been known to play important role in motility of several tumor cells, including human glioma cells. However molecular mechanisms associated with its effect on motility have not been studied in detail. Here we report the role of HA in motility and mechanisms. HA increased ROS, and blocking of ROS by N-acetyl-L-cysteine suppressed hyaluronic-acid-promoted adhesion and motility of melanoma cells. . HA increased phosphorylation of Akt, p38 MAPK, and IκB. Inhibition of PI3 kinase by LY294002, p38 MAPK by SB203580, and NF-κB by sulfasalazine suppressed HA-promoted adhesion and motility. Addition of H_2O_2 increased phosphorylation of Akt, p38 MAPK, and IkB. H_2O_2 enhanced motility of melanoma cells. These results suggest that ROS mediate HA-promoted motility. Sulfasalazine, an inhibitor of NF-κB, significantly suppressed ROS-promoted motility, suggesting that ROS require NF-κB for mediating HA-promoted motility. A synthetic peptide (GAHQFNALTVR; pep-1) that binds to and inhibit HA was shown to suppress HA-promoted motility by decreasing phosphorylation of Akt, p38 MAPK, and IkB. This suggests that CD44 is required for HA-promoted motility. Taken together, these results indicate that ROS mediate HA-promoted motility by activating, Akt, p38 MAPK, and NF-kB. The role of ROS in HA-promoted motility has not been studied.
Keywords: hyaluronic acid, ROS

H004

The Inhibitory Mechanism of Hyaluronic Acid on Allergic Inflammation

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Hyaluronic acid (HA), is an extracellular matrix glycosaminoglycan that regulates cell adhesion and motility. Mast cells play a critical role in the initiation and maintenance of inflammatory responses. However, the effects of HA on mast cell functions have not been studied. Here we investigated the effect of hyaluronic acid on allergic inflammation. Hyaluronic acid decreased secretion of β-hexosaminidase and histamine from antigen-stimulated RBL2H3 cells, in a dose-dependent manner. Hyaluronic acid significantly suppressed phosphorylation of ERK and p38 MAPK resulting from antigen stimulation. Hyaluronic acid significantly decreased ROS and calcium influx from antigen-stimulated RBL2H3 cells. Hyaluronic acid with various sizes (3MDa, 1MDa, 100KDa, 6KDa) showed similar effects on β-hexosaminidase secretion, histamine release, and ROS. The inhibition of GPCR by suramin did not restore decreased ROS, β-hexosaminidase secretion by HA. This suggests that GPCR is not required for anti-allergic effect by HA. HA did not increase cAMP even in the presence of IBMX, suggesting that anti-allergic effect by HA is not due to cAMP. Synthetic pep-1 that binds to inhibits function of HA prevented HA from decreasing phoshorylation of ERK, p38 MAPK, and ROS in RBL2H3 cells stimulated with DNP-HSA. This suggests that HA directly exert anti-allergic function. In conclusion, Hyaluronic acid exerts negative effect on allergic inflammation by inhibiting multiple signals and calcium influx.
Keywords: Hyaluronic acid, inflammatory

H005

Kaposi's Sarcoma-Associated Herpesvirus Viral IFN Regulatory Factor 1 Inhibits Transforming Growth Factor-Beta Signaling

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Kaposi's sarcoma-associated herpesvirus, also called human herpesvirus 8, has been implicated in the pathogenesis of Kaposi's sarcoma, body cavity-based primary effusion lymphoma, and some forms of multicentric Castleman's disease. The Kaposi's sarcoma-associated herpesvirus open reading frame K9 encodes viral IFN regulatory factor 1 (vIRF1), which functions as a repressor of IFN-mediated signal transduction. vIRF1 expression in NIH 3T3 cells leads to transformation and consequently induces malignant fibrosarcoma in nude mice, suggesting that vIRF1 is a strong oncoprotein. Here, we show that vIRF1 inhibited transforming growth factor-beta (TGF-beta) signaling via its targeting of Smad proteins. vIRF1 suppressed TGF-beta-mediated transcription and growth arrest. vIRF1 directly interacted with both Smad3 and Smad4, resulting in inhibition of their transactivation activity. Studies using vIRF1 deletion mutants showed that the central region of vIRF1 was required for vIRF1 association with Smad3 and Smad4 and that this region was also important for inhibition of TGF-beta signaling. In addition, we found that vIRF1 interfered with Smad3-Smad4 complex formation and inhibited Smad3/Smad4 complexes from binding to DNA. These results indicate that vIRF1 inhibits TGF-beta signaling via interaction with Smads. In addition, the data indicate the TGF-beta pathway is an important target for viral oncoproteins.
Keywords: KSHV, vIRF1, TGF-beta, Smad

H006

Contribution of Antigenic Lipoprotein, a *Salmonella* Surface Antigen in the Immune Response

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Salmonella typhimurium induces immune responses while it was infected in the host and has not been identified the antigens which contribute to induce strong immune responses. To investigate immunodominant *Salmonella* antigens, attenuated *S. typhimurium*(Δ*crp*) live vaccine was administrated into BALB/c mouse. The sera collected from immunized mice were used to detect the antigens in *S. typhimurium* cell lysates by immunoblot. An 6.9 kDa immuno-reactive protein band was detected by immunoblot. The protein purified from outer membrane fraction of *Salmonella* was analyzed to identify the protein through a MALDI-TOF assay. The protein was verified as Lpp which is major bacterial outer membrane lipoprotein component. The 5'-flanking and 3'-flanking regions of *lpp* gene were amplified by PCR, joined and cloned into a suicide plasmid, resulting in a recombinant suicide plasmid pBP109. A *S. typhimurium* mutant deleting *lpp* gene was constructed by allelic exchange with recombinant suicide plasmid pBP109, resulting in S. typhimurium CK23. The *lpp* gene deletion in CK23 was confirmed by DNA size comparison of *lpp* region and elimination of 6.9 kDa immuno-reactive protein in immunoblot. Lpp-specific polyclonal antibody was generated in a New Zealand White rabbit. With the use of anti-Lpp antibody, an immuno-reactive 6.9 kDa protein band was detected in wild-type strain but it was not detected in CK23, indicating that the polyclonal antibody is Lpp specific.
Keywords: Antigen, lipoprotein, Lpp

H007

Immune Stimulatory Effect of Partially Purified Compound from *Streptomyces* sp. CGS-1015

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Many secondary metabolites from *Streptomyces* spp. have immunomodulatory activities and antifungal activities. In this study, we tested immunomodulatory activities in culture broth of *Streptomyces* sp. CGS-1015 that were partially purified by DEAE-Sephacel ion exchange chromatography, and characterized it. The bioactive compound was partially purified in 0.4M NaCl by DEAE-Sephacel ion-exchange chromatography. And we have investigated the effect of these partial purified compounds (C-IE) on proliferation of mouse splenocytes, activation of mouse macrophage and expression of inflammatory mediator-proinflammatory cytokine, nitric oxide and transcription factor. we found that C-IE enhanced the activation of mouse macrophage - Raw 264.7 and peritoneal macrophage - by increasing of production of NO, expression of inflammatory cytokine and enhenced phagocytosis. Also the expression of these inflammatory mediators were controled by NF-kB and C-IE controled the expression of this transcription factor. And we identified CGS-1015 sp. These results imply that C-IE, partially purified immune stimulatory molecule, has the activity of enhancing lymphocyte mitosis, macrophage activation. And also we expect this molecule would have tumoricidal and antifungal effects.
Keyword : *streptomyces* sp.

H008

Reactive Oxygen Species-Mediated Activation of ASK1-p38 MAPK Pathways is Required for Proinflammatory Responses to the Mycobacterial PPD Antigen

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ROS have been shown to play an important role in the regulation of distinct signaling cascades, however, the intermediary role of intracellular ROS in macrophage activation upon mycobacterial protein(s) stimulation are largely unknown. In this study, we investigated the role of ROS in tuberculin PPD-induced activation of MAPKs with their upstream mediators. PPD stimulation induced a generation of superoxide and hydrogen peroxide in human monocytes and murine macrophages. The PPD-induced p38 MAPK activation and proinflammatory cytokine production were significantly attenuated by antioxidants such as NAC and PDTC. In addition, PPD-mediated ROS production leaded to activation of ASK 1 at upstream to p38 MAPK. Conversely, blockade of p38 MAPK or ASK1 nullified ROS synthesis, and proinflammatory cytokine production by PPD. The PPD-induced activation of ASK1 and p38 was significantly abrogated in TLR2-deficient macrophages. Further, TB patients showed enhanced ROS production, p38 phosphorylation, and up-regulation of proinflammatory cytokine expression in peripheral blood monocytes and alveolar macrophages, when compared with those by controls. Taken together, these results suggest that intracellular ROS mediated ASK1-p38 MAPK activation is necessary for PPD-induced proinflammatory responses in monocytes/macrophages via TLR2. The altered regulation of ROS synthesis and MAPK activation in response to the mycobacterial protein(s) could contribute to the pathogenesis of human TB.
Keywords: p38 MAPK, apoptosis-regulating-signal kinase (ASK) 1, Reactive oxygen species (ROS), purified protein derivative (PPD)

H009

Anti-Fungal Activity and Anti-Inflammatory Effects in Murine System of *Streptomyces* sp. SUS-0608 Isolated from Soil

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Of them active compound from *Streptomyces* sp. SUS-0608 was found to be an anti-fungal agent(AFA) and showed immunosuppressive activity on mouse splenocytes, mitogen stimulated splenocytes, macrophages viability and proliferation *in vitro* and *in vivo*. As an immediate host defense reaction, the innate immune response involves secretion of cytokines and other mediators leading to an inflammatory process that has antimicrobial effects. One important and widely-used transplantation factor that plays a pivotal role in many cellular responses is NF- κB. NF- κB is a DNA-binding protein that is important for maximal expression of many genes that are involved in inflammatory responses. As a result, production of NF- κB-mediated cytokine and radical that is important in inflammation is reduced to suppress immune response by AFA when macrophages are incubated with AFA for short culture time. AFA also induces apoptosis that is important in homeostasis in macrophages and splenocytes when cells are incubated with AFA for long culture time. The subcutaneous air-pouch has been found to be suitable for studying chronic granulomatous inflammation. And for the study related to rheumatic disease, it has the closest approximation to synovial tissue because it is only other blind connective tissue cavity which lacks a mesothelial basement membrane. The similarity of synovium was found be closet for 6 days after air infection.
Keywords: *Streptomyces* sp. SUS-0608, Anti-inflammatory

H010

The Effect of Deer [Sika Cervus Nippon (Temminck)] Antler on Morphological Alternation in Bone Marrow and Oxidative Stress-Induced Apoptosis

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In order to study morphological alteration and oxidative stress-induced apoptosis, ethyl alcohol extract was prepared from antler of *Sika Cervus nippon* (Temminck). It has been shown to differentiate into mature cells revealing morphological alteration by microscopy when cultured with DAE. This result suggested that DAE acts as multiple cytokine to support differentiation OF HSCs in Bone marrow. Furthermore, It has been known that enhanced apoptosis and elevated levels of reactive oxygen species (ROS) play a major role in aging. We investigated basal and ROS-induced levels of apoptotic lymphocytes derived from the spleen and thymus in mice. Apoptosis was reduced in vitro by treatment with DAE. The effect of DAE on apoptosis was proved by DNA fragmentation anaylasis, where the extent of DNA laddering was enhanced in the presence of dexamethasone.
Keywords: Sika Cervus nippon (Temminck), apoptosis

H011

Up-Regulation of Chemokine Ligand 20 in Human Tuberculosis

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Cytokines participate in protective and immunopathologic host responses during tuberculosis (TB). These effector molecules are produced transiently and locally controlling the amplitude and duration of the response. A variety of experiments has shown that excessive or insufficient production may significantly contribute to the pathophysiology of TB infection. Here we demonstrate that mycobacterial 30-kDa antigen or *Mycobacterium tuberculosis* actively induces chemokine macrophage inflammatory protein 3alpha/CC chemokine ligand 20 (CCL20) mRNA and proteins, by peripheral blood monocytes and mononuclear cells (PBMCs). A comparative analysis has revealed that the mRNA and protein expression of CCL20 was prominently up-regulated in sera, PBMCs, and monocytes from TB patients, compared with healthy controls. Of note, chronic TB patients showed more increased CCL20 expression in PBMCs than early TB did. In addition, the expression of cognate CC chemokine receptor 6 (CCR6) of CCL20 is up-regulated by the CD45RO+ T cells from TB patients. Blockade of tumor necrosis factor (TNF)-α, but not interleukin (IL)-10, significantly attenuated the production of CCL20 in PBMCs or monocytes, suggesting the modulation of CCL20 by TNF-α. Collectively, these results indicate that TB patients typically show CCL20 up-regulation *in vivo* or *ex vivo* in response to the mycobacterial antigen, and this effect is modulated by TNF-α. The exact role of CCL20/CCR6 during human TB is being investigated.
Keywords: chemokine ligand 20, tuberculosis (TB), Mycobacterium tuberculosis

H012

Ginsan Efficiently Enhance Mucosal Adaptive Immunity to Orally Administered Antigens

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Botanic polysaccharides show various immunomodulating effects. In this study, we screened for a polysaccharide that could enhance the migration of splenocytes to the Peyer's patch. Ginsan, extracted from *Panax ginseng*, specifically increased the migration of GFP positive splenocytes to the subepithelial dome of the Peyer's patch (PP). Next, expression of chemokines and chemokine receptors following ginsan treatment were screened. Ginsan treatment distinctively increased the expression of CCR1 in the spleen and its chemokine CCL3/MIP-1α in the PP. CCL3 expressing cells were CD8α-/CD11b+/CD11c+ dendritic cells which activates primarily T helper 2 cell. Next, signals that could regulate the expression of CCL3 were secreened. Ginsan treatment specifically increased the expression of COX-1 in the Peyer's Patch. When COX-1 was inhibited by either by SC-560 or acetylsalicylic acid, both the expression of CCL3 and the migration of GFP splenocyte was reverted to the control level. Finally, effect of ginsan on the adaptive immune response to oral antigen was determined. In ginsan treated group, antibody titers of both IgA in stool and IgG in serum were significantly higher than the control group. Moreover, acetylsalicylic acid reverted both the systemic and mucosal immune response enhanced by ginsan. Here, we report ginsan as a potent vaccine adjuvant for oral immunization.
Keywords: Ginsan, vaccine adjuvant, COX-1, CCL3, oral immunization

H013

Inhibitory Effects of *Houttuyniae cordata* Extracts on Cancer Cells

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Herba houttuyniae has been known to have anti-microbial, anti-inflammatory, anti-oxidant, and anti-cancer effects. however, its specific effect and mechanism on cancer cells is not well known yet. Therefore In the present study, we prepared houttuyniae cordata extracts by cold water extraction and investigated the effects on murine colon cancer cell line and other cancer cells. Treatment of Herba houttuyniae extract inhibited growth of CT26 cells and Human hepatoma HepG2 cells in a concentration and time dependent manner and showed morphological changes.

Keyword : houttuyniae cordata

H014

Mycobacterium tuberculosis eis is Critical for Inducing Macrophage Survival via MAPK Signalling Pathways through ROS Synthesis Modulation

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The *eis* gene of *Mycobacterium tuberculosis* (MTB) was reported to enhance intracellular survival of mycobacteria in macrophages, however, its involvement in host cell death and reactive oxygen species (ROS) signalling are largely unknown. In this study, we investigated the role of *eis* in the modulation of host cell apoptosis and ROS signalling during MTB infection. Apoptosis inducing activities were investigated in murine macrophages after infection with wild-type, *eis*-deletional (D*eis*), or complemented MTB strain. Macrophages infected with D*eis* showed significantly reduced cell viabilites, and increased apoptosis, and ROS synthesis, compared with those infected with wild-type strain did. Pro-apoptotic protein Bax was significantly increased, whereas anti-apoptotic protein Bcl(XL), was decreased, in macrophages infected with D*eis*. In addition, macrophages infected with D*eis* showed a significantly increased activation of MAPKs, as well as TNF-α than those infected with wild-type or complemented strain. Collectively, these data demonstrate that MTB *eis* is critical for inducing macrophage survival via MAPK signalling pathway, through ROS modulation, and thereby finding a safe haven for its growth and resist the host immune responses during TB infection.

Keywords: Mycobacterium tuberculosis, reactive oxygen species, apoptosis, infection, MAPK

H015

Effect of Ginsan in an Asthmatic Murine Model

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The morbidity and mortality of allergic asthma have increased in the past two decades. But corticosteroids therapy using frequently for asthma therapy have many side effects. Therefore development of new medicine is required. We studied to evaluate the effects of Ginsan, polysaccharide derived from Ginseng, to treat OVA induced allergic asthma mouse model. Mice were sensitized intraperitoneally and challenged aerosolized with OVA. Dexamethasone-treated, PBS- treated, and normal mice served as controls. The effects of Ginsan on airway inflammation, lung pathology, and cytokine and immunoglobulin production, were evaluated. To elucidate the mechanism of Ginsan, mRNA expressions of cycloxygenase (COX)-1 and COX-2 were determined by RT-PCR. Ginsan remarkably reduced airway hyperresponsiveness against methacholine challenge and reduced eosinophilia both in the bronchoalveolar lavage fluid and the lung. Also, airway remodeling was reduced compared to the PBS control group. Con A-activated splenocytes produced significantly lower IL-5 compared to the PBS control group while other cytokines were not altered. Ginsan treatment increased serum IgG2a while other immunoglobulin production including IgG1, IgA and IgE were not altered. Ginsan treatment upregulated COX-1 mRNA expression compared to PBS control group. We conclude that Ginsan was effective in treating OVA induced mouse asthma model by upregulating COX-1 gene expression in the lung rather than the systemic effect.

Keywords: Ginsan, asthma, COX, OVA

H016

Establishment of Cell Immune Response Testing Method to Validate the Efficacy of the HPV Vaccine Candidates (HPV-16 type)

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Several vaccine candidates against human papilomavirus will be in market soon. Therefore, to validate the efficacy of the HPV vaccine candidates methods of the efficacy test have to be established. In this study, we measured T cell proliferation and cytokine production to study cell mediated immunity of the vaccine and to establish standardized cell immune response testing method. The group (n=15) of Female Balb/c mice were immunized with purified VLPs expressed in Saccharomyces cerevisiae strain EGY48 and the other group (n=5) of mice were immunized with PBS as a control. In mouse spleens obtained after immunization, this splenocytes(2×10^5 cells/well) were seeded in 96well culture plate and incubated for 4,5 and 6days at 37℃ in presence of VLPs (1.5 μg/well). The MTT assay was applied to test the T cell proliferation and sandwich ELISA were performed to measure of cytokine, IFN-γ and IL-4 expressed in Th1 and Th2. Significant increased T cell proliferation and expression of cytokine were observed from the group immunized with VLPs. The results of this study suggest that HPV-16 L1 like particle expressed in *Saccharomyces cerevisiae* strain EGY48 be able to induce the cell mediate immune respones. Therefore, the T cell proliferation assay (MTT assay) and cytokine detection assay (sandwich ELISA) method could be applied to validate the efficacy of the HPV vaccine candidates.

Keywords: Human papilloma virus(HPV), virus-like particle(VLP), cell immune response, T cell proliferation assay, cytokine detection assay

H017

Expression of PRRSV Matrix Protein in Mammalian Cells

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The clinical signs of porcine reproductive and respiratory syndrome (PRRS) are characterized by reproductive failure in sows and respiratory illness in young pigs. Matrix (M) protein of PRRSV encoded by ORF 6 gene was known to have high immunogenicity among proteins of the virus. In this study, we identified the expression of mRNA and M protein in the mammalian cells transfected with DNA. The ORF 6 gene was cloned into TA cloning vector (Invitrogen) and pQE30 expression vector (Qiagen) after RT-PCR with viral RNA extracted from PRRSV-infected MARC 145 cells. The recombinant M protein was expressed in *E.coli* system and purified using a nickel-affinity column. Polyclonal antibody specific to PRRSV M protein was prepared from a rabbit immunized with the M protein. After the ORF 6 gene was subcloned into a mammalian expression vector pcDNA3.1 (Invitrogen), it was transfected into 3T3 cells. RT-PCR and Western blotting were used to identify the expression of viral mRNA and M protein in the 3T3 cells, respectively. The specificity of the polyclonal antibody was identified by Western blot. 525bp of ORF 6-specific PCR product was demonstrated in the transfected cells by RT-PCR. 19.3kDa of M protein was identified by Western blotting with the DNA-transfected cell lysate. These results indicate plasmid DNA encoding PRRSV M protein can be employed for the development of a PRRSV vaccine.

Keywords: PRRS, immunogenicity

H018

The PrrBA Signal Transduction Pathway is Controlled by Oxygen Tension via *cbb*$_3$-type Cytochrome *c* Oxidase in *Rhodobacter sphaeroides*

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The PrrBA two component system is one of the major regulatory systems that control expression of photosynthesis genes in response to changes in oxygen tension in the anoxygenic photosynthetic bacterium, *Rhodobacter sphaeroides*. We previously suggested that the *cbb*$_3$ cytochrome *c* oxidase has the dual function as a terminal oxidase and oxygen sensor. The *cbb*$_3$ cytochrome *c* oxidase encoded by the *ccoNOQP* operon is expressed primarily under microaerobic and anaerobic conditions. The catalytic subunit (CcoN) of the *R. sphaeroides cbb*$_3$ oxidase contains six conserved histidine residues and these histidines are strictly conserved in the catalytic subunit of members of the heme-copper oxidase superfamily. Alignments of the amino acid sequences of subunit I (CcoN or FixN) of the *cbb*$_3$-type oxidases show additional five conserved histidine residues (H214, H233, H303, H320 and H444). Interestingly, the H303A mutant form of the *cbb*$_3$ oxidase retains the catalytic function as a cytochrome *c* oxidase as compared to the wild-type oxidase, while it is defective in signaling function as an oxygen sensor. Aerobic derepression of PS genes in the strain containing this mutant form of the *cbb*$_3$ oxidase, strongly suggests that the PrrB activity is controlled by the *cbb*$_3$ oxidase rather than by the redox state of the electron transport chain. H303 appears to be implicated in either signal sensing or generation of the inhibitory signal which shifts the PrrB activity in favor of the phosphatase mode.

Keywords: cbb3 cytochrome c oxidase, photosynthesis, redox sensing, two-component system

H019

A Key Role of Inducible Nitric Oxide Modulated by Cholecalciferol and Interferon-Gamma in Human Macrophages through TLR2-Dependent Pathways

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The role of nitric oxide (NO) in human host defense remains unresolved. To assess the role of NO and inducible NO synthase (iNOS) in host defense during human tuberculosis (TB), the modulating factor(s) for iNOS induction in human macrophages are urgently elucidated. Here we show that combined treatment of 1,25-D3 and IFN-g together enhanced PPD-induced NO synthesis, nitrite release, and iNOS mRNA and protein expression, synergistically in human monocyte-derived macrophages (MDMs). Comparative *ex vivo* studies show that the NO synthesis and iNOS expression are markedly depressed in MDMs from chronic refractory tuberculosis (TB) patients, suggesting that 'canonical' iNOS suppression in MDMs is a mainly associated with pathogenesis in chronic phase of TB. Both NF-kB and MEK1-ERK1/2 pathways play an indispensable role in 1,25-D3 plus IFN-g-inducible iNOS expression in human MDM via TLR2. Further, the combined treatment of 1,25-D3 and IFN-g was more potent than either agent alone at inhibition of the intracellular MTB growth in human MDMs. Notably, this antimycobacterial effect was stronger than those with a known effector of 1,25-D3-induced cathelicidin, and counteracted by iNOS inhibitors. Taken together, these data provide a special emphasis on the key role of NO in host defense during human TB and the identification of novel modulating factors for iNOS induction in human macrophages.

Keywords: Human nitrite, macrophage, cholecalciferol, Interferon gamma

H020

Finding Nuclear Localization Signal in *Klebsiella*

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Members of the genus *Klebsiella* are commonly found in soil or water. Many isolates are capable of fixing nitrogen from the atmosphere, which has been proposed as being a nutritional advantage in isolated populations with little protein nitrogen in their diet. The species *Klebsiella pneumoniae* occasionally causes a serious from of pneumonia in humans. A nuclear localization signal(NLS) is a short stretch of amino acids that mediates the transport of nuclear proteins into the nucleus. NLS motifs are known as KxKK or KK/RRx$_{10\text{-}12}$K/R. We found an NLS motif in *Klebsiella* protein and examined the potential nuclear translocation of HsdM by fluorescence microscopy.

Keywords: *Klebsiella*, HsdM, NLS

H021

Development of a Sulfated Water-Soluble (1-3)-β-D-Glucan Biological Response Modifier Obtained from *Ganoderma lucidum*

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We describe characteristics for the solubilization of micro-particulate (1-3)-β-D-glucan obtained from *Ganoderma lucidum*. The β-D-glucan is a biological response modifier, but a major obstacle to the clinical utilization of β-glucan BRMs is their relative lack of solubility in aqueous media. Insoluble glucan is dissolved in methyl sulfoxide and urea and partially sulfated at 100℃. The resulting water-soluble product is called S-Ganoderan(SGL, sulfated-(1-3)-β-D-glucan from *Ganoderma lucidum*). The average yield of SGL from the insoluble glucan was 85%, and the solubility of SGL was above 95%. The monosugar contents of SGL was 34.9% of α-glucose and 35.9% of β -glucose. The molecular-weight averages of SGL was as single peak on the Sepharose CL-4B column chromatography, and their molecular-weight average was about 9,300 dalton. To confirm the type of interchain linkages associated with SGL, the ^{13}C NMR spectrum peaks shows good correspondence with laminarin, the β-(1-3)-linked triple-helical control.

Keywords: β-glucan, sulfated glucan, biological response modifier, Ganoderma lucidum

H022

Rat Kupffer Cell Activation of a Sulfated-(1-3)-β-D-Glucan, S-Ganoderan, Obtained from *Ganoderma lucidum*

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The effect of S-Ganoderan(SGL), a water soluble sulfated-(1-3)-β -D-glucan obtained from *Ganoderma lucidum*, on rat Kupffer cell (macrophage cell line) was examed. SGL was evaluated for its ability to induce formation of nitric oxide (NO), tumor necrosis factor-α (TNF-α) and transforming growth factor (TGF-β) from rat Kupffer cell *in vitro*. Hepatic macrophages activated by SGL did significantly elevated concentration of NO and TNF-α in cultured medium, but not significantly elevated TGF-β. SGL-activated Kupffer cells secrete 16.1 μM ($p<0.01$) of NO and 1,397ρg/㎖ ($p<0.01$) of TNF-α after 36 hrs of incubation at 37℃. The proliferation of SGL-activated Kupffer cells inhibited as compared with its negative control. These results indicate that the sulfated-β-D-glucan activates rat Kupffer cell and secretes NO and TNF-α.

Keywords: sulfated-β-glucan, Kupffer cell, Nitric oxide, macrophage activation, Tumor necrosis factor-α

H023

Clerodendron trichotomum Tunberg Leaves (CTL) Inhibits Pro-Inflammatory Gene Expression in LPS-Stimulated RAW 264.7 Macrophages by Suppressing NF-kB Activation

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Clerodendron trichotomum Tunberg Leaves (CTL) have been used for centuries in Chinese folk medicine for their anti-inflammatory properties. To investigate the molecular mechanism of anti-inflammation by CTL, we analyzed the regulation of TNF-α expression in RAW 264.7 cells, a key step in inflammation. The effect of CTL on the production and expression of tumor necrosis factor-α (TNF-α) was determined by enzyme-linked immunosorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR). CTL inhibited the production and expression of TNF-α in LPS-stimulated RAW 264.7 cells in a dose-dependent manner. In addition, activation of NF-κB, which controls TNF-α expression, was inhibited in LPS-stimulated RAW 264.7 cells by CTL in a dose-dependent manner, as demonstrated by an electrophoretic mobility shift assay (EMSA). Furthermore, CTL inhibited activation of NF-κB through inhibition of IκB degradation, as demonstrated by an western blot analysis of IκB-α. These results suggest that CTL inhibits the expression of the pro-inflammation gene through the inhibition of NF-κB dependent pathway in RAW 264.7 cells.

Keywords: Clerodendron trichotomum Tunberg Leaves (CTL), anti-inflammatory, tumor necrosis factor-α (TNF-α), nuclear factor-kappa B (NF-κB)

H024

Assessing the Serodiagnostic Potential of *Mycobacterium Tuberculosis* K-strain Specific Glycolipid

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Improved diagnostic reagents are needed for the detection of *Mycobacterium tuberculosis* infections, and the development of a serodiagnostic test would complement presently available diagnostic methods. *M. tuberculosis K-strain* is found to be most prevalent among the clinical isolates of *M. tuberculosis* in Korea. Total lipids extracted from *M. tuberculosis* H37Rv, K-strain, and *M. bovis* BCG were analyzed by thin layer chromatography (TLC). A specific glycolipid (KSL) in *Mycobacterium tuberculosis* K-strain was found and purified directly from TLC plate. The diagnostic potential of KSL was evaluated in active tuberculosis (TB) patients by ELISA and dot blot. The IgG and IgM antibody level to KSL were estimated in the sera from 188 TB patients and 94 healthy subjects. Sensitivities of 69% and 15% with specificities of 95% and 93% were obtained for the IgG and IgM, respectively.

Keywords: *M. tuberculosis* K-strain, glycolipid

H025

Immuno-Modulatory and Antitumor Effects of Crude Polysaccharides Isolated from *Lentinus giganteus*

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This study was initiated to evaluate immuno-modulatory and antitumor effects of *Lentinus giganteus*. Neutral salt soluble (0.9% NaCl), hot water soluble and methanol soluble substances (hereinafter referred to Fr. NaCl, Fr. HW and Fr. MeOH, respectively) were extracted from the mushroom. *In vitro* cytotoxicity tests indicated that Fr. HW and Fr. NaCl showed cytotoxicity against cancer cell lines such as Sarcoma 180, HepG2 and HT-29. Intraperitoneal injection with Fr. HW exhibited antitumor activity with life prolongation effect of 67.5% in ICR mice inoculated with Sarcoma 180. Fr. NaCl and Fr. MeOH improved proliferation of spleen cells and the immunopotentiating activity of B lymphocyte by increasing the number of spleen cells and alkaline phosphatase activity, respectively. The weight of spleen was increased slightly in the test group of ICR mice compared to the control.
Keywords: *Lentinus giganteus*, polysaccharide, antitumor, immuno-modulatory, Sarcoma 180

H026

Studies on Antitumor and Immuno-Modulatory Effects of Crude Polysaccharides Extracted from *Armillaria mellea*

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Armillaria mellea, one of edible and medicinal mushroom belongs to Tricholomataceae of Basidiomycota, has been known to have outstanding effects on the sarcoma 180 and Erhrlich carcinoma of mice. This study was initiate to evaluate immuno-modulatory and antitumor effects of *Armillaria mellea*. Neutral salt soluble (0.9% NaCl), hot water soluble and methanol soluble substances (hereinafter referred to Fr. NaCl, Fr. HW and Fr. MeOH, respectively) were isolated from the mushroom. Intraperitoneal injection with crude polysaccharides exhibited antitumor activity with life prolongation effect of 60~67.5% in mice inoculated with Sarcoma 180, respectively. Each of crude polysaccharide extract promote proliferation of spleen and the immunopotentiation activity by increasing the number of spleen cells. In case of Fr. NaCl, the numbers of peritoneal exudate cells and circulating leukocytes were increased by ten and two folds, respectively.
Keywords: Armillaria mellea, polysaccharide, antitumor, immuno-modulatory, Sarcoma 180

H027

Antitumor and Immuno-Modulatory Activities of Crude Polysaccharides Isolated from *Oudemansiella radicata*

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Oudemansiella radicata, one of edible and medicinal mushroom belongs to Tricholomataceae of Basidiomycota, has been known to exhibit outstanding curative effects on the hypertension caused by high blood pressure and inhibitory effect on the sarcoma 180. Neutral salt soluble (0.9% NaCl), hot water soluble and methanol soluble substances (hereinafter referred to Fr. NaCl, Fr. HW and Fr. MeOH, respectively) were extracted from the mushroom. Intraperitoneal injection with Fr. NaCl exhibited antitumor activity with life prolongation effect of 65~67.5% in mice inoculated with Sarcoma 180. Fr. NaCl and Fr. HW improved proliferation of spleen cells and the immunopotentiating activity of B lymphocyte by increasing the number of spleen cells and alkaline phosphatase activity by 1.7~2.4 and 2.2~8.7 folds, respectively. Intraperitoneal injection with Fr. NaCl increased the numbers of peritoneal exudate cells and circulating leukocytes by ten and two folds, respectively.
Keywords: Oudemansiella radicata, polysaccharide, antitumor, immuno-modulatory, Sarcoma 180

H028

In *vitro* Antivirus and Immunostimulation Activities of the Hot Water Extracts from Alaskan *Ganoderma applanatum* and *Phellinus pini*

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The crushed Alaskan *Ganoderma applanatum* and *Phellinus pini* fruiting bodies were extracted in boiling water for 4 h with the yield of 4.5 g/100 g and 20.5 g/100 g, respectively, in dry mass. Antiviral activity was examined by measuring the degree of inhibition of cytopathic effect of Coxsackie virus B3 (CVB3) on the host cells, HeLa, using MTT assay and crystal violet staining. Five mg each of the extracts of *Ganoderma applanatum* and *Phellinus pini* showed antiviral activity up to 60% and 30%, respectively. The immunostimulating activity of both extracts was determined by measuring TNF-α secretion from a mouse macrophage, Raw 264.7 cell, using an ELISA Reader at 450 nm. 500 ug of the *Ganoderma applanatum* extract and 31 ug of the *Phellinus pini* extract induced TNF-α secretion up to 31% and 15%, respectively. β-Glucans are well known to enhance the immune system. The crude β-glucans were obtained from the *Ganoderma applanatum* and *Phellinus pini* hot water extracts by ethanol precipitation with the yield of 0.525 g/4.5 g extract and 0.985 g/20.5 g extract, respectively, in dry mass. The primary structures of both β-glucan preparations were determined by enzymatic hydrolysis and TLC analysis. After the treatment of both β-glucans with laminarinase for 48 h, the TLC chromatograms showed only glucose and gentiobiose spots, clearly indicating that the β-glucans of Alaskan *Ganoderma applanatum* and *Phellinus pini* are those of β-1,3-glucans having β-1,6-linked glucose residues.
Keywords: Ganoderma applanatum, Phellinus pini, water extracts, Antiviral and immunostimulating activity, β-Glucan structure

H029

Identification of Immunodominant Antigen, OmpA in *Edwardsiella tarda*

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Edwardsiella tarda is the causative agent of the systemic disease, edwardsiellosis. There are 61 *E. tarda* serotypes according to the O-antigen, variations in serotype is one of the main factors that hinder the development of a practical vaccine. Current research is focusing on finding a common antigen among different serotypes of *E. tarda*. Up to date little is known about *E. tarda* protein antigen inducing strong immune response. Outer membrane protein A(OmpA) which is highly conserved protein in the Enterobacteriaceae has been known to induce strong immune response. Therefore, on the basis of the sequences of *ompA* from various enteric bacteria, the primers were designed and synthesized. The gene of *E. tarda* CK41 was amplified by PCR and a gene encoding 37 kDa protein was identified as an OmpA of *E. tarda.* OmpA-specific polyclonal antibody was produced and used for immunoblot assay. An immuno-reactive 37 kDa protein band was detected in *E. tarda* by anti-OmpA antibody. To investigate the role of OmpA in *E. tarda* pathogenesis, a suicide vector pBP421 containing a kanamycin resistant gene within *ompA* was constructed and an allelic exchange mutant *E. tarda* CK99 was confirmed by PCR. The *ompA* gene inactivation in *E. tarda* CK99 was verified by the absence of 37 kDa immuno-reactive protein band in immunoblot analysis. The search of the epitope within the OmpA is now under progress by serial deletion mutant experiment.

Keywords: *Edwardsiella tarda*, OmpA, antigen

H030

Effect of Immune Activities of Mycelium Cultural Extracts of Cultured Japanese Flounder, *Paralichthys olivaceus*

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The effects of mycelium cultural extract supplemented diet, hematology and disease resistance against *Vibrio anguillarum* in olive flounder, *Paralichthys olivaceus* were evaluated. Fish were fed the *Phellinus linteus* with *Coriolus militaris* versicolor mixed mycelium cultural extract supplemented diet , *Phellinus linteus* mycelium cultural extract supplemented diet and *Coriolus militaris* tmycelium cultural extract supplemented dieto a commercial diet for 12 week. The body weight and length gain from the fish fed on daily the phellinus with coriolus versicolor mixed mycelium cultural extract supplemented diet , phellinus linteus mycelium cultural extract supplemented diet and Coriolus versicolor mycelium cultural extract supplemented diet of each mycelium cultural extract were significantly higher than the control. The relative precent survival rate (RPS) after an artificial challenge with 7×10^5 cells of *Vibrio anguillarum* per fish was higher than the control.

Keywords: *Phellinus linteus, Coriolus militaris, Vibrio anguillarum*

H031

Probiotics-Mediated Hypo-Immune Response in Gut is Linked Withupregulation of CD4+Foxp3+ Regulatory T Cell Population *in vivo*

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Beneficial effects of probiotics intake on human health have been described in many diseases but the underlying mechanism associated with immune system is still not well known. We tested the effect of probiotics feeding on immune system of normal mice and on modulation of colitis. Oral administration of a mixture of five probiotics strains in Balb/c mice did not induce any harmful effect rather decreased lymphocyte infiltration in gut compared to PBS-fed group. Probiotics feeding reduced expression levels of all cytokines, and induced lymphocyte hypo-proliferation. But probiotics-feeding didn't induced apoptosis measured by FACS analysis, rather had slightly anti-apoptotic effect in spleen. We tested the potential involvement of CD4+Foxp3+ T cells in down-regulation of cytokine expression and lymphocyte hypo-proliferation in probiotics-fed group. Indeed, Foxp3 levels were increased in mRNA and protein levels respectively by CD4+ T cells of MLN of probiotics treated mice. The effect of in vivo enrichment of regulatory T cell by probiotics feeding was confirmed in TNBS-induced colitis model. Probiotics treatment effectively prevented development of TNBS-induced colitis.

Keywords: probiotics, IBS, regulatory T cell

H032

Recombinant Protein Vaccine for Avian Influenza Virus HA Induces Antigen-Specific Antibody Immune Responses

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The flu is a contagious respiratory illness caused by influenza viruses. Recently, an H5N1 avian influenza virus (AIV) has been found in wild birds from several countries. The infection rate of AIV is generally low to most people. However, H5N1 is one of the few AIVs having cross infection to humans and the most fatal among strains crossing the species barrier. So far effective preventive or therapeutic strategies for H5N1 infection are not available. Influenza type A viruses are divided into subtypes based on two surface proteins, hemagglutinin (HA) and neuraminidase (NA), of the virus. Because HA elicits a neutralizing antibody response, HA protein was focused for a development of preventive vaccine. HA coding gene was cloned into pET vector for bacteria recombinant protein expression. A purified HA protein was immunized into 5 weeks old BALB/c mice. 10μg of HA protein was immunized mucosally or intramuscularly. Ag-specific serum Ab response was detected by Ab ELISA. Ab titers in HA protein immunized group were higher than in PBS group. According to IgG isotyping analysis, Th2 type immune responses were induced following immunization with recombinant protein of HA, suggesting humoral immune responses against Ag had been generated. Whether these Abs have neutralizing activities need to be determined further. Our results suggest that both intranasal and intramuscular recombinant protein vaccine administration efficiently induce Ag-specific Ab immune responses.

Keywords: Avian influenza virus, H5N1, vaccine

I001

Polysaccharides from *Salicornia herbacea* Suppress Apoptotic Death of the Mouse Thymocytes in Culture

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Polysaccharides were prepared from *Salicornia herbacea* by extraction with hot steam water (CSP) and by further ultrafiltration (SP1). The crude aqueous extract (CSP) was fractionated over 1 kDa by ultrafiltration using 0.1-m^2 membranes and followed by lyophilization, resulting in the SP1 fraction. Both CSP and SP1 were shown to have immunomodulating activity on mouse lymphocyte in culture. Treatment of the mouse thymus cells with CSP or SP1 suppressed apoptosis of the cells as assayed quantitatively by Annexin V technique using flow cytometry. It is supposed that the immunomodulatory activity of the CSP or SP1 might come from suppression of the programmed cell death of the thymus cells.
Keywords: polysaccharide, *Salicornia herbacea*, immunomodulating activity, thymocyte, apoptosis

I002

Anti-diabetic Activity of Polysaccharides Derived from *Salicornia herbacea*

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Polysaccharides were prepared from *Salicornia herbacea* by extraction with hot steam water (CSP) and by further ultrafiltration (SP1). Polysaccharides, SP1 and whole plant powder diet (HP) showed remarkable anti-diabetic effect on streptozotocin-induced rats. Male sprague-Dawley rats were used as experimental animals and experimental groups were divided into four groups consisting of diabetic control group, normal control group, 2% SP1 group and 10% HP group. Animals were administrated with experimental drinks or dietary for 6 weeks. Glucose, triglyceride, total cholesterol and high density lipoprotein-cholesterol levels in serum were measured before and after intake of test materials. The levels of s-glucose, s-triglyceride and s-total cholesetrol in SP1 group and s-triglyceride and s-total cholesterol in HP group were significantly decreased as compared with those in control group. These results suggest that SP1 and HP both show antihyperglycemic, antihypertriglyceridemic, antihypercholesterolemic and anti-arteriosclerosis activity in diabetic rats. The SP1 exhibited higher anti-diabetic activity than HP.
Keywords: anti-diabetic activity, polysaccharide, *Salicornia herbacea*

I003

CYP102A5, a Self-Sufficient P450 Monooxygenase Catalyzing Hydroxylation of Fatty Acids

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Cytochrome P450s act on the inactive carbon-hydrogen bonds of alkanes, fatty acids, terpenes, and steroids; and some exhibit high regio and stereoselective monooxygenation activity. Therefore, cytochrome P450s are expected to be potential catalysts for fine chemical synthesis. A bacterial self sufficient P450s is a natural fusion of a P450 and a mammalian-like diflavin NADPH-P450 reductase. The gene encoding CYP102A5, a novel P450 monooxygenase from *Bacillus cereus*, was cloned and expressed in *Escherichia coli* BL21 using pET expression system. The expressed recombinant enzyme was purified by Ni-NTA affinity chromatography and characterized. CYP102A5 is a 120 kDa (1065 aa) self-sufficient monooxygenase which belongs to Class III, consisting of an FMN/FAD-containing reductase domain and a heme domain. The deduced amino acid sequence of CYP102A5 exhibits a high level of identity with amino acid sequences of CYP102A1 (60%) from *Bacillus megaterium* and CYP102A2 (75%), CYP102A3 (59%) from *Bacillus subtilis*[1]. In reduced, CO-bound form[2], the enzyme shows a typical Soret band at 449 nm. It catalyses the oxidation of alkanes, alkanoic acids and unsaturated fatty acids. Further investigations of this interesting novel P450 monooxygenase are now in progress.
Keywords: P450, fatty acid, hydroxylation

I004

Superporous Agarose Bead-Packed Microfluidic Platform for Immunoassays

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We report the development of microfluidic immunoassay platform for the detection of goat IgG. The microfluidic device was fabricated with a glass slide and PDMS (polydimethyl siloxane) by transferring a pattern of a microchannel from a SU-8 master to PDMS and later binding the patterned polymer to a glass slide. Protein A-immobilized superporous agarose beads were introduced and packed into the microchannel. Superporous agarose beads which contain both normal diffusion pores and wide flow pores provide the channel with large surface area, thereby enhancing the binding chance between antigens and antibodies and also allowing sample solution to easily flow through the channel. This macroporous structure in the channel offers the chip increased sensitivity and wider range of detection. To test the feasibility of our platform, α-goat IgG, goat IgG, AP-conjugated α-goat IgG, BCIP/NBT substrate were introduced into the michrochannel by flow in a sequential manner. The resulting antigen-antibodies complex performed mainly on the surface of superporous was ultimately detected with the purplish-brown color of the precipitates formed. This microfluidic platform successfully detected an analyte with sensitivity down to 100 pg/ml. the platform is feasible for application in rapid type immunoassays due to its short analysis time, high sensitivity, easy procedure and can be further used as a field-portable biosensor because it would not need extra detection equipments .
Keywords: superporous agarose, Enzyme-linked immunoassay, microfluidics, poly(dimethyl siloxane), protein A

I005

Screening Biofilm Inhibitors of *E. coli* O157:H7 in the Microfluidic System

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Biofilm represents a context in which bacterial cells can, through physical and chemical interactions, establish and maintain long-term relationships with each other. Especially, bacterial cells embedded in biofilm are more resistant to environmental stresses, such as sanitizers than planktonic peers. Conventional methods use for observing biofilm are lack of real-time monitoring capability, cumbersome sample processing, etc. Herein, we developed a microfluidic platform for monitoring biofilm formation and also screening for biofilm inhibitors. To increase the surface to volume ratio in a microfluidic device, superporous agarose beads which contain both normal diffusion pores and very wide flow were packed in the channel of a microfluidic device. Increase in the surface-to-volume ratios provide bacteria with higher binding chance, resulting in increased biofilm formation in the device. Since flow is efficiently controlled in the microfluidic device, sample loading, washing, and staining procedures were easier and performed in a more controlled manner. Also, real-time monitoring is possible in this device because of a thin and clear window for observation. It is suggested by these results that the microfluidic platform is feasible for application in screening inhibitors and monitoring biofilm formation.This work was supported by Korea Research Foundation Grant funded by Korea Government (MOEHRD, Basic Research Promotion Fund) (KRF-2005-003D00320).

Keywords: Microfluidic, Biofilm, Superporous agarose, Poly phenol

I006

Sequence Analysis of Plasmid-pILR091 Genome of *Lactobacillus reuteri* L09 Isolate

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The genus *Lactobacillus* is the largest of the genera included in LAB but a few *Lactobacillus* plasmid-encoded functions have been discovered and have been used. In this study, we isolated plasmid DNA from *L. reuteri* isolate and described the characteristics and its relationship with other plasmids. After restriction enzyme analysis of plasmid DNA extracted *L. reuteri* isolate, pILR091 digested with *Sal*I was cloned in pQE-30Xa vector and sequenced. The complete sequence was confirmed by the re-sequencing of PCR products and analyzed with Genbank database. The copy number and stability were determined by quantitative-PCR. The complete sequence of *L. reuteri* showed the 7,185 nucleotides with one cut site by only two enzymes, *Sa*lI and *Hin*dIII and 39% of G-C content. The *ori* sequence of pC194-RCR family with little different sequence (TTTATATTGAT) was located in 63bp upstream of replication protein sequence, OFR 1. A total of 5 ORFs was identified and the coding sequence represented a total of 4,966 nucleotides (70.4%). ORF1, replication protein of pILR091 had the low similarity with replication protein sequence of pTE44. Other ORFs also showed low homology and *E*-value. The average G-C content of pILR091 was 39% and similar with that of genomic DNA. The copy number of pILR091 was determined as about 24 to 25 molecules per genomic DNA. These results suggested that pILR091 might be a good candidate to construct a vector which could be used for cloning and expression in *lactobacilli*.

Keywords: plasmid DNA, Replication, Open reading frame, G-C content

I007

Improved Translational Efficiency by Optimization of Sequence Context around Initiator Methionine in Pichia pastoris

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Commercially available expression vector kits normally contain multiple cloning site between the promoter and the initiation codon for facilitated cloning procedure. However, it is known that the translation of the mRNA is affected by the 5'-untranslated leader sequence (5'-untranslated region, 5'-UTR). It has been reported that the translational efficiency of human serum albumin was increased up to 50-fold by adjusting the 5'-UTR to be identical to that of the highly expressed alcohol oxidase mRNA in Pichia pastoris. This was tested for other promoters in *P. pastoris*, the frequently used GAPDH promoter and the newly isolated TEF promoter. The restoration of the exact sequence of the 5'UTR by removal of restriction sites resulted in 1.8 fold and 2.5 fold increase, respectively, in the expression level of the lipase B of *Candida antarctica* (CalB). The importance of the sequence context around the initiation codon was also exemplified by the optimization of the sequence immediate downstream of initiator methionine. Insertion of Ala or Gly codon just downstream of Met codon of mating factor α prepro leader sequence resulted in the increase in the expression level of CalB by 2 fold and 2.5 fold, respectively. In another signal sequence (TFP3), however, the same manipulation did not improve the expression level. Therefore, optimization of the sequence immediate downstream of initiator methionine may require further optimization depending on the sequence of interest.

Keywords: Optimization, *Pichia pastoris*, *Candida antarctica* lipase B (CalB)

I008

Finding Two Different Bacterial Lipases with Opposite Enantioselectivities toward Ethyl 4-Chloro-3-Hydroxybutyrate from Intertidal Flat

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Ethyl (S)-4-chloro-3-hydroxybutyrate (ECHB) is a chiral intermediate for the synthesis of Atorvastatin, a drug for hypercholesterolemia. (S)-ECHB could be produced by the enantioselective enzymatic resolution of racemic ECHB. In this research, we screened bacterial lipases able to hydrolyze one chiral form of ECHB preferentially as follows. Many tricaprylin-hydrolyzing psychrophilic microorganisms were isolated from the intertidal samples collected around Ganghwa Island. Among them, 18 different strains were screened by comparison of their cellular protein patterns using SDS-PAGE. Hydrolytic activities of their culture broth toward (R)- and (S)-form of p-nitrophenyl 4-chloro-3-hydroxybutyrate were measured spectrophotometrically. Strain 8-2 showed 3 times high hydrolytic activity toward (S)-form than (R)-form, whereas, on the contrary, strain 19-2 had 3 times high activity toward (R)-form than (S)-form. With these two lipases, hydrolytic reactions toward racemic ECHB were performed and their enantioselectivities were measured by chiral gas chromatography.

Keywords: lipase, ethyl 4-chloro-3-hydroxybutyrate, enantioselectivity

I009

Cloning and Sequencing Analysis of αGal cDNA

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αα Restriction endonucleases and other enzymes were used in accordance with the manufacture's instructions. Total RNA was prepared from mycelia by the phenol-chloroform method, and poly(A)+ RNA was purified with an oligo(dT)-cellulose column. A DNA fragment encoding a portion of the *Penicillium* sp. αGal gene was amplified by the reverse transcription(RT)-PCR method with a set of P1 and P2 primers designed from the N-pep and V-pep sequences. To determine the nucleotide sequence of the full-length cDNA coding for *Penicillium* sp. αGal, the 5' and 3' RACE technique was used. To obtain the 3' RACE products, the primary PCR using primers P5 and AP1 was followed by a nested PCR using primers P6 and AP2. The 5' and 3' RACE products were cloned into the pCRII vector, and sequence analysis of both strands of the cloned genes was performed by using the 373 DNA sequencer.

Keywords: AlphaGal cDNA,, Penicillium sp.

I010

Some Properties of Recombinant *Mortierella vinacea* Galactosidase II Expressed in *Saccharomyces cerevisiae*

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α-Galactosidase is of particular interest in view of its biotechnological applications. α-Gal from *Mortierella vinacea* has been used in the sugar refining process to increase the sucrose yield by elimination raffinose which prevents normal crystallization of beet sugar. The cDNAs coding for *Mortierella vinacea* α-galactosidase II were expressed in *Saccharomyces cerevisiae* under the control of the yeast *GAL10* promoter. The recombinant enzymes purified to homogeneity from the culture filtrate were glycosylated, and had properties identical to those of the native enzymes except for improving the hear stability of α -galactosidase II and decreasing the specific activities of both enzymes.

Keywords: Recombinant Mortierella vinacea, *Saccharomyces cerevisiae,* Expression

I011

The Preparation of β-1,4-Mannobiose and β-1,4-Mannotriose by Yeast Fermentation and Enzyme System

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α-Galactosidase is of particular interest in view of its biotechnological applications. α-Galactosidase from coffee beans demonstrates a relatively broad substrate specificity, cleaving a variety of terminal α -galactosyl residues. Cyamopsis tetragonoloba α-galactosidase effectively liberates the α-galactosyl residue of galactomannan. Removal of a quantitative proportion of galactose moieties from guar gum by α -galactosidase improves the gelling properties of the polysaccharide and makes them comparable to those of locust bean gum. To apply the specific character of the enzyme and to carry out the preparation of mannobiose and mannotriose using the combined process. The combined process consists of namely hydrolyzing the substrate by the crude enzyme and eliminating monosaccharides from the resulting hydrolysate with a yeast.

Keywords: mannobiose, mannotriose, yeast fermentation

I012

Isolation of Novel Astaxanthin-Producing Bacterial Strains

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Astaxanthin is a xanthophyll carotenoid with the most powerful antioxidant activity known to occur naturally. Therefore, commercial interest in astaxanthin production is increasing. We isolated several orange-colored bacterial strains from the surface water in Eulsukdo island near Busan. The isolates were screened for the presence of astaxanthin and carotenoid composition by HPLC analysis. Out of 50 isolates tested, 16 strains were confirmed to produce astaxanthin as a major carotenoid. These isolates were further characterized by 16S rDNA sequence analysis. The majority of the isolates belonged to the genus *Deinococcus* sp. exhibiting an identity level of 99.00-99.69 to *Deinococcus aquaticus, Deinococcus grandis* and *Deinococcus* sp. MBIC 3950. Astaxanthin is synthesized from β-carotene by a concerted action of the ketolase (*crtW*) and the hydroxylase (*crtZ*). Attempts to clone the β-carotene ketolase gene from these isolates by using the degenerate primers based on the consensus sequence of the reported *crtW* genes failed to produce the expected PCR procducts. This observation implied the presence of different type(s) of ketolase gene in these isolates. This was supported by the recent report describing the occurrence of *crtO* type β-carotene ketolase gene in *Deinococcus* species. In *Deinococcus radiodurans* R1, a *crtO* homolog showed β-carotene ketolase activity in spite of no significant homology to *crtW*-type ketolases.

Keywords: astaxanthin, β-carotene ketolase

I013

Development of Immunomagnetic Separation and Receptor-Mediated Methods for Noroviruses

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Norovirus is one of the most important foodborne pathogens causing more than 90% of nonbactarial gastroenteritis worldwide. Among 5 different genogroups(GI-GV) of noroviruses, both GI and GII noroviruses were known to infect human. Due to the inability to culture norovirus in vitro, molecular characteristics and immune response to norovirus have been insufficient known. Recently, there was the recent food poisoning outbreak affecting more than 2800 students in metropolitan Seoul area. Despite of its importance in public health, diagnostic techniques for detecting norovirus in food were poorly developed. We developed immunomagnetic separation(IMS) methods for detecting noroviruses in food and other complex environmental media. Both GI and GII noroviruses were obtained from KCDC. Then, the capsid regions (ORF2) of both GI and GII norovirus were cloned into pGEX-4T-1 expression vectors and expressed in Escherichia coli. host strain BL21 star DE3 through IPTG induction. The expressed protein was purified by GST columns to be injected into rabbits for obtaining both monoclonal and polyclonal antibodies. The IMS method was developed and characterized using commercially available antibody and Lewis^b antigen of human histoblood group antigens that is known as a human receptor to norovirus. Our developed IMS methods could detect as low as several hundred norovirus particles. This IMS method can be very useful tool for detecting norovirus in food and other complex media.

Keywords: Norovirus, Immunomagnetic Separation(IMS), Human Histoblood group antigens(HBGAs)

I014

Deproteinization of Natural and Acid-Treated Crab Shell Wastes by a Commercial Proteolytic Enzyme

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Delvolase® was screened from the commercial enzymes such as Delvolase®, MaxazymeTH NNP, Ccllupulin MG, Cytolase, Econase MP 1000, and Econase CEPi, as the high proteolytic enzyme for deproteinization of crab shells to extract chitin. Delvolase® was stable between pH 5.0 and 10.0, and the optimum pH was 8.5. The optimum temperature was 60 ℃. Deproteinization of acid-treated crab shells was dependent on the enzyme concentration. Deproteinization of natural and acid-treated crab shells with 1% Delvolase® was 90% and 81%, respectively, after 5 days treatment at 60 ℃, suggesting the applicability of the Delvolase® in the process for bio-extraction of chitin from the crustacean sehlls. Ash content was little affected by the protease treatment.

Keywords: Deproteinization, Proteolytic enzymes, Delvolase®, Demineralization, Red crab shell

I015

Acyl–CoA Carboxylase of *Streptomyces toxytricini*

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An oligonucleotide probe was designed from conserved sequences in carboxyltransferase (CT) or β-subunit of ACCs of actinomycetes origin. Based on the restriction pattern derived from hybridization data, the 7.5kb *Bam*HI fragment was selected and cloned into λBlueStar phage vector. Subsequent *Pst*I digestion of the purified 7.5kb *Bam*HI insert gave three fragments with size 1.2kb, 2.6kb, and 3.7kb respectively. They were subcloned in pGEM3-Zf(+) vector. Sequencing analysis revealed two ORFs, ORF1 (*accD1*) and ORF2 (*accA1*), identified within 4kb DNA fragment and the initiation codon of ORF1 is located in proximity to *Bam*HI restriction site. The genes were designated as and, respectively. The distance between them is only 30bp, which implies that these two genes are co-transcribed. The *accA1* and *accD1* genes encode 538 amino acids for a protein of 58.1kDa and 673 amino acids for a protein of 74kDa, respectively. The protein encoded by *accD1* gene (AccD1) showed strikingly high homology with the carboxyltransferase subunit (ACC, βsubunit) of several propionyl– and acetyl–CoA carboxylases of actinomycetes origin. The *accA1* gene encodes a protein (AccA1) similar to biotin carboxylase subunits (ACC αsubunit) with different microbial origin. Based on the alignment of the data obtained in this work, we propose that the identified *accD1* and *accA1* genes represent a and b subunits of ACC in *S. toxytricini*.

Keywords: Acyl-CoA carboxylase, AccA1, AccD1, *Streptomyces toxytricini*

I016

Probing Conserved Helical Modules of Portal Complexes by Mass Spectrometry based Hydrogen/ deuterium Exchange

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The dsDNA bacteriophage P22 has a ring shaped dodecameric complex of the 84 kDa portal protein which forms the central channel of the DNA packaging motor.CryoEM reconstruction of the C-terminally truncated P22 portal (1-602) complexes at ~8Å has revealed a ring-like morphology shared by portal complexes of phi-29, SPP1, T3 and herpes simplex virus. The secondary structure prediction of P22 portal protein and its threading onto the crystal structure of the phi-29 portal complexes suggested that P22 portal protein complex shares conserved helical modules which were found in the dodecameric interfaces of the phi-29 portal complex. Although a cryoEM reconstruction at this resolution can provide global structural information, it lacks sufficient resolution to directly identify the amino acids involved in subunit-subunit contacts.To identify the amino acids involved in subunit-subunit contacts in the P22 portal ring complexes and validate threading model, we performed comparative hydrogen/deuterium exchange analysis of monomeric and dodecameric portal proteins of P22 and phi-29 using FT-ICR mass spectrometry (LTQ-FT). Hydrogen/deuterium exchange experiments provided evidence of inter-subunit interactions of P22 portal complex at regions which were predicted to be conserved helical modules based on threading using the crystal structure of the phi-29 portal complexes.

Keywords: virus, FT-ICR mass spectrometry, Hydrogen/deuterium exchange

I017

Microbial Cyclosophoraose as a Novel Catalytic Carbohydrate for Methanolysis and Transesterification of Esters of Amino Acid Derivatives

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Methanolysis and transesterification of esters of amino acid derivatives can be induced by a natural microbial cyclooligosaccharide, a cyclosophoraose (cyclic-(1->2)-β-D-glucan), which is a member of a family of unbranched cyclooligosaccharides produced as *intra*- and *extra*oligosaccharides by soil microorganisms of the genus, *Rhizobium*. Cyclosophoraose catalyzed the methanolysis and transesterification for *N*-acetyl-L-phenylalanine ethyl ester, *N*-acetyl-L-phenylalanine *p*-nitrophenyl ester, *N*-acetyl-L-tyrosine ethyl ester, *N*-acetyl-L-tryptophan ethyl ester and ethyl phenyl acetate. Transesterification was investigated in nine nonaqueous solvents. The ratios of K_{cat_Cys} to K_{uncat} were determined by nuclear magnetic resonance (NMR) spectroscopy for methanolysis and high performance liquid chromatography (HPLC) for transesterification. A characteristic of cyclosophoraose-induced catalysis was higher affinity for esters of *N*-acetyl-L-phenylalanine than other substrates in both reactions, suggesting that cyclosophoraose catalyzed the methanolysis and transesterification with regioselectivity.
Keywords: Microbial cyclooligosaccharide, Cyclosophoraose, Methanolysis, Transesterification, Amino acid esters, Rhizobium meliloti

I018

Isolation of *Actinomycetales* sp. KLM-2, a Novel Agar-Degrading Bacterium, from Brown Seaweed

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An agar-degrading bacterium, strain KLM-2, was isolated from brown seaweed, Undaria pinnatifida, using M9 agarose agar plate. Strain KLM-2 produced both intracellular and extracellular agarase, and grew utilizing agar or agarose in the culure medium as sole carbon source. The subsequent BLAST analysis on sequence similarity indicated that the closest relatives of the strain KLM-2 were unclassified Actinomycetales bacterium and Streptomyces griseorubens. On the basis of phylogeny analysis it was concluded that strain KLM-2 belongs to the genus Actinomycetales or Streptomyces, because 16S rDNA sequences of A. bacterium and S. griseorubens in GenBank were same each other. This work was financially supported by the Region Innovation System Program, Ministry of Commerce, Industry and Energy, Republic of Korea.
Keywords: Actinomycetales, Marine bacteria, 16S rDNA, Agarase activity

I019

L-Arabinose Isomerase Expression System in GRAS Hosts

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Arabinose isomerase (AI, E.C. 5.3.1.4), a commercial enzyme for the edible tagatose production *in vitro* in an immobilized enzyme system, has been studied using the enzymes expressed in *E. coli* system, which might cause noxious by-product in food. To ensure food safety tagatose manufacturing process, we have developed AI expression system in GRAS hosts. The thermostable AI genes from *Thermotoga neapolitana*(TNAI) and *Geobacillus stearothermophilus*(GSAI) were subcloned into a *Corynebacterium - E. coli* shuttle vector (pMT1) and a *B. subtilis – E. coli* shuttle vector (pHPS3133) resulted in pMT1/TNAI, pMT1/GSAI, pHPS3133/TNAI and pHPS3133/GSAI, respectively. The restriction deficient *Corynebacterium glutamicum* AS019-E12 and *Bacillus subtilis* 168 were used for GRAS expression hosts. The TNAI and GSAI showed no activity in the *Corynebacterium* host containing pMT1/TNAI, pMT1/GSAI. The GSAI activity was found in the *Bacillus* host containing pHPS3133/GSAI, while no TNAI activity was found. Further study on the high expression of GSAI in *Bacillus* host is on the way.
Keywords: expression system, *Corynebacterium, Bacillus*, pMT1, pHPS3133

I020

Deacetylation of Chitin by Crude Enzyme from a Newly Screened *Absidia corymbifera* DY-9

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Among more than a hundred colonies of fungi isolated from soil samples, DY-9 has been screened as an extracellular chitin deacetylase (CDA) producer. The isolate was further identified as *Absidia corymbifera*, based on the morphological properties and the nucleotide sequence of 18S rRNA gene. The fungus was screened in yeast peptone glucose (YPD) liquid medium but exhibited maximal growth in yeast peptone chitin (YPC) liquid medium containing 2% of chitin instead of glucose at pH 9.0 and 28℃ with 150 rpm. The CDA activity of DY-9 was maximal on the 5th day of culture in YPD liquid medium. The CDA was inducible by addition of chitin powder and swollen chitin. This enzyme showed a maximal activity at pH 6.5 and 55℃. In addition, it had a pH stability range of 7.5-8.5 and a temperature stability range of 4-45℃. When water soluble chitin (WSCT-50, degree of deacetylation, DD, 50%) was used as a substrate, the crude enzyme activity of DY-9 showed 0.91 U/ml at pH 4.5 and 50℃. The enzyme was enhanced in the presence of Mg^{2+} but inhibited by Co^{2+}. Among various substrates tested, WSCT-50, glycol chitin and crab chitosan (DD 71-88%) can be handled by the CDA.
Keywords: Chitin deacetylase, WSCT-50, Absidia corymbifera DY-9

I021

Characterization of Chitin Deacetylase Produced by *Mortierella* sp. DY-52

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Chitin deacetylase (CDA), the enzyme that catalyzes the hydrolysis of acetamido groups of N-acetylglucosamine in chitin, has been isolated from culture filtrate of the fungus *Mortierella* sp. DY-52 and further characterized in this report. The molecular mass of the enzyme was 54 kDa and 59 kDa as judged by SDS-PAGE, suggesting that the enzyme exists as a dimmer. The CDA was inducible by addition of glucose and chitin. This enzyme showed a maximal activity at pH 4.5 and 60℃. In addition, it had a pH stability range of 4.5-8.0 and a temperature stability range of 4-40℃. When water soluble chitin (WSCT-50, degree of deacetylation, DD, 50%) was used as a substrate, the crude enzyme activity of DY-52 showed 0.6 U/ml at pH 4.5 and 50℃. The enzyme was enhanced in the presence of Co^{2+} and Ca^{2+}. The enzyme was active toward WSCT-50, glycol chitin and crab chitosan (DD 71-88%), but was inactive with nature crystalline chitin.
Keywords: Chitin deacetylase, WSCT-50

I022

Production of N,N'-Diacetylchitobiose from Chitin Using the Crude Enzyme from *Aeromonas* sp. GJ-18

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A bacterium, *Aeromonas* sp. GJ-18, having strong chitinolytic activity was used for crude enzyme preparation which contained *N*-acetyl-D-glucosaminidase and *N,N'*-diacetylchitobiohydrolase. *N,N'*-Diacetylchitobiose was produced from chitin as a major hydrolytic product by utilizing the temperature sensitivities of the chitin degradation enzymes in the crude enzyme preparation. Among various temperatures, the highest yield(32.7%) of *N,N'*-diacetylchitobiose was abtained at 50℃ with the substrate of 3% swollen chitin during 7 d. Among various subtrates, the highest yield(56.5%) of *N,N'*-diacetylchitobiose was obtained from 2% swollen chitin during 7 d. The final production ratio of *N*-acetyl-D-glucosamine to *N,N'*-diacetylchitobiose was 0.21 when 25 ml of crude enzyme was added to the swollen chitin at one time. However, when the same amount of enzyme was divided into two times, 15 ml of that was added before reaction and 10 ml of that added after 3 days of incubation, the final ratio was 1.1 during 7 d.
Keywords: N,N'-Diacetylchitobiose, *Aeromonas* sp. GJ-18, Chitin

I023

Development of Water-Quality Monitoring System Using an Electrochemically Active Bacteria-Based Biosensor

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The biosensor, based on electrochemically-active bacteria (EAB) in combination with 3-electrode electrochemical cell, has been applied for measurements of biochemical oxygen demand (BOD) and detection of toxic materials. The positively poised working electrode in the electrochemical cell was used as an electron acceptor of the EAB. The generated current pattern from biosensor and its Coulombic yield were found to be dependent on the BOD_5 value of the wastewater. When toxic solution was fed to the biosensor, significant signal was observed from the amperometric measuring system of the biosensor. These results indicate that the EAB based biosensor in this study can be applied to the monitoring of water-quality, especially BOD and toxicity.
Keywords: electrochemical enrichment, electrochemically-active bacteria, three-electrode electrochemical cell

I024

Measurement of Decolorizing Rate of Dyes in the Presence of *S. aureus* or *E. coli*

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S. aureus, and *E. coli* were examined their ability to reduction (decolorization) of dyes using cyclic voltammetry, chronoampherometry and spectrophotometry. Reduction potential of various dyes was found using cyclic voltametry for further study. When the dyes were reduced at -1.0 V that was lower than reduction potential of the dyes using chronoampherometry, decolorization of the dyes was observed. When *S. aureus*, and *E. coli* reduced the dyes under the anaerobic condition, decolorizing rate of dyes by *E. coli* was different from decolorizing rate of dyes by *S. aureus*. These results showed that the ability to decolorize the dyes is based on the characteristics of each bacterial species.
Keywords: dye, decolorization, *S. aureus, E. coli*

I025

Submerged Culture Conditions for the Production of Water Souble Exo- and Endo-Polysaccharides by Lentinus lepideus

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Lentinus lepideus, a Basidomycete fungus, is an edible mushroom and belongs to the family of Pleurotaceae. The water-soluble glycan extract from *L. lepideus* is known as strong immunomodulating substance which is induces a variety of cytokines in the human peripheral lymphocyte system and hematopoiesis of granulocyte lineage *in vivo*. Submerged cultures of fungus using bioreactors has been used to produce mycelial biomass as bioactive compounds from the culture. However, despite their potential usefulness, *L. lepideus* has not been extensively tested for their production of potential polysaccharides in submerged culture. In the present study, we tested various culturing factors that might influence the production of mycelial biomass, water soluble endopolysaccharides (PPS) and water soluble exopolysaccharides (EPS) from *L. lepideus* and determined optimal conditions for the PPS and EPS production. High yield of the EPS required moderate culture temperature (25℃) and long culture period (16-20 days). In contrast, PPS production required higher temperature (35℃) and short culture period (8 days). The most suitable bioreactor for the production of cell biomass and polysaccharides was balloon type air bubble bioreactor. In the optimal conditions, 8 g/ℓ of mycelial biomass, 9% of PPS and 1.6 g/ℓ of EPS could be obtained from submerged culture of *L. lepideus*.

Keywords: *Lentinus lepideus*, exopolysaccharides, endopolysaccharides, mycelial culture

I026

Engineerering the Astaxanthin Synthetic Pathway in *Eschreichia coli*

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Astaxanthin, a carotenoid pigment, is one of the most powerful biological antioxidant that has been known to occur naturally. In a previous study, we isolated the astaxanthin biosynthetic gene clusters containing the β -carotene ketolase (*crtW*) and the hydoroxylase (*crtZ*) from marine bacteria. In this study, these two genes were expressed in *Escherichia coli* strains that have been engineered to produce β-carotene. The expression cassette contained two different *crtEIBY* clusters, Fos2 or Fos3, for the production of β-carotene and *crtWZ* operon for production of astaxanthin from β-carotene. The expression cassette was constructed in pBAD promoter vector system. The kanamycin resistance selection marker was also tested for potential improvment in plasmid stability in place of ampicillin resistance marker. Initial testing of these constucts resulted in a minimal level of astaxanthin production. A dramatic improvement of astaxanthin production was achieved through expression of genes (*mvaK1*, *mvaK2*, *mvaD*) required for production of isopentenyl pyrophosphate from mevalonate. The vector also contained *idi* gene that has been reported to be essential for proper production of carotenoid in *E. coli*. Further optimization of the expression cassette was performed by replacing the *crtE* gene of the clusters, the probable rate-determining step in carotenoid biosynthesis, with several other *crtE* genes. The astaxanthin productivity and carotenoid profile was assayed by HPLC of the cell extract.

Keywords: astaxanthin, carotenoid, mevalonate

I027

The Influence of Submerged Culture Conditions of *Lentinus lepideus* on Immunomodulating Activities of Endo- and Exo-Polysaccharides Obtained from them

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Polysaccharides of *Lentinus lepideus* have strong immunomodulating activities. As the production of polysaccharide from mycelia is more efficient than that from fruit bodies, the influence of culture conditions from submerged cultures of mushroom has drown much attention. This study deal with culturing factors affecting immunomodulating activities of endo-polysaccharides (PPS) and exo-polysaccharides (EPS) obtained from submerged cultures of *L. lepideus*. The mycelia were cultured under various culture conditions. PPS and EPS were extracted and their immunomodulating activities were compared by measuring level of TNF-α. TNF-α level by EPS varied depending on mycelial cell lines, carbon sources or culture periods. Highest amounts of TNF-α was produced when glucose was used as a carbon source and cultured for 4 days by EPS. Meanwhile, PPS derived from culture under various culture conditions except for culture period did not give any difference in TNF-α amounts. PPS obtained from 16 days culture induced highest level of TNF-α. The results suggest that immunomodulating activitiy of PPS is less affected by culture conditions than that of EPS in *L. lepideus*.

Keywords: *Lentinus lepideus*, immunomodulating activities, endopolysaccharides, exopolysaccharides

I028

Glycosylation Analysis and Pharmacokinetics of Cytotoxic T-lymphocyte Antigen 4 Immunoglobulin (CTLA4-Ig) Produced in Rice Cells

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Cytotoxic T-lymphocyte antigen 4 immunoglobulin (CTLA4-Ig) is a recombinant human fusion protein consisting of the extracellular domain of human CTLA4 and theFc region. It has demonstrated immunosuppressive activity and the ability to induce immunogenic tolerance in several in vivo animal models. It has been produced in different cell types, with different properties that depend on the production process used. We investigated the terminal sialylation and evaluated the pharmacokinetics of CTLA4-Ig produced in rice cells (rrhCTLA4-Ig). α2,3-terminal sialylation and α2,6 terminal sialylation of rrhCTLA4-Ig were detected by *Maakia Amurensis* (MAL 2) and *Sambucus Nigra* (SNA). After intravenous administration to rat, pharmacokinetics of rrhCTLA4-Ig, crhCTLA4-Ig (CHO cells derived GM-CSF) as well as deglycosylated rrhCTLA4-Ig and crhCTLA4-Ig were evaluated. The serum samples were kinetically obtained in the rats and the CTLA4-Ig level in serum were measured using sandwich ELISA. rrhCTLA4-Ig does not have the terminal sialylation and was cleared from the blood circulation faster than the crhCTLA4-Ig. Moreover, the deglycosylated rrhCTLA4-Ig and crhCTLA4-Ig were cleared faster than native forms. These results suggest that the presences of glycans on rrhCTLA4-Ig and crhCTA4-Ig are important in vivo stability of CTLA4-Ig and that terminal sialylation of glycans on CTLA4-Ig increase the *in vivo* stability of CTLA4-Ig.

Keywords: Cytotoxic T-lymphocyte antigen 4 immunoglobulin (CTLA4-Ig), Terminal sialylation, Maakia Amurensis (MAL 2), Sambucus Nigra (SNA)

I029

A Comparative Study of TiO_2 Thin Films on Antibacterial Orthodontic Wires and Bracket

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TiO_2 thin films were deposited on orthodontic wires and bracket using sol-gel, CVD(Chemical Vapor Deposition) and PE-CVD(Plasma Enhanced-CVD) methods. Physical properties and Antibacterial effects of TiO_2-coated surfaces were investigated. Methylene blue degradation test was carried out to evaluate the photocatalytic activity of TiO_2 and corrosion resistance of TiO_2 thin films against fluoride solution was also analyzed by observing the surfaces of TiO_2-coated wires and brackets via SEM after immersion in sodium fluoride solution. Through the comparison of property and photocatalytic activity of TiO_2 thin films according to coating method, following results were obtained. Adhesive strength and Photocatalytic activity of TiO_2 thin films were highest in PE-CVD and gradually low in order of CVD, sol-gel method. However, corrosion resistance of TiO_2 thin films against fluoride solution was stronger in CVD or PE-CVD methods than sol-gel method. These results suggest that the CVD or PE-CVD methods would rather appropriate than sol-gel method for the TiO_2 coating on orthodontic wires and bracket.
Keywords: Antibacterial, TiO_2, Orthodontic Wire

I030

One-Step Purification of *Streptomyces* Phospholipase D by Immunoaffinity Chromatography using Peptide Antibodies

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S*treptomyces* phospholipase D(PLD, EC3.1.4.4) has a commercial importance for its conversion of phosphatidylcholine to several other phospholipids of great value. *Streptomyces* PLDs have been purified by conventional chromatography, which gave low activity yields and required somewhat cumbersome procedures. In this study, we tried one-step purification of a *Streptomyces* PLD by the preparation of immunoaffinity resins to overcome the purification problems. By using structural information of a *S.* species PLD previously reported and protein structure prediction progrms, we selected five different epitopes from the protein sequences of *S. somaliensis* PLD, prepared their anti-peptide antibodies, and purified PLD by using immunoaffinity resins. We will discussed the purification of PLD related with the purification yields and purity and the enzymatic characterization of purified PLD.
Keywords: *Streptomyces*, phospholipase D, phospholipids, purification, affinity chromatography

I031

Screening of Monoclonal Antibodies Against a Viral Protein Using Protein Chip Technology

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Oyster mushroom spherical virus (OMSV) is a causative agent of Die-back disease in oyster mushroom, *Pleurotus ostreatus.* Biochemical characterization of OMSV has revealed that it consisted of a single-stranded RNA genome that encodes at least 7 open reading frames including a viral coat protein and an RNA-dependent RNA polymerase (RDRP). RDRP is the crucial and specific catalytic enzyme for viral replication. To further study the viral replication process in its host mushroom cells, we have produced monoclonal antibodies (MAbs) against a fragmented RDRP protein. For the rapid screening of MAb, a protein chip technology based on Alexa-488(A488) dye labeling method was introduced. Eighty seven monoclonal antibodies (MAbs) against the fragmented RDRP protein (F7) were generated from mouse hybridoma cells. The F7 protein was chemically coupled onto an amine-modified slide glass. The MAbs were spotted onto the F7-coupled slide glass. The amounts of bound MAbs were measured by binding of A488-modified secondary antibody using a fluorescent image scanner. Five out of 87 MAbs have chosen by the signal intensity. The specificity of the selected MAbs in detecting OMSV RDRP was further justified by an immunoblot analysis and a surface plasmon resonance biosensor assay.
Keywords: MAb screening, Protein chip, Virus, Detection, Mushroom, RNA polymerase

I032

The Effect of *Auricularia auricula* Fruiting Body on Oral Microorganisms and *Helicobacter pylori*

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Helicobacter pylori is an important cause of chronic gastric and peptic ulceration, infected 82% on Korean. *Helicobacter pylori* probably does not in itself induced gastric cancer, but it may promote either the production of carcinogens or mutagenic events and WHO regards it as first grade carcinogens. The effect of *Auricularia auricula* fruiting body extracts on oral microorganisms and *Helicobacter pylori* were examined using a Muller-Hinton agar diffusion method. The diethyl ether extracts of *Auricularia auricula* fruiting body showed highest as 19 ㎜ inhibition zone among chloroform, ethyl acetate and ethanol. The top spot after TLC analysis(Aa1) from *Auricularia auricula* fruiting body showed high inhibition activity against *H. pylori*. The effect of *Auricularia auricula* fruiting body extracts on *Streptococcus mutans* an oral microorganisms was as MIC 0.33 mg from diethyl ether extracts of 1 g mushroom, and Aa1 fraction after purified by TLC was identified in two bands from 4 bands.
Keywords: *Auricularia auricula, Helicobacter pylori, Streptococcus mutans*, Dental caries

I033

Strain Improvement for Lipase Display on *Escherichia coli* Cell Surface Based on Transcriptome Analysis

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Cell surface display is a technique for expressing target proteins fused with anchoring motif on the surface of host cells. This technique has a various application fields including the development of live vaccines, peptide · antibody libraries, bioremediation and biosensor. The efficiency of surface display systems is totally different depending on the used target proteins, anchoring motifs and host strains. To date, the best condition has been determined by trial and error without considering the reason. In this study, the effects of lipase cell surface system on the cellular physiology of *Escherichia coli* were examined at transcriptome level using DNA microarray, followed by real-time PCR analyses. Based on these analyses, the specific gene targets were selected and manipulated (deleted or amplified) for confirming their effects. As a result, the strain for efficient cell surface display could be improved by rationally manipulating several genes identified by transcriptome analysis. Additionally, it was investigated why these genes effect cell surface display at gene expression and protein levels using real-time PCR, Western blot, and mass spectrometry analyses. [This work was supported by the Korean Systems Biology Research Grant from the Ministry of Science and Technology. Further supports by LG Chem Chair Professorship, Microsoft and IBM SUR program are appreciated.]
Keywords: cell surface display, lipase, transcriptome, strain improvement, *Escherichia coli*

I034

Isolation and Biochemical Characteristics of Biosurfactant-Producing *Bacillus subtilis* TBM 3101(3)-9

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A biosurfactant-producing strain, TBM 3101(3)-9 was isolated from the soil sample of Taebaek Mountain through an antifungal test and emulsification assessment. The isolate was identified as Bacillus subtilis by the API Staph kit and Bergey's manual of systematic bacteriology. The strain, designated as B. subtilis TBM 3101(3)-9 was assessed, regarding to the microbial growth by the physical and chemical test, surface tension, emulsification activity and stability. The surface tension of the isolate sharply decreased to the minimum 29 mN/m at 18 h growth. Of note, its emulsification was highest stabilized when tributyrin was utilized as a substrate, indicating that in comparison with a variety of synthetic surfactant, the biosurfactant produced by the isolate was significantly similar to a synthetic surfactant, Tween 20. In addition, the biosurfactant showed a high emulsification activity when soybean oil, crude oil and tetradecane were used as a substrate. Thus, these studies could contribute to the detection and development of biosurfactant beneficial to the environment and humans.
Keywords: *Bacillus subtilis* TBM 3101(3)-9, biosurfactant, emulsifying activity, emulsifying stability

I035

Isolation and Characterization of Lipopeptide Biosufactant Produced by *Micrococcus* sp. GBM 3309(3)-11

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A strain producing biosurfactant, GBM 3309(3)-11 was isolated from the soil sample of Kyebang Mountain by the antifungal and emulsification assessment. The isolate was identified as *Micrococcus* sp. by the API Staph kit and Bergey's manual of systematic bacteriology. The growth, surface tension, emulsifying activity and stability of the isolate were investigated by physical and chemical tests. The surface tension dramatically decreased to the minimum 31.98 mN/m after a 42h culture. The emulsifying stability reached to a highest degree when tributyrin was used as a substrate, suggesting that it did likewise as Tween 20 and Tween 40, compared with a variety of synthetic surfactant. The high emulsifying activity was also obtained when soybean oil and crude oil were used as the substrate. These findings, thus, could derive the development of sustaining biosurfactant benign to the environment.
Keywords: *Micrococcus* sp. GBM 3309(3)-11, biosurfactant, emulsifying activity, emulsifying stability

I036

Antiradical and Antioxidant Activities of the Crude Extracts from *Alnus* sp.

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Abstract In the current study, we investigated the possible antiradical and antioxidant activities of crude extracts of *Alnus*. sp using different antioxidant tests: 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, Β-carotene linoleate model system, ferric thiocyanate (FTC) method, reducing power and metal chelating activities. Those various antioxidant activities were compared with standard antioxidants such as L-ascorbic acid, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). In the assessment of the free radical scavenging, the methanol and ethyl acetate extracts showed strong antioxidative activity with 87.5 ㎍/㎖ and 65.8 ㎍/㎖ of IC_{50} values, respectively. This antioxidant property depended on the concentration and augmented by the increasing amount of the extract. In addition, total phenolic compounds in the methanol and acetate extracts of *Alnus* sp. were determined to be gallic acid equivalents. The results obtained in the present study indicated that *Alnus* sp. is a potential source of natural antioxidant. In addition, the crude extracts were found to possess antimicrobial activity against several bacteria and yeast.
Keywords: *Alnus* sp., Antioxidant activity, Antiradical activity

I037

Antimicrobial and Antioxidant Activities of the Solvent Extracts from *Tetragonia tetragonioides*

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Tetragonia tetragonioides has long been used as a traditional remedy for stomach cancer and furuncle. We investigated antimicrobial and antioxidant activity of the solvent extracts of *Tetragonia tetragonioides*. The solvent fractions were extracted by 100% methanol (MeOH) and successively extracted by n-hexane, methyl chloride(CH_2Cl_2) and ethyl acetate (EtOAc). Antioxidant activites of solvent fractions from *Tetragonia tetragonioides* were examined by the ferric thiocyanate method, reducing power, metal chelating activity and free radical scavenging assay. The CH_2Cl_2 and EtOAc extracts of *Tetragonia tetragonioides* was found to exhibit a distinctive antioxidant activity. Antimicrobial activity of the extracts was investigated against several microorganisms. The antibacterial activity was determined by an agar-well diffusion method and expressed as the average diameter of the zone inhibiting bacterial growth around the wells. The minimum inhibitory concentration (MIC) of the active extracts was determined by using the micro-plate dilution assay. The EtOAc extracts exhibited a significant antibacterial activity against both Gram-positive and Gram-negative bacteria.
Keywords: *Tetragonia tetragonioides*, Antimicrobial, Antioxidant

I038

Pathogen-Host Cell Adhesion and Inhibition (I): Role of Traditional Medicinal Plant Polysaccharides

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Bacteria specifically adhere to host cells, mainly based on carbohydrate-protein interactions. In an effort to characterize the inhibitory effects against pathogenic bacteria, most notably *Helicobacter pylori*, *Porphyromonas gingivalis*, *Propionibacterium acnes*, and *Staphylococcus aureus*, we investigated polysaccharides from *Artemisia capillaries* (AC), *Camellia sinensis* (CS) and the *Panax ginseng* (PG), using human and animal cell lines. PG-F2 and CS-F2 showed marked inhibitory activity against the bacteria-mediated hemagglutination with a minimum inhibitory concentration (MIC) between 0.01 and 0.1 mg/mL. Notably, CS-F2 showed higher activity than PG-F2 and AC-F2 *against H. pylori, P. acnes,* and *S. aureus.* Furthermore, the inhibitory effects of CS-F2 and PG-F2 on the adhesion of *H. pylori* to AGS gastric epithelial cells, of *P. gingivalis* and *A. actinomycetecomitans* to KB oral squamous cells, and of *P. acnes* and *S. aureus* to NIH 3T3 fibroblast cells were further assessed, resulting in MIC values 0.063 - 0.13 mg/mL and 0.125 - 0.5 mg/mL, respectively. Probably more importantly, these polysaccharides were found to exert no inhibitory effects against beneficial bacteria such as *Lactobacillus acidophilus*, *Escherichia coli*, or *Staphylococcus epidermidis*. Our results strongly suggest that the acidic polysaccharides from the medicinal plants may have a selective anti-adhesive effect against pathogenic bacteria, while having no effects on beneficial or commensal bacteria.
Keywords: bacterial adhesion, antiadhesive activity, *Helicobacter pylori, Porphyromonas gingivalis, Propionibacterium acnes, Staphylococcus aureus*

I039

Pathogen-host Cell Adhesion and Inhibition (II): Analysis of Traditional Medicinal Plant Polysaccharides

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In an effort to obtain a more through understanding of the anti-adhesive mechanism against pathogens, purified acidic polysaccharides from medicinal plants were examined. The acidic polysaccharides were purified from *Artemisia capillaries*, *Camellia sinensis*, and *Panax ginseng* and characterized. CS-F2 and PG-F2 may be a pectin like arabinogalactan-type polysaccharide, which are mainly composed of galacturonic and glucuronic acids, with a molecular weight of approximately $8.0x10^4$ Da and $1.2x10^4$ Da, respectively, along with rhamnose, arabinose, and galactose as minor components. The complete hydrolysis of PG-F2 via chemical or enzymatic treatment resulted in the abrogation of its anti- adhesive activity. However, limited hydrolysis via treatment with pectolyase yielded an oligosaccharide fraction with activity comparable to the precursor acidic polysaccharide PG-F2. The purified oligosaccharides were determined to inhibit the attachment of gastric, oral, and skin pathogenic bacteria, in a range of 0.01-0.5 mg/mL. Our results suggest that the oligosaccharides also play an inhibitory role on bacterial adhesion to host cells and that our established purification protocol can be applied to obtain active acidic polysaccharides from other medicinal plants. Further purification using recycling HPLC resulted in several oligosaccharides PG-O2, PG-O4, and PG-O5 which were found to have approximately 3-5 monosaccharide units, of which the structure determination is in progress using NMR.
Keywords: Antiadhesive, *Artemisia capillaries, Camellia sinensis, Panax ginseng*, arabinogalactan type, polysaccharide, oligosaccharide

I040

Construction of Engineered Indigoid-producing Oxygenase Enzymes Using Combined Methods of Site-saturation and Site-directed Mutagenesis Techniques

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A novel indigo-producing oxygenase gene, designated *ipoA* (1,197 bp), was isolated and characterized from *Rhodococcus* sp. strain T104. The *ipoA* gene has no close homologs in the GenBank database. Blastn analysis failed to detect sequence similarity to the *ipoA* nucleotide sequence although when analyzed by blastp its deduced amino acid sequence showed at most 35% identity with an indole oxygenase from an uncultured bacterium JEC54. The *ipoA* gene including the putative RBS was amplified by PCR, cloned into the pCR-Blunt vector, and transformed into *Escherichia coli* strain XL1-Red, which is deficient in three of the primary DNA repair pathways. Loss of the blue pigment indigo-producing ability (white colonies) was initially screened on LB plates containing kanamycin. A total of ten mutant clones were identified by subsequent DNA sequencing: A58V, P59L, G128S, G251D, I40N, M292V/M328T, H372Y, N370D, P59S and A125P. The substitution A58V was selected for further study, primarily because alanine and valine have relatively similar side chains. Saturation mutagenesis was performed at position A58 and revertant clones were isolated for forming blue colonies. DNA sequencing analysis identified five different revertants among a total of eighteen: A58Q, A58G, A58M, A58C, and A58S. It is interesting to observe that rather dramatic amino acid changes result in an active enzyme. This suggests that the alanine residue at position 58 plays a unique role in the IpoA enzyme activity.
Keywords: indigo, oxygenase, *Rhodococcus*, protein engineering

I041

Microbial Community Analysis of Hydrogen Producing Dominant Species in Food Wastes Continuous Reactor

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Hydrogen is known as a clean renewable energy. Hydrogen production by anaerobic microorganisms has attracted much of attention as they can use various carbon-sources for efficient hydrogen production. There have been abundant food wastes generated from human activities, and it is prohibited direct landfill, therefore, this has become an environmental concern in recent years. But these food wastes can serve as potential energy substrates for hydrogen production because of its high organic content.Here, a continuous hydrogen-producing reactor(CSTR) packed with anaerobic mixed microbial culture and food wastes and monitored community changes in the reactor over a period of incubation. The hydrogen production rate was measured periodically and the point at which the maximum hydrogen production achieved was chosen for sampling to analyze microbial community changes. Analysis of microbial community and dominant species in the food waste CSTR was performed by 16S rDNA clone library construction. Our restriction fragment length polymorphism(RFLP) yielded 30 different clones belonged to three major types of microbial population. Out of the total 30 distinct clones, 16 clones were represented *Clostridia* sp. (67%), four clones for *g-Proteobacteria* sp. (17%), and two each clones (8%) for *Bacilli* and *Bacteriodete*, respectively. Our results revealed that the *Clostridia* sp. are the dominant species that engaged in maximum hydrogen production in CSTR.
Keywords: biohydrogen production, microbial community, clone library

I042

Optimization of TGE (Transmissible Gastroenteritis) Virus Vaccine Production Using Swine Testicle (ST) Cell

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TGE(Transmissible Gastroenteritis) is caused by a virus belonging to coronavirus, that causes an acute infection of the small intestine of the pig. No drugs are effective against TGE virus in swine. Thus, treatment must be directed at the effects of the virus rather than at the virus itself. The optimum operation variables such as oxygen concentration, multiplicity of infection (MOI), infection time, and harvest time were investigated for the vaccine production by immobilized ST cells. And, the optimum operation strategy was searched for the efficient production of TGE virus vaccine in bioreactor. Three operation modes including batch, fed-batch, and perfusion were tried and their performances were evaluated in terms of final virus titer. Fed-batch culture was tried only at virus production phase by feeding glucose and glutamine. Perfusion was tried at both cell growth phase and virus production phase by medium exchange with specific intervals. Finally, approximately 7.5 fold increase of virus titer was observed in the optimum condition tried.
Keywords: cell culture, virus, vaccine

I043

Adaptation of Recombinant CHO Cells Producing IDS to Serum-Free Suspension Culture

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Iduronate 2-sulphatase (IDS) is a lysosomal enzyme involved in the sequential degradation of the glycosaminoglycans heparan sulphate and dermatan sulphate. Mucopolysaccharidosis type II (MPSII, Hunter syndrome) is caused by a deficiency of IDS. For the industrial production of recombinant protein IDS, the establishment of high productivity cell line and the optimization of culture condition is required to enhance IDS productivity. Serum is a potential source of bacterial, mycoplasmal and viral contamination, it has a possibility of the introduction of serum proteins and pyrogens into the final product etc. And suspension culture is preferred for its simplicity and scalability. Thus it is current trend to employ suspension culture in serum free media in large scale animal cell culture process. In this study, recombinant CHO cells producing IDS were adapted to serum free media and suspension culture condition in Erlenmeyer flask, and finally one liter suspension culture was tried in bioreactor. The maximum cell density was 1.3×10^6 cells/mL and IDS concentration was 5 mg/L.
Keywords: CHO cell, IDS, Serum-free culture

I044

Heterologous Expression of the Modular Polyketide Synthase, Erythromycin DEBS1-TE, in *Pseudomonas putida* KT2440

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Polyketide family is a class of structurally diverse natural products. Many of polyketides have potent antifungal activity against plant pathogenic fungi and the producer actinomycetes have potential as a biological control agent for soil-borne plant diseases. However, actinomycetes have limited ability in colonizing the rhizosphere of plants where the infection by plant pathogen occurs, which may diminish their potential as biocontrol agents. On the other hand, rhizobacteria have innate ability to establish their population in the rhizosphere, although they produce only limited numbers of antifungal agents. We reasoned that an engineered rhizobacteria producing diverse polyketide antibiotics might be a potent biocontrol agent equipped with both chemical weapons of actinomycetes and root colonizing ability of rhizobacteria. In order to examine the possibility of transplanting the polyketide biosynthetic machinery of actinomycetes into rhizobacteria, we tried heterologous expression of a fusion protein DEBS1-thioesterase (DEBS1-TE) of *Saccharopolyspora erythrea* in a rhizobacteria strain. DEBS1-TE was cloned into an *E. coli-Pseudomonas* shuttle vector and transferred into *Pseudomonas putida* KT2440. GC-MS analysis of the culture extract of the recombinant strain revealed that DEBS1-TE was successfully expressed to produce triketides in *P. putida.* This is the first report on heterologous expression of the polyketide synthase originating from actinomycetes in *Pseudomonas* sp.
Keywords: polyketide, heterologous expression, *Pseudomonas* sp., biocontrol agent, plant disease

I045

Antitumorigenic Activity of *Paecilomyces* spp. Cultivated on Soybean on DMH-Induced Colon Carcinogenesis in F344 Rat

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Soybean Dongchunghacho supplementation induced antigenotoxic effect on DMH induced DNA damage in the colon cell (% fluorescence in tail : 68.5 ± 2.3 vs. 55.1 ± 3.4), but no antigenotoxic effect was observed in the blood cell. DMH-induced colon tumors had significantly greater COX-2, iNOS, γ-GCS, GST-P and GST-M2 gene expression than the corresponding normal mucosa. However, treatment with Soybean Dongchunghacho induced inhibitory effect on the colon tumor overexpression of COX-2, iNOS, γ-GCS, GST-P and GST-M2. Our results provide evidence that Soybean Dongchunghacho has a protective effect on the process of colon carcinogenesis, suppressing the development of preneoplastic lesions, possibly by modulating COX-2, iNOS, γ-GCS, GST-P and GST-M2 gene expression in tumors, suggesting that Soybean Dongchunghacho has chemotherapeutic activity.

Keywords: *Paecilmyces* spp, Antitumprenic activity, Soybean dongchunghacho

I046

Studies on the Culture Medium for Large Production of Spores of *Bacillus Subtilis*

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The genus *Bacillus subtilis* is considered as a model endospore-forming Gram-positive bacterium for prokayotic developmental studies and also used widely for industrial purpose. Under conditions of extreme nutrient limitation, *Bacillus subtilis* undergoes a differentiation process that converts the rod-shaped bacterial cell into a dormant spore. In this research, we have investigated optimal condition of culture medium for industrial purpose to give high yield spore number and sporulation efficiency in *Bacillus subtilis*. We found that the highest total cell number and spore number was obtained at the glucose concentration of 1g/l with the *Bacillus subtilis* strain we investigated. Also, three kinds of organic nitrogen sources (yeast extract, peptone, casein acid hydrolysate) were investigated at the various concentrations on *Bacillus subtilis* with the carbon source fixed. The best result regarding nitrogen source was obtained with yeast extract. Studies of the cultivation conditions led to the maximum concentration of total cell from 5×10^7 to 7×10^7 cells ml^{-1} and the spore concentration from 3.1×10^7 to 5.6×10^7 cells ml^{-1}. And the sporulation efficiency was 68%- 80% in the *Bacillus subtilis* strain we investigated. When we determine the enzyme activities of supernatant of *Bacillus subtilis* in different medium, we found that protease activities and xylanase activities are not changed much. However, the activity of amylase increased with the concentration of glucose increased

Keywords: *Bacillus subtilis*, sporulation efficiency, total cell number, spore number

I047

Comparison of Antitumor Activities Elicited by Inoculation with *Salmonella typhimurium* Harboring Eukaryotic Expressing Plamid or Prokaryotic Expressing Plasmid of TNF-alpha

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To evaluate the *Salmonella typhimurium* based TNF-alpha cytokine therapy as a tumor treatment mice were inoculated subcutaneously with *S. typhimurium* harboring eukaryotic expressing plasmid or prokaryotic expressing plasmid, which were compared for their ability to induce tumor inhibition and survival rates. The *S. typhimurium* harboring prokaryotic expressing plasmid induced tumor growth inhibition more than did *S. typhimurium* harboring prokaryotic expressing plasmid. The results also suggested that *S. typhimurium* harboring prokaryotic expressing plasmid induced more prolonged survivals in tumor bearing mice. These results suggest that *S. typhimurium* secreting TNF-α by prokaryotic expressing route are more efficient tumoricidal agent than by eukaryotic expressing route.

Keywords: TNF-alpha, *Salmonella typhimurium*, TTSS

I048

Binding Study of AfsK, a Ser/Thr Kinase from *Streptomyces coelicolor* A3(2) and *S*-adenosyl-L-methionine

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The phenomenon that exogenous addition of *S*-adenosyl-L-methionine (SAM) in *Streptomyces coelicolor* A3(2) enhances actinorhodin production suggests that SAM may initiate actinorhodin biosynthesis. Because AfsK is the first protein that influences actinorhodin production, SAM may interact with AfsK. Although the three- dimensional structure of AfsK has not been determined yet, the differences between nuclear magnetic resonance (NMR) signals obtained from the free form of SAM and those from SAM-protein complex can help us determine whether SAM binds the C-terminal of AfsK or not. In the present study, the analysis of the data supports that SAM binds Afsk based on NMR spectroscopy.

Keywords: NMR, AfsK, S-adenosyl-L-methionine, actinorhodin

I049

Microorganisms Against *Plasmodiophora brassicae*

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In order to discover microorganisms showing anti-fungal activities against *P. brassicae*, many microorganisms were isolated from Korean salt-fermented fishery products, jeotkal because they are more relatively safe for human than soil microorganisms. Jeotkal samples tested was produced from *Ammodytes personatus*. Thirty-eight strains were isolated from *A.personatus*. Their fermentation broths were centrifuged. The supernatants were collected and kept for the activity test against *P. brassicae* at 4℃. The crop used for testing was *Brassica campestris* subsp. napus var. pekinensis, cv Hukjinju. Among 38 samples screened, 8 fermented broths showed 100% control value. Eight strains showing 100% of the control value were identified on the basis of partial 16S rDNA and their images obtained from SEM, and the evolution trees were constructed using PHYDIT program.
Keywords: *P. brassicae, A.personatus*, biopesticide

I050

Mg^{2+}-dependent *o*-Methyltransferase from *Streptomyces avermitilis* MA-4680: Cloning, Expression, Purification, and Enzyme Characterization

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Streptomyces avermitilis is one of *Streptomyces* species to be able to methylate on hydroxyl group of compounds. Based on the sequence homologous search, *S. avermitilis* have several genes which contained methyltransferase domain. Among them, one gene, namely, saomt5 was cloned into pET-15b expression vector by PCR using sequence-specific oligonucleotide primers. Identification of methylating activities, and expression and purification of enzyme revealed that the SaOMT5 was an *S*-adenosyl-L-methionine dependent *O*-methyltransferase. The purified SaOMT5 was reacted with several compounds as substrates, so that SaOMT5 catalyzed *O*-methylation of flavonoids such as 6,7-dihydroxyflavone, 3'4'-dihydroxyflavone, quercetin, 7',8'-dihydroxy-flavone, and 2',3'-dihydroxyflavone, and caffeic acid. The reaction products were analyzed by TLC, HPLC, and NMR spectrometer.
Keywords: *Streptomyces avermitilis*, Identification, O-methyltransferase

I051

Enrichment, Performance and Microbial Diversity of Microbial Fuel Cell Fed with Formate

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Formate is known as a non-fermentable single carbon substrate which is present in anaerobic ecosystem. This fatty acid acts as an electron donor to anaerobic respiratory bacteria and also is involved in bacterial metabolism as intermetabolite. In this study, mediator-less microbial fuel cells (MFCs) have been used to enrich microbes oxidizing formate with the concomitant of electricity generation. The artificial wastewater containing formate (20 mM) was fed to MFCs. The MFCs showed around 10 mA of current in 4 months. Over 90% of formate was oxidized but only 30% of electron available from formate oxidation was recovered as an electricity. The performance of these MFCs was optimized in terms of various operating conditions such as pH and dissolve oxygen concentration in cathode compartment. The analyses of anode effluent were shown that the oxidation of formate in MFCs was recognized as not only oxidation by electrochemically active bacterium (EAB) to generate electricity. At the same time, acetate and butyrate were detected. The data of denaturing gradient gel electrophoresis (DGGE) showed that the DNA extracted from formate-enriched MFCs had different patterns from those of inoculum. Accordingly, we also compared the performance and the DGGE patterns of formate-enriched MFCs with those of acetate-enriched MFCs at the same conditions which converted up to 70% electron available from acetate consumption to electricity.
Keywords: microbial fuel cell, formate, electricity, DGGE

I052

Substrate Specificity of the Recombinant *Thermus* sp. α-Galactosidase

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α-Galactosidase(α-Gals) are known to occur widely in microorganisms, plants, and animals, and some of them have been purified and characterized. α-Gals catalyze the hydrolysis of 1,6-linked α-galactose residues from oligosaccharides and polymeric galactomannans. Hydrolysis of the galacto- oligosaccharides (such as melibiose, raffinose, and stachyose) and of the galactomanno-oligosaccharides(such as Gal^3Man_3, Gal^3Man_4) by the purified α -Gal was done at pH 6.0(0.1M sodium phosphate buffer) and 70°C. The sugar sample obtained after the enzyme reaction was analyzed by thin-layer chromatography (TLC) for the characterization of the hydrolysis products. The rwaction products were developed with 1-propanol-nitromethane- water(5:2:3,vol/vol). The sugars on the plate were detected by heating at 140°C for 5min after spraying with sulfuric acid.
Keywords: Gal^3Man_3, Gal^3Man_4, Substrate specificity

I053

Characterization of the Recombinant *Penicillium* sp. α-Galactosidase

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α-Galactosidase(α-Gal)(EC 3.2.1.22) is of particular interest in view of its biotechnollogical applications. αGal from coffee beans demonstrates a relatively broad substrate spccificity, cleaving a variety of terminal α-galactosyl residues, including blood group B antigens on the erythrocyte surface. Treatment of type B erythrocytes with coffee bean αGal results in specific removal of the terminal α-galactosyl residues, thus generating serological type O erythrocytes. Cyamopsis tetragonoloba(guar) αGal effectively liberates the α-galactosyl residue of galactomannan. Removal of a quantitative proportion of galactose moieties from guar gum by αGal improve the gelling properties of the polysaccharide and make them comparable to those of locust bean gum. Here we describe the cloning of *Penicillium* sp. αGal cDNA, its expression in *Saccharomyces cerevisiae*, and the purification and characterization of the recombinant enzyme.

Keywords: High-Level expression, Galactosidase

054

Antioxidant Activity of Extracted Astaxanthin from *Haematococcus pluvialis* Using Ultrasonification

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The freshwater green microalga *Haematococcus pluvialis* is widely studied as one of the best sources of astaxanthin (3,3'-dihydroxy-β,β'-carotene-4,4'-dione). Astaxanthin is not only used as a pigmentation source in the diets of fish and animals, but also has potential clinical applications due to its higher antioxidant activity than β-carotene and vitamin E. In this study, astaxanthin was extracted from *H. pluvialis* using ultrasonification. After adding distilled water with the weight ratio of 10 (distilled water) to 1 (cell), ultrasonification extraction was performed with various power (0 to 200 W) and extraction time (0 to 60 min). The antioxidant activity of extracted astaxanthin was measured by DPPH free radical scavenging activity. Also, the morphology of pretreated cell was compared with non-pretreated cell by scanning electron microscope. In conclusion, the extracted astaxanthin from *H. pluvialis* using ultrasonification would be widely used in the food industry, health products, and medical fields.

Keywords: Astaxanthin, Ultrasonification, *Haematococcus pluvialis*

I055

Process Development for the Recovery of Catechin Compounds from Green Tea Using Glass Column Chromatography

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Catechin compounds are bioflavonoids, polyphenols and powerful antioxidants, and the green tea is one of the best catechin sources: epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG). In this study, the process for the recovery of catechins from the green tea was developed by using the chromatographic method. The green tea leafs, farmed on the flanks of Mt. Halla (Jeju, Korea), were prepared and extracted with water at 80℃ during 1 hour (pH 7). Then the leaf extract was eluted into the glass chromatography column (outer diameter = 2.7 cm, length = 40 cm) packed with silica gel (Waters Co. 34 Maple street, Milford,MA 01757: Prep C18 125A 55-105 mm: 10 g). The eluates were collected and injected into the HPLC system (Waters: 2696 separations module and 299 photodiode array detector, Column: Symmetry RP_{18} 5m:4.6 X 250mm) after syringe filtration. As a control experiment, the leaf extract was also partitioned with chloroform and then with ethyl acetate. These solvents were used for remove caffeine impurity and purify the catechins. This comparative study clearly showed that the chromatographic method in this study can simplify the purifying process and reduce the harmful solvent. Furthermore the column chromatographic method is a good candidate to separate catechins selectively on a commercial scale.

Keywords: Catechin, Green tea, EGCG, Column chromatography

I056

Process Development of Recovery and Purification of Immunosuppressant Mycophnolic Acid

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Mycophenolic acid(MPA) blocking the synthesis of xanthosine monophosphate is a nonnucleoside inhibitor of inosine monophosphate dehydrogenase. Therefore mycopholoic acid is a drug currently used for immunosuppressive agent such as in transplantation of heart, kidney and liver. And MPA is produced by fungus *Penicillium brevicompactum*. In addition, mycophenolic acid be able to used as antiviral, anti bacterial, antifungal, anti autoimmune diseases and anticancer agent with broad range. In this study, the fermentation process of MPA was developed for the higher yield and massive production. Media compositions such as carbon and nitrogen sources were optimized. Nonionic surfactant Tween 80, vegetable oil and oleic acid increase the productivity as membrane permeability enhancer, wall growth hindrance, safety antifoamer, and carbon source. The recovery and purification process of MPA is designed using MeOH extraction, resin adsorption and elution, solvent extract. And the respective unit operation processes are optimized such as optimal concentration of MeOH for extraction from broth and elution from HP-20 resin, optimal pH of aqueous phase for ethyl acetate extraction, optimal condition of vacuum concentration for crystallization and so on.

Keywords: Immunosuppressant, Mycophenolic acid, Recovery and Purification

I057

Effects of *N*-glycosylation on Secretion of VEGF in *Saccharomyces cerevisiae*

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Vascular endothelial growth factor (VEGF) is a specific mitogen for vascular endothelial cells *in vitro* and a potent angiogenic and vascular permeability enhancing factor *in vivo*. VEGF is a homodimeric glycoprotein that contains a single N-glycosylation site at amino acid position 75. In this study, the homodimeric forms of glycosylated VEGF121 and VEGF165 were efficiently secreted from *Saccharomyces cerevisiae*. The *S. cerevisiae* a factor pre-sequence was found to be a better secretion leader for the VEGF proteins than the a prepro-sequence. The purified VEGF proteins were shown to be biologically active when tested by rat-aortic ring assay. To investigate the relationship between *N*-glycosylation and secretion of VEGF in *S. cerevisiae*, we eliminated the single *N*-glycosylation site in VEGF by site-directed mutagenesis. The mutated VEGF proteins were not secreted when guided by the a factor pre-sequence. Interestingly, however, the a factor prepro-sequence, which has three *N*-glycosylation sites in the pro-region, could direct the secretion of non-glycosylated VEGF proteins, albeit to a lesser degree. Taken together, these results suggest that *N*-glycosylation, especially the core glycosylation occurring in the ER, may be important for secretion of VEGF in *S. cerevisiae*.

Keywords: *Saccharomyces cerevisiae*, VEGF, N-glycosylation, secretion

I058

Biological Carbon Dioxide Conversion to Methane by Hydrogenotrophic Methanogens in a Hollow-Fiber Membrane Biofilm Reactor

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Carbon dioxide is one of the global warming gases. A utilization of a sustainable energy is one of the effective technologies for a mitigation of the CO_2 accumulation in the atmosphere. If renewable energy can be used for methane synthesis, H_2 is converted to methane with reducing CO_2. We investigated the conversion of CO_2 to CH_4 using a novel hollow-fiber membrane biofilm reactor. We have converted CO_2 to CH_4 with anaerobic methanogens using CO_2 and H_2. All gases diffused into water through the membrane, resulting I almost 100% transfer efficiency. We have successfully operated the Hf-MBR for stable methane production from CO_2 and H_2 under continuous operations for 100 days. Methane ratio of produced gas reached 80~90% after 16 days. Methane produced from 8 days since the reactor was operated. The production contents of methane gas were 482ml/day on average from 16 days to 46 days, 635ml/day on average from 47 days to 75 days and 641ml/day on average from 76 days to 100 days respectively. We have performed DGGE for bacterial and archaeal 16S rDNA to analysis of microbial community in the reactor.

Keywords: hydrogen, carbon dioxide, methane, hollow-fiber membrane, hydrogenotrophic methanogen, DGGE

I059

Yield Optimization of Herbal Medicine Extracts for Anti-arthritis

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This study was undertaken with developing optimum extraction condition for three herbal medicines which showed the strongest hyaluronidase (HAse) inhibitory and Nitric oxide (NO) inhibitory activity. A central composite design was applied to investigate the effects of the four independent variables, grain size (mash, X_1), concentration of alcohol (%, X_2), extraction time (hr, X_3), and extraction temperature (℃, X_4) , on three dependent variables, soluble solid (Y_1), HAase inhibitory (Y_2) and NO production inhibitory activity (Y_3). As the results, soluble solids increased in proportion to grain size and alcohol concentration. The optimum range of extraction conditions for maximized physicochemical properties were above 12 h and 30℃, respectively. The extraction yield of soluble solids under optimum condition was increased to 3-45 times and NO production from LPS treated macrophage was significantly inhibited by herbal medicine mixtures. Consequently, these strongly suggest that the optimum extraction condition for mixture of three herbal medicines (AR, SF and PG) expected to be useful in the development of functional source against inflammatory as well as arthritis diseases.

Keywords: hyaluronidase, Nitric oxide

I060

A Double Deletion of *phoP* and *rpoS* Genes Greatly Attenuate the Virulence of *Salmonella enterica* Serova Strain Typhi, but Protective Immune Response Against Wild-type Ty2 was not Altered

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Both *phoP* and *rpoS* genes are known to be the important factors for virulence of *Salmonella enterica* Serovar strain Typhi (*S. typhi*). Thus, many oral live vaccine candidates (including Ty800 and Ty21a) were developed with these genes mutation and used as oral live vaccine worldwide. However, it had not been studied yet for the effect of double deletion in both *rpoS* and *phoP* loci of *S. typhi*. We assumed that both virulence gene deletion greatly attenuate *S. typhi*, and concerned about the result in immune response. Here, we constructed three *S. typhi* deletion mutants (*rpoS, phoP, rpoS-phoP* deletion) and characterized the abilities of these mutants for invasiveness, virulence and protective immune response compared to wild-type Ty2 and each other mutants. In this study, we found that *phoP* deleted *S. typhi* is less invasive to HT-29 cell than their wild-type Ty2 and *rpoS* deleted strain. The number of invasive organs was significantly decreased in the HT-29 cell infected with *phoP* and *phoP-rpoS* deleted *S. typhi* compared to those with wild-type Ty2 and *rpoS* deleted. The LD_{50} of peritoneal infection to mice was higher in *phoP* than *rpoS* and highest in the *phoP-rpoS* deleted *S. typhi*. When the mice were nasally immunized with each *S. typhi* mutants for 8 weeks at 4 weeks interval after immunization, all *S. typhi* mutants increased Specific serum IgG levels for wild type *S. typhi* Ty2 and showed protection against wild-type Ty2 challenge at 8 weeks in BALB/c mice.

Keywords: rpoS, phoP, attenuation, invasiveness, *Salmonella typhi*

J001

Screening Anti-Pathogenic Agents Against the Infection of *Escherichia coli* O157 Using a Surrogate Disease Model, *Caenorhabditis elegans*

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Escherichia coli O157:H7 is an important foodborne pathogen. The study of the molecular pathogenesis of *E. coli* O157:H7 has been hampered by the lack of genetically tractable, convenient animal models. Here we report a surrogate disease model, *Caenorhabditis elegans*, for the study of *E. coli* O157:H7 pathogenesis and high throughput screening (HTS) of anti-pathogenic agents. Nematodes fed wild-type *E. coli* O157:H7 strains were killed faster than nematodes fed non-pathogenic strains, such as *E. coli* OP50. To determine which virulence factors are responsible for this killing mode, quorum sensing gene such as *luxS* (encoding AI-2 synthase), which are known to contributes to the pathogenesis of many bacterial parasites in other hosts including a human, were deleted. The mortality of *C. elegans* fed *luxS* mutants was lower than that of nematodes fed wild type. Using the current surrogate model, anti-pathogenic agents were screened by feeding nematodes *E. coli* O157:H7 with green tea polyphenols. The mortality of *C. elegans* fed *E. coli* O157:H7 with green tea polyphenols was lower than that of nematodes fed only *E. coli* O157:H7. *C. elegans* is a useful animal model for the study of *E. coli* O157:H7 pathogenesis and also HTS of anti-pathogenic agents. This work was supported by Korea Research Foundation Grant funded by Korea Government (MOEHRD, Basic Research Promotion Fund) (KRF-2005-003D00320).
Keywords: *Escherichia coli* O157:H7, *Caenorhabditis elegans*, polyphenols

J002

The Screening of Actinomycetes and Identification Antimicrobial Activity against Bacteria Causing Rusty Ginseng Roots

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Rusty root in ginseng is that ginsengs turned to red to brown. It severely decreases the quality of ginseng and causes significant loss of product value. Recent study indicated that rusty roots can be caused by bacteria isolated in ginseng (ref-Choi prof.). In this study we pursued to isolate Actinomycetes that is antagonistic to the rusty-root-causing bacteria. CG20126 (*Agrobacterium tumefaciens*) out of 32 rusty-root-related bacteria, was used to isolate a proper Actinomycetes. Antibacterial activity with 932 cultures of Actinomycetes in G.S.S medium (soluble starch, glucose, soybean meal, beef extract, yeast extract, NaCl, K2HPO4,CaCo3, D.W in pH 7.2) was measured. Twenty eight Actinomycetes strains were selected and the strongest antibacterial strain (ATO4O104) was classified based on 16S rDNA sequence. The Actinomycetes strain, ATO4O104, isolated in soil of USA volcano national park was identified as *Streptomyces adephospholyticus*. To test plant toxicity, radish seeds were sprouted with the culture of *S. adephospholyticus* but it did not show any harmful effect. The butanolpartition out of n-hexane, ethyl acetate, butanol, and water showed the highest antibacterial activity.
Keywords: Rusty ginseng roots, Actinomycetes, Antibacterial activity

J003

Isolation of Tannin-Degrading Bacteria from Korean Fermented Vegetables

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Tannins are plant polyphenolic compounds present in both animal and human diets, which are divided into two groups based on chemical structure: hydrolysable tannins and condensed tannins (proanthocyanidins). Both groups tend to have similar biological properties Hydrolyzable tannins, such as gallotannin and ellagitannin, are widely distributed in the plant kingdom These tannins bind readily with proteins to form indigestible complexes, and the complexes are considered to be resistant to degradation within the guts of mammals Tannase specifically breaks the galloyl ester bonds of tannins, thereby inhibiting their protein-binding properties Tannase finds widespread application in food and beverage processing The enzyme is common in some species of fungi and bacteria including lactobacilli species We isolated some lactic acid bacteria degrading tannin from several Korean fermented vegetables in order to use in the food industry and evaluated its diversity in Korean fermented vegetables.
Keywords: tannin, lactic acid bacteria, Korean fermented food

J004

Efficient Utilization of Starch by Industrial Strains of *Saccharomyces cerevisiae* Expressing the Glucoamylase and α-Amylase Genes from *Debaryomyces occidentalis*

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Debaryomyces occidentalis glucoamylase gene (*GAM1*) was placed under the control of the *Saccharomyces cerevisiae* alcohol dehydrogenase gene promoter (*ADC1p*) and introduced into the chromosomes of three industrial polyploid strains o *Saccharomyces cerevisiae* (ATCC 4126, ATCC 9763 and ATCC 24858). Yeast transformation was carried out by an integrative process targeted to δ-sequences. To obtain strains eligible for commercial use, an integrative cassette devoid of bacterial DNA sequences was constructed that contains the *GAM1* gene and δ -sequences. The glucoamylase activity of *S. cerevisiae* ATCC 9763 transformed with this integrative cassette was 3.7 times higher than that of *D. occidentalis*. This new strain expressing the *GAM1* gene and ATCC 9763 expressing *D. occidentalis* α-amylase gene (*AMY*) were mixed-cultured to produce both glucoamylase and α-amylase for efficient utilization of starch. The growth, substrate utilization and enzyme activity of these strains are described.
Keywords: *Debaryomyces occidentalis* glucoamylase gene (GAM1), industrial polyploid strains of *Saccharomyces cerevisiae, D. occidentalis* α-amylase gene (AMY)

J005

Analysis of Enzyme Activities in *Bacillus* spp. Isolated from Chungkookjangs

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Chungkookjang is a traditional soybean-fermented food and fermented mainly by *Bacillus* spp. In this study, Chungkookjangs were obtained from Gyeonggi and Gangwon provinces and twenty-two *Bacillus* spp. were isolated and identified by using 16S rDNA analysis *B. subtilis* and *B. licheniformis* are dominant in the Gyeonggi area and *B. licheniformis*, in the Gangwon area Enzyme activities-protease, amylase, cellulase and fibrinolytic enzymes-were analyzed with the isolated strains Higher enzyme activities were obtained from the strains isolated in the Gyeonggi area Based on the results of enzyme activities, Anseong-2, Yongin-1 and -2, Kwangjoo-1 and -2 and Odaesan-1 strains are possible candidates for the production of high qualities of Chungkookjangs.

Keywords: Chungkookjang, *Bacillus* spp., protease, amylase, cellulase, fibrinolytic enzyme

J006

Antitumor Effects of *Kluyveromyces marxianus* TFM-7 Isolated from Kefir

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The objective of this study was to investigate antitumor activity and some characteristics of strain TFM-7, isolated from Kefir. The strain TFM-7, which has the antitumor effect, was identified based on results of API 50 CHL kit and 26S rDNA sequencing analysis. The strain TFM-7 was identified as *Kluyveromyces marxianus*. *K. marxianus* TFM-7 was cultured with potato dextrose broth medium at 27℃ for 72 h, and the inhibitory effects of the proliferation against seven typical tumor cell lines and a normal cell line were detected using MTT assay. The antitumor effect and some characteristics of *K. marxianus* TFM-7 were investigated during culture period for 7 days. The proliferation inhibitory effects of *K. marxianus* TFM-7 was found to inhibit the growth of culture period for 6 days more than 30%, except the 7th day. On the 3rd day, except A 549 cell line, other six tumor cell lines were suppressed by the highest inhibition rate, 69.9, 52.7, 89.4, 78.3, 59.2, and 68.7% after 72 h of treatment with the vegetative cells of *K. marxianus* TFM-7 at 10 mg/ml, respectively. *K. marxianus* TFM-7 was found stronger antitumor effect of HEp-2 cell line 89.4 % than any other tumor cell in a dose dependent at 10 mg/ml, respectively. The NIH/3T3 normal cell showed weaker cytotoxic effect even at the high concentrations of 10 mg/ml, which inhibited the growth by less than 35%. Therefore, *K. marxianus* TFM-7 showed the more effective cytotoxicity to tumor cell line than normal cell line.

Keywords: kefir, 26S rDNA, *Kluyveromyces marxianus*, MTT assay, antitumor

J007

Protective Effects of Astaxanthin on the Hepatocytes Rat Treated with Carbon Tetrachloride

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Nowadays, people is exposed to stress, drinking, smoking and drugs. These affect the liver negatively and may cause fatal threat to human. A functional compound that protects liver from harmful damage but is safe would be helpful. Carotenoids are known that is present in fruit. Beta-carotene is the most well-known carotenoid that is a precursor of vitamin A. Color of carotenoids is yellow, orange, or red and they are hydrophobic. Astaxanthin is a strong antioxidant that stabilizes unstable oxygen or removes free radicals. It can potentially protect the liver because of its antioxidant effect. To determine this, white rats were used in this study. After treatment of astaxanthin 100ppm and CCl_4 0.2ml/100g, the effect of astaxanthin on the liver protection was measured. After feeding for 1 month, the rats were bisected and the liver was brought out. To measure the effect of liver protection, catalase activity and fat peroxide content were measured. The treatment of CCl_4 alone increased catalase activity 13.88 mmole/mg protein/mi and increased fat peroxide 4.62 μmole/ml, compared to the control 17.62 and 12.67 respectively. The treatment of astaxanthin+ CCl_4 kept the level of catalase activity 8.89 mmole/mg protein/min from increase and the concentration of lipid peroxide 2.31 μmole/ml from increase. In this study, we showed that astaxanthin could protect the liver from harmful effect of CCl_4 and it could be used for a liver protector from peroxidation.

Keywords: antioxidant, astaxanthin, free radical, river, CCl_4

J008

Effect o Lactic Acid Bacteria on Lipogenesis and Cholesterol Metabolism Relate Gene Expression via LXR Signaling Pathway

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For many years, it has been recognized that elevated serum cholesterol is a risk factor associated with atherosclerosis and coronary heart disease. Ingestion of probiotic lactic acid bacteria (LAB) would be a more natural method to decrease serum cholesterol in human. In previous studies, we have demonstrated that heat killed *L. plantarum K518* has hypocholesterolemic effect in animal model. In this study, we have investigated whether heat killed LAB exert hypocholesterolemic effect through liver X receptor (LXR) signaling pathway. *L. plantarum* K518 and *L. casei* MK10 strains significantly increase the LXR target genes such as ABCA1 and ABCG1 in THP-1 and $CaCO_2$ cell lines. Moreover, *L. casei* MK10, *St. thermophilus* MK52 and *St. thermophilus* MK55 strains significantly increase LXR related gene, ApoE expression in THP-1 cell line. Especially, lipoprotein lipase (LPL) expression was significantly reduced by administration of *Bf. logum* MK20 in THP-1 cell line. It is known that overproduction of LPL in monocyte accelerates atherosclerosis. *L. plantarum* K518, *L. casei* MK10 and *Bf. logum* MK20 strains increased by 13%~20% for ABCA1 or ABCG1 mediated cholesterol efflux in THP-1 cell line. These results suggest that the regulation of LXR target genes and LXR related genes by LAB is prophylactic and/or therapeutic tool to induce hypocholesterolemic effect.

Keywords: Lactic acid bacteria, Cholesterol, LXR, ABCA1, ABCG1, Atherosclerosis, Hyperlipidemia

J009

Purification and Characterization of an Antitumor Substance Produced by *Kluyveromyces marxianus* TFM-7

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The objectives of this study were to purify the antitumor substance, isolated from *Kluyveromyces marxianus* TFM-7 and to investigate the inhibitory effects of the proliferation of active compounds. The antitumor substance from *K. marxianus* TFM-7 was purified by HP-20 column chromatography, solvent extraction, silica gel column chromatography, thin layer chromatography, and high performance liquid chromatography (HPLC). The active compounds was identified as hexahydro pyrrolo[1,2-a]pyrazine-1,4-dione by comparing with GC-MS, ^{1}H - and ^{13}C - NMR, UV, and FT-IR data. The proliferation inhibitory effects of hexahydro pyrrolo[1,2-a]pyrazine-1,4-dione from the PDB cultures of *K. marxianus* TFM-7 was found to inhibit the growth more than 50% against all tumor cell lines studied at 500 μg/ml and Hep-2 tumor cell lines was suppressed by the higher inhibition rate 80.1 and 58.2% than any other tumor cell lines after 72 h of treatment with 200 and 100 μg/ml of hexahydro pyrrolo[1,2-a]pyrazine-1,4-dione, respectively. However, NIH/3T3 normal cell showed weaker cytotoxic effect at the same concentrations, which inhibited the growth by less than 10%. Therefore, hexahydro pyrrolo[1,2-a]pyrazine-1,4-dione showed the more effective cytotoxicity to tumor cell line than normal cell line.
Keywords: *Kluyveromyces marxianus*, MTT assay, antitumor, purification, hexahydro pyrrolo[1,2-a]pyrazine-1,4-dione

J010

Antimicrobial Resistance and Virulence of *Enterococcus* spp. Isolated from Foods and Patients

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Probiotics are generally regarded as safe (GRAS). However, there have been reports about probiotics with pathogenecity and possible antimicrobial resistance. Especially in the case of probiotic enterococcus, its pathogenecity and antimicrobial resistance must be checked before used in foods. In this work, 16 food isolates and 8 clinical isolates were tested with PCR for the presence of virulence genes and antimicrobial resistance genes. These were genes for Esp surface protein, gelatinase, endocarditis antigen, aggregation, haemolysin and genes for vancomycin-resistance. Among tested genes, *vanA, vanB, esp, agg,* and *cylA* were found only in clinical isolates not in food isolates. In case of *efaA* (endocarditis antigen), it was detected in both clinical and food isolates. This result suggests that *vanA, vanB, esp, agg* and *cylA* in probiotics need to be checked for safety.
Keywords: probiotic, enterococcus, antimicrobial resistance, virulence factor

J011

Development and Characterization of a New Cloning Vector, pUCC3α Using Cryptic Plasmid from *Kimchi* Lactic Acid Bacteria

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The nucleotide sequence of a cryptic plasmid pCC3 from *Leuconostoc* sp. isolated from kimchi, was determined. Sequence analysis of the pCC3 revealed that it contained 3,386 bp, 33.2% G+C content and nine open reading frames(ORFs). One putative ORF, OFR6(138 amino acids) revealed 97% similarity with replication initiation protein of pTXL1, a stable theta replicating plasmid from *Leuconostoc mesenteroides* subsp. *mesenteroides* Y110. A new cloning vector pUCC3α was constructed using pCC3, pUC18 and α-amylase gene(3.0 kb) from *Bacillus licheniformis.*
Keywords: cloning vector, cryptic plasmid

J012

Production of Isomaltooligosaccaride by Dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F

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Dextransucrase(EC 2,4,1,5) secreted by *Leuconostoc mesenteroides* NRRL B-512F transfers the glucosyl moiety of sucrose to from dextran. In addition to catalyzing the synthesis of dextran from dextransucrase also catalyzes the glucose transfer reaction from sucrose to maltose synthesizing isomaltooligosaccharide(IMO) inclding panose as a major component. In order to produce IMO at the large quantity, we carried out optimization of substrate concentration by ascending manner of sucrose-maltose contents. Enzyme was reacted with sucrose-maltose mixture and the synthesized IMO was quantitatively analyzed by TLC and HPLC system. When 20%(w/v) of sucrose and 10%(w/v) of maltose were reacted IMO(including maltose) content among total sugar was 65.7%, and when contents of sucrose and maltose were both 20%, The IMO content was 54.4%. The IMO produced by this enzyme reaction contained high amount of fructose as a by product and this gave sweet from this experiments. We obtained data to modulate IMO contents by combining sucrose-maltose ratio to fit the consumer's physicochemical requirement for spectic food uses.
Keywords: Dextransucrase, *Leuconostoc mesenteroides* NRRL B-512F, isomaltooligosaccharide

J013

Identification and Characterization of Fibrinolytic Enzyme from Korean Fermented Fish, Jeot-gal

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Jeot-gal is a kind of the korean fermented sea food and used for seasoning. We isolated 188 strains from shrimp, anchovy and yellow corvina jeot-gal depepnding on the salt concentration. Sixteen strains was screened for fibrinolytic activities using fibrin plate, and one strain showing the largest halo zone on fibrin plate was chosen and characterized. The strain was identified as Bacillus lichemifomis using 16S rRNA sequencing and API 50CHB kit. The fibrinolytic enzyme was purified through ammonium sulfate precipitation, DEAE ion-exchange chromatography, and gel filteration chromatography. The molecular weight of the purified fibrinolytic enzyme was estimated approximately 37 kDa by SDS-PAGE and fibrin-zymography assay. The enzyme is serine-type protease because it was inhihited by PMSF. The enzyme degraded fibinogen, thrombin, and fibrin directly but not bovine serum albumin or skim milk. The internal amino acid sequence of the enzyme has determined by tryptic digestion mapping, and the result indicates that the enzyme is close to bacillopeptidase F. The molecular cloning of the gene encoding this enzyme is under progress.

Keywords: fibrinolytic, *Bacillus licheniformis*, bacillopeptidase F, fibrin

J014

DNA Sequences of a Cryptic Plasmid pYC2 from *Lactobacillus sakei* Isolated from Kimchi

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The complete nucleotide sequence of a cryptic plasmid from *Lactobacillus sakei* isolated from Kimchi has been determined. The plasmid designated as pYC2 is a 1,970 bp long circular molecule with a G+C content of 33.96%. DNA sequence analysis of pYC2 revealed two open reading frames (ORFs). One putative ORF, ORF2 (226 amino acids) that revealed 95.6% similarity with the replication protein of plasmid pLS141-1 from *Lb. sakei*. The double strand origin (*dso*) and the single strand origin (*sso*) of rolling circle replicating (RCR) plasmids were founded in nucleotide sequence of pYC2. Based on sequence homology, it is deduced that plasmid pYC2 replicates by rolling circle mechanism.

Keywords: *Lactobacillus sakei*, Plasmid, Kimchi

J015

Complete DNA Sequence and Analysis of a Cryptic Plasmid pMC5 Isolated from *Lactobacillus plantarum*

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A cryptic plasmid designated pMC5 was isolated from *Lactobacillus plantarum* and its complete 4,305 bp nucleotide sequence was determined with a 43.6% G+C content. The structural organization of pMC5 was highly(80.5%) similar to pLJ42 from *Lactobacillus plantarum* and sequence analysis revealed five putative open reading frames(ORFs). Based on sequence similarity, it is deduced that ORF1 and ORF2 codes for a putative replication protein(*Rep*A), and ORF3, ORF4 and ORF5 codes for a putative mobilization protein.

Keywords: *Lactobacillus plantarum*, cryptic plasmid

J016

Antioxidative and Antimicrobial of Extracts from Different Mushroom Myclium Culture Extracts Cultivated in the Synthetic Liquid Medium and Citrus Extracts

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In this study, we waso investigate the antioxidative and antimicrobial activities of usage of mycelium cultural extract from mushroom. Mushroom mycelium was grow in a defind synthetic liquid medium and citrus extracts, and the culture extracts were examined for antioxidant andantibacterial activity. Myceliums of *Phellinus llinus linteus, Coriolus versicolor, Sparassic crispa, agaricus blazei, lnonotus obliquus, lentinus edodes, hericium erinacium, gonoderma lucidium* in 10% citrus extract supplemented medium and synthesis medium were iccubator (120 rpm, 24~30℃) for 7~15 days. The synthetic liquid medium and citrus extracts Culture extracts , showed high Antimicrobial activity of *Phellinus llinus linteus, Coriolus versicolor, Sparassic crispa.* The Culture extracts obtained from the synthetic liquid mediu showed 30 -93% of the 1,1-diphenyl-2-picrylhydrazyl radical scavenger activity, the culture extracts obtained from the citrus extracts medium exhibited antioxidant activity up to 55%.

Keywords: Antibacterial, antioxidant, synthetic liquid medium, citrus extracts

J017

Antimicrobial Effect of *Salvia miltiorrhiza* Bunge on Methicillin Resistance *Staphylococcus aureus*

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MRSA (Methicillin resistant *Staphylococcus aureus*) are bacteria which can be found in our normal daily life. They can be easily ingested through milk, vegetables, meats, etc. MRSA emerged in many part of the world and increased complex clinical problems. Therefore, new agents are needed to treat the MRSA. Thus, in this study we evaluated the antimicrobial effect of *Salvia miltiorrhiza Bunge. Salvia miltiorrhiza Bunge* was extracted by 80% MeOH and then antimicrobial effect of the extract was investigated against various kinds of MRSA stains (KCCM 11812, 40510, 40511, 40512). It was determined the antimicrobial effect of extract with disc diffusion method, MIC and MRSA gene expression investigation(RT-PCR, Western blot). Therefore, *Salvia miltiorrhiza Bunge* showed inhibition zone against MRSA (KCCM 11812, 40510, 40511, 40512) as 15 mm at 5 mg/disc. Also, revealed *MecA, MecI, MecR1*, and *FemA* were the most highly manifested MSRA genes. *Salvia miltiorrhiza Bunge* showed inhibition MRSA genes expression at 2mg/ml. These results suggest *Salvia miltiorrhiza Bunge* that can be used in antimicrobial effect and functional material.

Keywords: antimicrobial effect, *Salvia miltiorrhiza* Bunge, MRSA

K001

Database of Korean Patent Bio-Sequences

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We have built a database server called Patome which contains the annotation information for patented bio-sequences from the Korean Intellectual Property Office (KIPO). The aims of the Patome are to annotate Korean patent bio-sequences and to provide information on patent relationship of public database entries. The patent sequences were annotated with Reference Sequence (RefSeq) database. Through the annotation, we found that nearly 2.6% of human genes were associated with Korean patenting, compared to 20% of human genes in the U.S. patent. The raw patent data and the annotated data were stored in the database. Annotation information can be used to determine whether a particular RefSeq number is related to Korean patent. Patome infrastructure consists of three components – the database itself, a sequence data loader, and an online database query interface. The database can be queried using submission number, organism, title, applicant name, or accession number. Patome can be accessed at http://www.patome.net. The information will be updated every two months.

Keywords: Korean patent sequence, Patome

K002

Identification of Bacteria Attached to Human Scalp Hair and Effect of Detergent Treatment

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The importance of human hair as a potential source of a bacterial contamination has been overlooked by the majority of people who tends the sick. The colonization of bacteria is promoted by surface adhesins, some of which bind to extracellular matrix molecules. We collected hair samples from patients of intensive care units and habitants of rehabilitation center and isolated 14 bacterial strains by placing the hair on Brain Heart Infusion agar plates. When we treated hair with detergent or shampoo, 5 strains of attached bacteria were not completely removed. Isolated strains were grouped by the nucleotide sequence of 16S rRNA genes. Scanning electron micrographs of hair specimens showed that bacteria remained on the surface of the cuticles of the hair even after treatment of detergent. These results indicates that some of bacteria which adhere to scalp hair is hardly removed by conventional shampoo. Therefore shampoo with bactericidal agent is required to decontaminate the attached bacteria on patient hair.

Keywords: Decontamination of hair, adhesin, human scalp hair

K003

Eztaxon: a Web-Based Integrated Environment for Phylogeny and Taxonomy

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Eztaxon is a software program that was designed to assist molecular phylogeny process from DNA sequences. As Eztaxon is web programming, it is able to be operated to various systems and doesn't need to set up in personal computers. The program was used to the 16S ribosomal RNA sequence databases that were formatted in open databases. Therefore, it is profitable as analyzing of 16S ribosomal RNA sequence. The program provided integration of nucleotide sequence blasting, pair wise alignment, diversely information links and increased efficiency of analyzing rRNA sequences for molecular evolution.

Keyword : Eztaxon

K004

Studies on a New Antibiotic Substance Produced by *Paenibacillus polymyxa* DY1 Strain Isolated from Korean Soil

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Because of wide abuse of antibiotics in clinical practice, the continuous advent of various kinds of new multi-drug resistant (MDR) bacteria becomes an important medical issue. The increase of MDR bacteria is expected to make it more difficult to control infectious disease with currently available antibiotics. Therefore, the development of new antibiotics has drawn more attention lately In this study, a new strain of bacteria, DY1, which showed a promising inhibitory capability on various MDR bacteria, was isolated from the mountain forest soil in Korea. This strain was identified as a member of *Paenibacillus* spp. by an array of biochemical and morphological tests. In the blast search with 16S rDNA, this strain showed the highest match with *Paenibacillus polymixa* with 99.8 % similarity (1,413 bp/1,416 bp) In disc diffusion tests, DY1 showed significant level of growth inhibition on enterohemolytric *E. coli* strains, enteropathogenic *E. coli* strains, MDR *Salmonella* strains, MDR *Shigella* strains, and pathogenic *Vibrio* strains. The active substance was found from the supernatant of the DY1 culture. It was heat resistant, and was non-polar low molecular substance.

Keywords: MDR(multi-drug resistant), *Paenibacillus polymyxa*

K005

PHYVIEW: A Java-Based Tool for Comparing the Phylogenetic Trees Using Different Phylogenetic Methods

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Comparing the phylogenetic trees was used for genome function and evolution. PHYVIEW was designed at java-based tool for comparing the phylogenetic trees using different phylogenetic methods. The algorithm was that each branch pairs up in one phylogeny with a matching branch in each phylogeny, and it is found the optimal map between branches in several phylogenetic trees. The software enables the user to explore the corresponding mapping between the phylogenies interactively, and clearly highlights those parts of the trees that differ, both in terms of topology and branch length. The software tool was also a unified viewing tool for consensus trees. The essential work for finding optimal phylogenetic tree can be accomplished efficiently by this application.

Keywords: Phyview, Phylogenetic tree

K006

Anaerobic Fungi, *Chitridiomycetes*, Digesting Fiber and its Diversity

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Chytridiomycetes are the only known true obligatory anaerobic rumen fungi and their population usually varies between 10^3 and 10^5 zoospores ml^{-1} of rumen contents. This study is to isolate rumen anaerobic fungi digesting fiber and see their diversity of ribosomal ITS1 and 18S rRNA. Among isolated anaerobic fungi, the 12 strains showed apparent degradability of fiber (filter paper, Whatman® No 1). It was clearly observed fungal colonization and development on filter paper strip, a sole energy source. Generally, the fungal sporangia, thalli or rhizoid were present on exposed surfaces and cut edges of the strips. A reduced fibrous mass, a decreased fiber cohesion and a weakened fiber structure by the fugal strains were detected in 5 days of culture using ground filter paper Their diversity ranged from 37.4 to 100% in similarity of pairwise sequence comparison. A high degree of similarity was observed between the isolates in three groups (> 80%). The fungal strains produced a reduced sugar which was presumably catalyzed from cellulose of filter paper during the incubation. The amounts of reduced sugar were increased in fungal strain inducing higher enzyme activity such as cellulose, xylanase and β-glucanase.

Keywords: Anaerobic fungi, Rumen, *Chitridiomycetes*

K007

Photopolymerization of Diacetylene Lipid Bilayers

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Photopolymerization of diacetylene-containing phospholipids 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine (DiynePC) in substrate-supported planar bilayers (SPBs) has been studied for the biomimetic membrane systems. Diacetylene lipid, ruptured by vesicle fusion, was lithographically polymerized by uv irradiation using a physical mask. Because the polymerized bilayers were resisted detergent solubilization, SPB pattern was established by removal nonpolymerized region using ethanol. Also, phosphatidylcholine from egg yolk (egg-PC) which can contain membrane bound molecules, such as membrane protein, and receptors, were incorporated into the lipid-free regions. This functionalized lipid bilayers will lead to construct complex and versatile biomimetic model membrane arrays.

Keywords: photopolymerization, Diacetylene Lipid Bilayers

K008

Bacterial Shot Hole of Japanese Apricot (Prunus mume) Caused by *Xanthomonas campestris* pv. *pruni*

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Bacterial shot hole of Japanese apricot (Prunus mume) was found in all cultivating area in Korea. The disease caused by phytopathogenic bacteria is economically important one resulting in severe damage of Japanese apricot production. Typical symptoms were characterized by red spots formed on fruits and leaves, which turned to brown color and then finally resulted in necrosis. Isolations from diseased leaves and fruits on yeast extract-dextrose-calcium carbonate agar (YDC) yielded nearly pure culture of a yellow-pigmented bacterium typical of a Xanthomonad. Forty strains isolates from 18 different areas were identified on the basis of physiological and biochemical characteristics and also on the basis of 16S rDNA sequences. Pathogenicity tests confirmed that bactrerial shot hole of Japanese apricot in Korea is caused by *Xanthomonas campestris* pv. pruni(*X. arboricola* pv. pruni). Forty strains were classified into six groups according to the number and size of plasmids.

Keywords: *Xanthomonas campestris* pv. pruni, Bacterial shot hole, Prunus mume

K009

Application of Insoluble Silver-Alginate Complex Beads for Sludge Solubilization and Pathogen Reduction

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Silver-alginate complex beads (SACB) were prepared by the reaction of sodium alginate with silver nitrate, followed by crosslinking with $CaCl_2$, and the effects of SACB for the solubilization of waste activated sludge (WAS) and the removal of pathogen were investigated. FTIR analysis showed that the complex formed mainly by the covalent bonds between hydroxyl groups of alginate and silver. Treatment of WAS with SACB resulted in a significant increase of soluble chemical oxygen demand (COD) concentration. At pH 7.0 and a temperature o 25℃, the released soluble COD concentration in SACB-treated WAS (total solid = 2.0%) reached up to 4400 mg/L within 2 days. Moreover, treatment of culture suspensions of *Escherichia coli* and *Staphylococcus aureus* with 5% (v/v) SACB for 5 min resulted in the reduction of cell counts by two to three magnitudes. These results suggest that SACB might be useful for sludge reduction process and inactivation of pathogenic microorganisms in the water.

Keywords: Silver-alginate complex, Sludge solubilization, Antimicrobial activity

K010

Detection of Polymorphisms Sites in Prion Protein Gene of Sika Deer Living in Korea

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Chronic wasting disease (CWD) is an emerging transmissible spongiform encephalopathy (prion disease) of North American cervids such as mule deer, white-tailed deer, and elk.To characterize the *PRNP* polymorphisms in sika deer living in Korea, we analyzed the *PRNP* of 23 sika deer. Blood samples were provided from the Seoul Grand Park Zoo and DNAs extracted from blood were used as templates for PCR. The PCR products were purified by PCR purification kit. Sequencing reactions were performed in a MJ Research PTC-225 Peltier Thermal Cycler using a ABI PRISM BigDye ™ Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (FS enzyme) (Applied Biosystems).We found two single nucleotide polymorphism (SNPs) in sika deer (C408T and G676C). Allele frequencies of these polymorphisms in sika deer were 41.3% for the C allele of the 408 SNP and 56.52% for the G allele of the 676 SNP. The analysis of amino acid sequences of sika deer PrP indicated that they had the same amino acid residues indentified in CWD-infected cervid.In this study, we found two SNPs in sika deer *PRNP*. In addition, sika deer had the same amino acid residues found in CWD-susceptible elk, mule deer and white tailed deer.

Keywords: CWD, Prion protein, Single nucleotide polymorphism

K011

Improved Proteome Profiling Method Using Small Heat Shock Proteins by Inhibiting Proteolysis

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Proteolytic degradation is one of the critical problems in two-dimensional electrophoresis (2-DE). We report that small heat shock proteins (sHsps), including $IbpA_{Ec}$ and $IbpB_{Ec}$ from *Escherichia coli* and $Hsp26_{Sc}$ from *Saccharomyces cerevisiae*, are able to protect proteins *in vitro* from proteolytic degradation. Addition of sHsps during 2-DE of human serum or whole cell extracts of *E. coli*, *Mannheimia succiniproducens*, *Arabidopsis thaliana*, and human kidney cells allowed detection of up to 50% more protein spots than those obtainable with currently available protease inhibitors. Therefore, the use of sHsps during 2-DE significantly improves proteome profiling by generally enabling the detection of many more protein spots that could not be seen previously. [This work was supported by a Korean Systems Biology Research Grant from the Korean Ministry of Science and Technology. Additional support was provided by the LG Chem Chair Professorship, the Brain Korea 21 project, and the Center for Ultramicrochemical Process Systems.]

Keywords: sHsps, proteome, proteolysis

K012

Effects of Gingko Leaf Meal on Growth and Change of Ginkgo Flavon Glycosides in *Pleurotus eryngii*

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A mushroom cultivation medium containing gingko leaf meal and its manufacturing process was proposed in this study. Leaves of gingko tree containing ginkgo flavon glycosides were dried and ground to the meal. In this work, gingko meal (10～30% v/v) added to the mixture of sawdust and wheat bran was considered to help the production of mushroom fruiting body. Mushroom yields were increased 3 to 12% when the mushrooms were cultivated with this medium. Also the hardness of fruiting body was increased, therefore it was useful for storage and distribution of this mushroom. Furthermore mushroom cultivated with this medium may contained ginkgo flavon glycosides(GFG) such as kaempferol, isorhamnetin, and quercetin having the effect of improving blood circulation.

Keywords: gingko, *Pleurotus eryngii*, fruiting body

K013

Identification for Protein(s) Interacting with EB1 (end-binding 1) Protein in *Giardia lamblia*

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Giardia lamblia, a human pathogen causing diarrheal disease, is an interesting microorganism regarding its phylogenetical position as a member of the earliest lineage of the *Eucarya*. Trophozoite, one of the two phases comprising its life cycle, is an active form able to multiply by binary fission. A cDNA clone encoding a putative EB1 (end-binding) homologous protein was identified in *G. lamblia*. The EB1 protein is one of the key factors in determining cellular polarity, which is conserved in various eukaryotic cells from yeast to humans. Thus, role of this putative EB1 protein was examined in *G. lamblia* having a polarity. Recombinant EB1 protein was prepared, and used to make the polyclonal antibodies by immunizing rats. Western blot analysis and immunolocalization using the EB1 protein-specific antibodies indicate that EB1 protein expresses in a constitutive mode during life cycle of *G. lamblia*, i.e., trophozoites and encysting cells, and localizes in boundaries of the adhesive disc and between nuclei. Based on a hypothesis that this putative EB1 homologue may function in controlling microtubule assembly, yeast two hybrid assay was performed to identify protein(s) that interact with EB1. Thirteen colonies with interaction-positive phenotypes were selected, and identified to be genes for γ-giardin, neurogenic locus Notch protein precursor, and three hypothetical proteins. Interaction of these 5 clones with the EB1 protein was confirmed by coimmunoprecipitation.
Keywords: *Giardia lamblia*, Microtubules, End-binding protein 1

K014

Genetic Analysis of the Invariant Residue G791 in *Escherichia coli* 16S rRNA Reveals a Functional Role of RelA in Ribosome Function

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A base substitution was introduced at the position 791 in *E. coli* 16S rRNA, which was previously identified as an invariant residue for ribosome function. These ribosomal RNA mutations reduced the ability of ribosomes to carry out protein synthesis by more than 70%. Selecting genomic library clones that restore the ability of protein synthesis to these mutant ribosomes identified *relA* whose product interacts with ribosome and triggers the stringent response. Over-production of RelA assisted the mutant ribosomes to synthesize approximately two times more CAT protein and consequently renders cells expressing the mutant ribosomes more resistant to chloramphenicol. Bacterial cells over-producing RelA showed the typical effects of elevated guanosine 3',5'-bispyrophosphate (ppGpp) levels including a slowing of growth and an inhibition of cellular *rrn* P1 promoter activities. However, the degree of distribution of mutant rRNA in total rRNA was not affected by RelA over-production indicating that the partial restoration of protein synthesis ability to the mutant ribosomes was not stemmed from the stringent response triggered by over-production of RelA. These findings imply the existence of a previously unrecognized role played by RelA in ribosome function.
Keywords: *E. coli* 16S rRNA, ribosomal RNA, relA, guanosine 3',5'-bispyrophosphate (ppGpp)

K015

Rapid and Specific Analysis of Biological Agents Using PCR and Real-Time PCR

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According to analysis data on biological agents, many countries are suspected to have biological warfare programs. To prepare for biological warfare and terror, rapid detection of targeted agents is required. It is important to detect and identify the biological agents as soon as possible to reduce casualties of service man and civilians. The condition of PCR and real-time PCR on some kinds of bio-agents was established and coordinated to minimize analysis time for unknown bio-samples, respectively. The primers for PCR analysis were designed for particular 20 ~ 24 bases in specific pathogen-related genes of 9 microorganisms. The detection limit of PCR was shown as 100 pg ~ 5 ng of total DNA per reaction. The primers for real-time PCR analysis were designed for particular 19 ~ 26 bases in specific pathogen-related genes of 8 microorganisms. The detection limit of PCR was shown as 20 pg ~ 1 ng of total DNA per reaction. The results will enable quick, accurate cross-over analysis on unknown biological agents and contribute to preserving social systems.
Keywords: biological agent, PCR, real-time PCR

K016

Inhibitory Effects of Traditional Herbal Medicines on the Production of Nitric Oxide Synthesis

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Nitric oxide (NO) is synthesized via the oxidation of arginine by a family of nitric oxide synthesis (NOS). An inflammatory mediator presently undergoing intense study is nitric oxide. The overproduction of nitric oxide plays a vital role in the regulation of inflammation and immunity. For the screening of anti-inflammation, ethanolic extract of 59 species of traditional herbal medicines were examined their inhibitory effect, and we confirmed that 3 herbs (CJC, DH, KK) possessed inhibitory effects on NO production particularly. The dried DH roots were extracted with the ethanol and were fractionated sequentially with n-hexane, chloroform, ethyl acetate, n-butanol and aqueous fractions. As a result, chloroform soluble fractions have high nitric oxide synthesis inhibitory effect, respectively. We also investigated the effect of DH on expression of iNOS in LPS treated-macrophage cells by reverse transcription polymerase chain reaction (RT-PCR). To isolate the active compound from DH, the chloroform fraction from DH ethanolic extract was subjected to column chromatography on a silica gel using a step-wise gradient solvent system of chloroform containing increasing amounts of methanol. As the results, Fr. II fraction showed the strongest inhibitory effects on nitric oxide production.
Keyword : Nitric Oxide

K017

Development and Evaluation of Attenuated Vaccines of Avian Infectious Bronchitis Virus Based on Molecular Epidemiology Studies

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Infectious bronchitis (IB), caused by infectious bronchitis virus (IBV), is one of the most serious diseases in poultry. Through the use of attenuated live as well as inactivated vaccines, economic losses due to this disease have been significantly reduced. However, cross-protection between different serotypes is variable or poor and therefore the choice of vaccine strain should be carefully considered. Since 1990, nephropathogenic type IBV has become a major concern in Korea, however respiratory type IBV infection still issued. It may reflect recent epidemiological situation of IB in East Asia characterized by increasing of nephropathogenic type IBV infection. Through the genetic analysis of recent IBV Korean isolates, we compared them with Japanese and Chinese isolates as well as old IBV Korean isolates. Japanese and Chinese IBVs showed close relationship with Korean IBV isolates. Especially, recent nephropathogenic IBVs was getting closer to Chinese IBVs than preexistent IBVs. Furthermore, we attenuated nephropathogenic type Korean IBV K2/01 isolate and evaluated them as a vaccine candidate. In addition to examining alteration on the pathogenicity between old and recent IBV isolates, efficacy of the K2/01 against IBV infection on SPF chickens was evaluated. Finally, the K2/01 showed wide protection against recent and old nephropathogenic type IBVs as well as respiratory type IBVs. The K2/01 seemed to be an effective vaccine candidate for controlling IB in East Asia.
Keywords: infectious bronchitis virus, vaccines, Infectious bronchitis, nephropathogenic

K018

Cytotoxic Effects of Nanoparticles Assessed *in vitro*

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This study examined the biological effect of nanoparticles. For this work, we used nanoparticles such as silver (Ag), zinc (Zn), iron (Fe) and silicon dioxide (SiO_2: 10-20, 40-50, 90-110nm). We also assessed the toxicity of relatively larger particles of silicon dioxide (SiO_2;45㎛), silver (Ag;2-3.5㎛) to compare the cellular toxic responses with respect to the different sizes of nanoparticles. We used several human cell lines such as brain (A-172), kidney (293 cell), liver (HuH-7), stomach (MKN-1) and pancreas (AsPC-1) to observe the acute toxic effect. For toxicity evaluations, DNA concentration, mitochondrial activity and permeability of the plasma membrane are assessed under the following exposure conditions; 2400ppb, 240ppb, 24ppb, 2.4ppb, 0.24ppb, for 72 hours. DNA concentration, mitochondrial activity were moderately affected by the nanoparticles in cell culture. The nanoparticles did not affect membrane permeability. There was no difference seen dependent on the size of the nanoparticles.
Keywords: nanoparticle, cytotoxicity

K019

Effect of Substitution-Mutated SicA on Type III Secretion of *Salmonella typhimurium*

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Salmonella typhimurium encodes a type III secretion system (T3SS) to transport several proteins across the bacterial cell and into eukaryotic cells to interfere with host-cell signal transduction pathway. T3SS is made up of more than 50 proteins, each expression is tightly regulated by temporal and hierarchy system, among which chaperones play a central role as partitioning factors or secretion pilots. Although class-II chaperones for T3SS translocon has little conserved sequence, their conformational function categorized themselves into three tetratricopeptide repeats (TPRs) family. In *S. typhimurium*, SicA is known for class-II chaperone of SipB/C translocon protecting from their premature association. To elucidate the importance for SicA on secretion of SipB, we disrupted chromosomal sicA using the phage lambda Red recombinase which allows recombination with the short(-40nt) PCR product in one-step, and we substituted TPRs' canonical residues of SicA using site-directed mutagenesis (Y41A, A44E, Y45A, Y41A&Y45A, F60A, L77A). We found that introduction of substituted mutation into sicA mutant background resulted in the lack of SipB, indicating that substitution on conserved canonical residues from TPR of SicA render conformational change and mutated SicA no more bound SipB in *S. typhimurium* cytosol..
Keywords: *Salmonella* SicA chaperone TPRs

K020

Essential Oils are Effective to Control Storage Disease Caused by *Botrytis cinerea* on Strawberry Fruits

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Importance of substances extracted from natural resources including plants is enhancing based on the demand of consumers and scientists who know well side-effects of agrochemicals in foods as well as crops. This research was performed for environmental-friendly control of gray mold, *Botrytis cinerea*, using natural products during storage of harvested strawberry fruits. Antifungal substance was isolated and identified from *Asarum sieboldii* selected among 31 medicinal plants, and formulation and novel method were considered for agricultural application practically. Antifungal substance extracted from *Asarum sieboldii* was identified to be Methyl eugenol, a kind of essential oils. Emulsification of essential oils at 300ppm concentration with tween 20 and 600ppm concentration with ethanol, enhanced the control effect even at low concentration and, in contrast, reduced phytotoxity. Achieving 76.1% control value with 100ppm of Methyl eugenol, period of storage for the strawberry fruit was prolonged by treatment one day before harvest. It should be possible to control several plant diseases caused by *B. cinerea* concerning storage environmental-friendly, throughout formulation which enhances a control efficacy of essential oils for agricultural application based on determining available concentration, and on developing various application methods.
Keywords: *Botrytis cinerea*, Methyl eugenol, essential oil

K021

Growth Hormone Releaser Attenuates Memory Impairments via Phosphorylation of AKT/GSK3b and Activation of Muscarinic M1 Receptor in the Genetic Model of Aging Gene Klotho Mice

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It has been recognized that a defect in *Klotho* gene expression accelerates the degeneration of multiple age-sensitive traits. Accumlating evidence indicating that aging is associated with declines in the cognitive function and the activity of the growth hormone (GH)/insulin-like growth factor-1(IGF-1). *Klotho* mutant mice receiving GH releaser increased hippocampal phosphorylation of Akt/GSK3β and ERKs, while decrease phosphorylation of JNKs as compared with wild type case Memory enhancing effects of GH releaser was more pronounced in the mutant mice than in the wild type mice Consistently, changes in the levels of the malondialdehyde, protein carbonyl, and acetylcholine, and in the expressions of Bcl-2, Bax, and cleaved caspase-3, and in the bindings of muscarinic M1 receptor were attenuated in the presence of GH releaser. These attenuations of GH releaser were more accentuated in the brain of the mutant mice than that of wild type mice. The results suggest that GH releaser-induced activations in the Akt/GSK3β signaling and muscarinic M1 receptor binding plays a crucial role in preventing cognitive dysfunction and oxidative stress as shown in *Klotho* mutant mice.

Keywords: *Klotho*, aging, growth hormone, IGF-1, Akt/GSK3β

K022

Functiona Expression of the Carotenoid Biosynthesis Gene from *Paracoccus haeundaensis*

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Carotenoids are important natural pigments produced by many microorganisms and plants. Previously, we reported that isolation of a new marine bacterium, *Paracoccus haeundaensis*, which produces carotenoids, mainly astaxanthin, and an astaxanthin biosynthesis gene cluster was cloned and characterized from this organism. The astaxanthin biosynthesis gene cluster in *Paracoccus haeundaensis* consists of six genes: crtW, crtZ, crtY, crtI, crtB, and crtE contain 726, 486, 1158, 1503, 912, and 879 base pairs, respectively. Here, high-level expressions of carotenoid biosynthesis genes from *P. haeundaensis* were achieved by introducing these genes into *E. coli.* The recombinant proteins were produced in the cytoplasm of the transformed cells. We describe the purification and characterization of expressed proteins from the transformed cells with each gene encoding the carotenoid biosynthetic enzymes from *P. haeundaensis*. These results will provide a wider knowledge of the primary structures of the astaxanthin biosynthesis enzymes at the molecular level and facilitate the biotechnological applications of carotenoids.

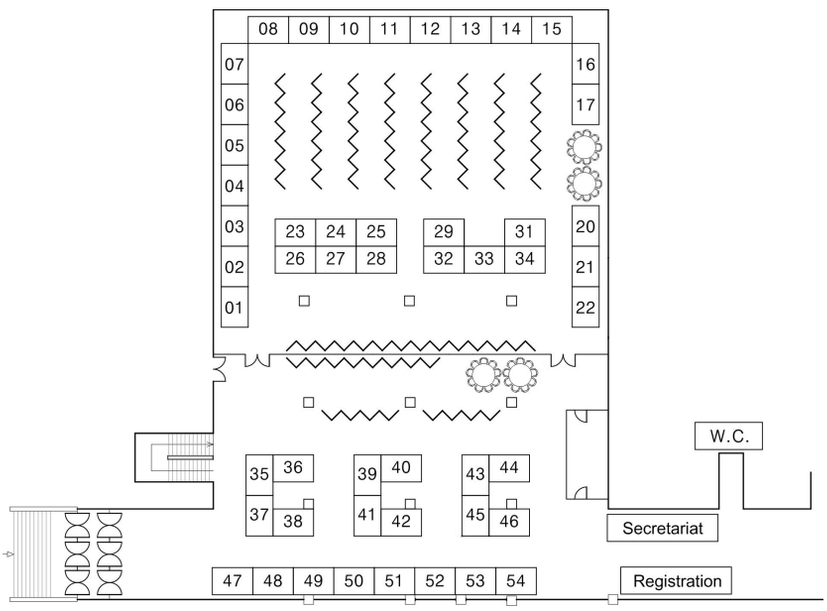
Keywords: Carotenoids, *Paracoccus haeundaensis*, crtW, crtZ, crtY, crtI, crtB, crtE, marine bacterium

Instrument Exhibition

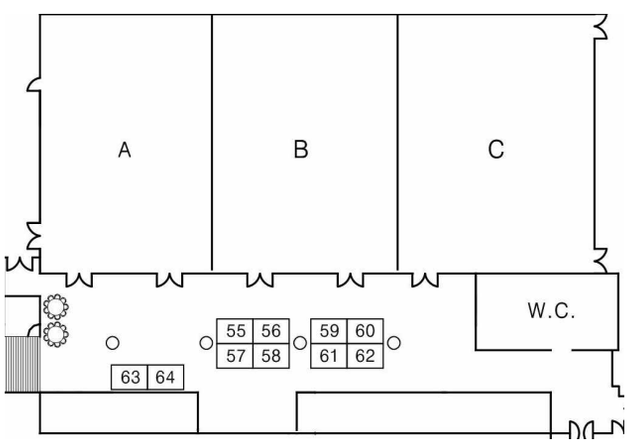
백지

Plan for Exhibitors

▶ 2nd Floor (Gayageum Hall & Lobby)



▶ 3rd Floor (Guhmoongo Hall Lobby)



Company	Booth No.	Company	Booth No.	Company	Booth No.
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CHAYON	16	Human Corporation	25	Roche Diagnostics Korea Co., Ltd.	63, 64
Chemical Technology Corporation	2, 3	INNERVIEW Co., Ltd.	42	Sartorius Korea Biotech	55, 56, 57, 58, 59, 60, 61,62
CMI Bio Co., Ltd.	15	INSUNG CHROMA-TECH CO., LTD.	1	Seolin Tech	11, 12
DAIHAN Scientific, Co., Ltd.	51, 52, 53, 54	iNtRON Biotechnology, INC.	28	Shinhan Scientific	39, 41
Daiki Sciences., Ltd.	38	JinSung Uni-Tech	33, 34	ShinYoung Corporation	31
Dawinbio Inc.	32	KBT (Korea Bio-Tech)	5, 6	SIWON Optical Technology	35
Dionex Korea	40	Koma Biotech Inc.	23	Sulim	37
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Filterpia Corperation	24	Macrotech Corporation	46	TechSan Microsystem	36
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Exhibitiors

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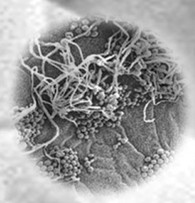
Main Technology & Products	OXOID: Microbiology-related Product (Media, Assay kit, etc.) AB: Molecular biology and Biochemistry-related Product (PCR, Real-Time PCR, etc.) AnaSpec: Proteomic, Immunology-related Product (Peptides, Assay kits, Antibodies, etc.)				

CHAYON					
CEO	Sunki KIM	TEL FAX	+82-2-3471-4100 +82-2-3471-0040	Homepage E-mail	www.chayon.co.kr info@chayon.co.kr
Address	829 Yeoksam-dong, Gangnam-gu, Seoul 135-936, Korea				
Main Technology & Products	Plate reader, Proteomics system, Cell imaging, In vivo imaging				

Chemical Technology Corporation					
CEO	Jonghoon SHIN	TEL FAX	+82-2-3437-5163 +82-2-3437-5165	Homepage E-mail	www.chemtechcorp.co.kr chemtech@chemtechcorp.co.kr
Address	3-310 Hyundai Goldentel, 14 Kwangjang-dong, Kwangjin-gu 143-800, Korea				
Main Technology & Products	Microbiology Solution * Auto Colony counter: Flash & Go(IUL) * Stomacher: Masticator(IUL) * Incubator: Incucell(MMM) * Evaporator: Strike 202(Steroglass) * Vortex Mixer: Vortex Classic & Wizard* Multi Stirrer15				

CMI BIO Co., Ltd.					
CEO	Eun-Suk CHOI	TEL FAX	+82-2-444-7101 +82-2-444-7201	Homepage E-mail	www.cmibio.com cmibio@cmibio.com
Address	#302, 221-6 Guui-dong, Kwangjin-gu, Seoul 143-200, Korea				
Main Technology & Products	Cansera FBS (Cansera) ABM PCR related enzymes, Tag antibody, Safeview, agarose, cell-based assay, Virus purification kit, antibodies, ELISA kits, etc. (ABM, Cell Biolabs, Antigenix, Cell sciences,…)				

DAIHAN Scientific, Co., Ltd.					
CEO	Eun-Taek SHU Jae-woong LEE	TEL FAX	+82-2-967-5235 +82-2-967-5032	Homepage E-mail	www.DAIHAN-Sci.com sblee@daihansci.co.kr
Address	24-4, Sangwolgok-dong, Sungbuk-gu, Seoul 136-120, Korea				
Main Technology & Products	PCR and Cell Culture Related Reagents, Consumable Plastics, Incubator, Dry Oven, Orbital Shaker, Rocker, Heat Block, Hot plate Stirrer, Autoclave, Homogenizer, Growth Chamber, Water Bath, Circulation Bath, Shaking Water Bath, LN2 Tank, Gel Documentation System, UV Transilluminator, All laboratory Equipment				



Daiki Science Co., Ltd.					
CEO	Jeong-Lok RHEE	TEL FAX	+82-2-597-8213 +82-2-597-8214	Homepage E-mail	www.daiki-sci.com res@daiki-sci.com
Address	4F DAIKI B/D, 1031-7 Bangbae-dong, Seocho-gu, Seoul 137-060, Korea				
Main Technology & Products	Charm II, Hypoxia Workstation and Micro Aerophilic Work Station, CO_2 Incubator, Haematology Analyzer, Chemical Analyzer, Automatic colony counter, Automatic glassware washer, Spectrophotometer, EISA Reader, Automatic Media Preparation, Air Sampler, Formalintec				

Dawinbio Inc.					
CEO	Sun Ha RYOO	TEL FAX	+82-2-422-4915 +82-2-422-4916	Homepage E-mail	www.dawinbio.com hslee@dawinbio.com
Address	#302 Doorim B/D, 49-4 Bangi-dong, Songpa-gu, Seoul 138-828, Korea				
Main Technology & Products	1. Antibody 2. Molecular biology related product 3. Cell culture related product 4. Apparatus and Equipment 5. Various sterile syringe filter, Bottle top filter, PVDF membrane, NC membrane, UF membrane and Protein concentration/purification system				

Dionex Korea					
CEO	Hak Soo KIM	TEL FAX	+82-2-2653-2580 +82-2-2653-2508	Homepage E-mail	www.dionexkorea.com dionex@dionexkorea.com
Address	Yangcheon Bldg, 330-11 Shinjung 7-dong, Yangcheon-gu, Seoul 158-742, Korea				
Main Technology & Products	Ion Chromatography, HPLC, Capillary & Nano LC, ASE (Accelerated Solvent Extraction), Data Management System				

DisGene Co., Ltd.					
CEO	S. K. Kim	TEL FAX	+82-2-3471-4600 +82-2-3454-1176	Homepage E-mail	www.disgene.co.kr disgene@disgene.co.kr
Address	8th Fl., BMS bldg, Yeoksam-dong, Gangnam-gu, Seoul 135-936, Korea				
Main Technology & Products	Biomolecule Purification System (Lab & Process scale) Charged Aerosol HPLC detector				

Dong-In Biotech					
CEO	Gil Nam CHO	TEL FAX	+82-2-431-7375 +82-2-3402-3725	Homepage E-mail	www.donginbio.com info@donginbiotech.co.kr
Address	19-9, Geoyeo-dong, Songpa-gu, Seoul 138-130, Korea				
Main Technology & Products	**Molecular part : RBC / Duchefa / Qbiogene / Bioworld / Elpis biotech** : Competent cell, DNA & RNA prep. Kit, Cloning kit, PCR polymerase Microbial Media, Antibiotics, Molecular biochemicals, Molecular & cell biology research products, **Immunology part : Assay designs / stressgen / Echelon / ICT / sapphire bioscience** : ELISA kit, Detection kit, Antibodies, Proteins, Cell & lipid signaling, Apoptosis				

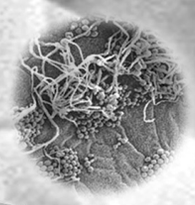
Elsevier Korea					
CEO	John KWAK	TEL FAX	+82-2-725-4698 +82-2-725-4388	Homepage E-mail	www.elsevier.com k.park@elsevier.com
Address	404 Hongik Bldg, 194 Insa-dong, Chongro-gu, Seoul 110-290, Korea				
Main Technology & Products	Science Technology & Medical Books, Journals & Online Services				

Filterpia Corperation					
CEO	Hee Song CHOI	TEL FAX	+82-2-402-0308 +82-2-402-4698	Homepage E-mail	www.begerow.co.kr szzang@begerow.co.kr
Address	Rm 201, 79-15 Oguem-dong, Songpa-gu, Seoul 138-858, Korea				
Main Technology & Products	Filtration, Seperation & Clarification Filterpress Sheet filters & Module				

HANA SCIENTIFIC INC.					
CEO	Chul YOON	TEL FAX	+82-2-3473-6060 +82-2-3473-6670	Homepage E-mail	www.hanasc.co.kr hanasc@korea.com
Address	1628-52 Seocho-dong, Seocho-gu, Seoul 137-879, Korea				
Main Technology & Products	Cell Viability Analyzer Cell & Particle sizing and Counting Analyzer Cell Counter				

Hanil Science Industrial Co., Ltd.					
CEO	Un Ho CHANG	TEL FAX	+82-32-543-6906 +82-32-542-7124	Homepage E-mail	www.ihanil.com hanil@ihanil.com
Address	853-19 Jackeon-dong, Bupyeong-gu, Incheon 403-030, Korea				
Main Technology & Products	Centrifuge 1. High Speed Centrifuge 2. Micro Centrifuge 3. Large Capacity Centrifuge 4. General Gentrifuge				

Hansol Science & Medical Co., Ltd.					
CEO	Seung Deok YU	TEL FAX	+82-32-671-6663 +82-32-671-6606	Homepage E-mail	www.hansolsm.com hansolsm@hansolsm.com
Address	#406 Dawoo Techno Town, 344 Nae-dong, Ojeong-gu, Bucheon-si, Gyeonggi-do 421-807, Korea				
Main Technology & Products	Auto Clave, Deep Freezer, B.S.C, Centrifuge				



Human Corporation					
CEO	Hyungil KIM	TEL FAX	+82-2-418-4182 +82-2-423-6430	Homepage E-mail	www.humancorp.co.kr info@humancorp.co.kr
Address	Eunsung Building, 304-19 Jamsilbon-dong, Songpa-gu, Seoul 138-865, Korea				
Main Technology & Products	Water Purification System UV/VIS Spectrophotometer Washing Machine Stomacher Auto Colony Counter				

INNERVIEW Co., Ltd.					
CEO	Charles W. CHOI	TEL FAX	+82-2-583-3845 +82-2-583-3846	Homepage E-mail	www.inner-view.co.kr francesco@inner-view.co.kr
Address	825 Hyundai Venture-vill 713, Suseo-dong, Gangnam-gu, Seoul 135-539, Korea				
Main Technology & Products	Microscope, Image Analyzer, Microscope Digital Camera				

INSUNG CHROMA-TECH CO., LTD.					
CEO	G. S. YOON	TEL FAX	+82-2-2644-1991 +82-2-2644-1996	Homepage E-mail	www.insung.net is@insung.net
Address	89-106 Sinjeong 2-dong, Yangcheon-gu, Seoul 158-849, Korea				
Main Technology & Products	Microbial Identification System, Phenotype Microarray, Chromatography, Mass Spectrometry, Column & Consumable, LabChip System, Drug Discovery System, Liquid Handler, Sample Preparation System, Electron Nose, Electron Tongue				

iNtRON Biotechnology, INC.					
CEO	Sung Jun YOUN	TEL FAX	+82-31-778-7890 +82-31-736-7245	Homepage E-mail	www.intron.co.kr kskim1026@intron.co.kr
Address	206 Sungnam Venture Building, 587 Sujin 1-Dong, Sufeong-gu, Sungnam-si, Gyeonggi-do, 461-804, Korea				
Main Technology & Products	1. DNA/RNA Related Products: DNA-spin, MEGAquick-spin, MEGA-spin, PCR-spin, G-spin, easy-spin, RNA-spin, Viral Gene-spin, G-DEX, easy-BLUE, PROBER 2. Protein Related Products: PRO-PREP, WEST-one, WEST-zol, PRO-Measure, PRO-stain 3. PCR Related Products: I-Taq Series, I-PFU, Maxime PCR Premix, Maxime RT-PCR PreMix, power cDNA Kit, ONE-STEP RT-PCR PreMix, e-Mycoplasma Detection Kit, dNTP 4. Others: Muta-Direct Mutagenesis Kit, Agarose, PRE-made Agarose Gel, DNA/Protein Size Marker, Cloning Reagents, Enzymes, LPS Extraction Kit, NO Detection Kit, Beta-Gal Staining Kit				

JinSung Uni-Tech					
CEO	Sung Bin JUN	TEL FAX	+82-2-2164-2271 +82-2-2164-2277	Homepage E-mail	www.jsunitech.com info@jsunitech.com
Address	#906 CenterPlus, 39 Moonre-dong 1ga, Youngdeungpo-gu, Seoul 150-091, Korea				
Main Technology & Products	Culture Media, Microbial Identification kit, Foodborne pathogen detection kit, Anaerobic workstation, Spiral plater, Stomacher, Pulsifier, Mycotoxin detection kit, Air sampler, Nasco whirl-pak, ATP monitoring				

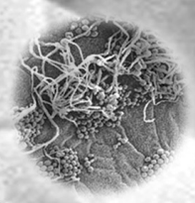
KBT (Korea Bio-Tech)					
CEO	HyeongKil KIM	TEL FAX	+82-31-716-6052 +82-31-716-6056	Homepage E-mail	www.kbt.co.kr kbtbio@kbt.co.kr
Address	98-1 Dongwon-dong, Bundang-gu, Sungnam-si, Gyeonggi-do 463-490, Korea				
Main Technology & Products	Gel Documentation Sys, Electrophoresis Sys. Microplate reader & Washer, Luminimeter, Electroporation & Cell Fusion Sys, PCR, Evaporation Sys, Freeze Dryer, Low Temp Incubator, Oven, Furnace, Homogenizer, Vacuum Pump, Aspirator, Pump, Pure Water Sys, pH meter, Shaker, Mechanical Stirrer, Organic Synthersizer				

Koma Biotech Inc.					
CEO	SangHoon MOON	TEL FAX	+82-2-579-8787 +82-2-578-7042	Homepage E-mail	www.komabiotech.co.kr koma@komabiotech.co.kr
Address	#301, Gayang Technotown, 1487 Gayang 3-dong, Gangseo-gu, Seoul 157-793, Korea				
Main Technology & Products	Luminex, Imaging system(Vilber), CO_2 Incubator, Nitrogen Tank, PCR, Centrifuge, Electrophoresis kit & Reagents, Cell culture Products(FBS, disposable pipette etc), Antibody, Antigen, ELISA kit, Assay kit for cell biology, SNAP-Tag, Fluorescent dyes, Neurochemicals, peptides, Taq & premix				

KOS Inc.					
CEO	Dae hee CHOI	TEL FAX	+82-2-486-7930 +82-2-486-7931	Homepage E-mail	www.kosinc.co.kr hojoon@kosinc.co.kr
Address	425-1 Gil-dong, Gangdong-gu, Seoul 134-834, Korea				
Main Technology & Products	Fluorescence / Brightfield Real-Time microscope Imaging Acquisition System, MetaMorph Image analysis software, Real-Time Confocal Microscope System, Invivo animal Fluorescence Luminescence Imaging System				

KRIBB BRC					
CEO	Hee mock OH	TEL FAX	+82-42-860-4600 +82-42-860-4625	Homepage E-mail	www.brc.re.kr sales@kribb.re.kr
Address	52 Eoeun-dong, Yuseong-gu, Daejeon 305-806, Korea				
Main Technology & Products	Distribution Service Patent Deposit and Distribution				

MACROGEN, Inc.					
CEO	ByongHwa LEE	TEL FAX	+82-2-2113-7000 +82-2-2113-7120	Homepage E-mail	www.macrogen.com webmaster@macrogen.com
Address	10F, World Meridian Center, 60-24 Gasan-dong, Kumchun-gu, Seoul 153-801, Korea				
Main Technology & Products	* Sequencing Service: Normal & Whole genome Sequencing, Library construction, 16s rRNA * BIO Chip Service: Customized Chip, Expression Chip, Array CGH, Genotyping * Genetically Engineered Mice: Knockout & Transgenic Mouse * Lentivirus Vector Service: Gene Expression, shRNA Lentivirus Vector System				



Macrotech Corporation					
CEO	Ju Hyong LEE	TEL FAX	+82-31-903-3093 +82-31-903-3096	Homepage E-mail	www.macrotech.co.kr macro@macrotech.co.kr
Address	#802, Central Plaza, 846 Janghang-dong, Ilsan-gu, Goyang-si, Gyeonggi-do 411-380, Korea				
Main Technology & Products	* Sorvall & Heraeus centrifuge : Microcentrifuge, Refrigerated Tabletop Centrifuge. Large Capacity Cenrtrifuge, Highspeed centrifuge, Ultracentrifuge * Nikon : Biological Microscopes(Inverted, Upright, Stereo), Confocal Microscopes				

Philekorea Technology, Inc.					
CEO	Minbong PARK	TEL FAX	+82-1544-3137 +82-1644-3137	Homepage E-mail	www.philekorea.co.kr support@philekorea.co.kr
Address	119-15 Shinseong-dong, Yuseong-gu, Daejeon 305-804, Korea				
Main Technology & Products	Corbett Life Science QIAGEN DNR Bio-Imaging Systems				

PROTAGEN					
CEO	Duk-Soo YOON	TEL FAX	+82-2-438-5740 +82-2-438-5743	Homepage E-mail	www.protagen.co.kr protagen@empal.com
Address	4F, Soo B/D, 561-1 Junggok-dong, Kwangjin-gu, Seoul 143-909, Korea				
Main Technology & Products	- In-vivo Imaging System - Multispectral Imaging System - Transfection Reagent				

Roche Diagnostics Korea Co., Ltd.					
CEO	Hun Koo LEE	TEL FAX	+82-2-550-5539 +82-2-568-1878	Homepage E-mail	www.roche-applied-science.com dohyun.myung@roche.com
Address	15F, Samhwa Bldg., 144-17 Samsung-dong, Gangnam-gu, Seoul 135-745, Korea				
Main Technology & Products	Molecular Biology Instrument and biochemicals				

Sartorius Korea Biotech					
CEO	D. S. KIM	TEL FAX	+82-31-782-7011 +82-31-782-7090	Homepage E-mail	www.sartorius.or.kr info@sartorius.or.kr
Address	431 Michelan Chereville, 180 Jungja-dong, Bundang-gu, Sungnam-si, Gyeonggi-do 463-811, Korea				
Main Technology & Products	"Total Solution Provider in BioPharmaceutical Development & Manufacturing" BioLab: Filtration Products, Centrifuge, Lab Water Systems, Protein Purification Kits, Upstream: Fermenter/Cell Culture Bioreactor/Disposable Bioreactor and Disposable Bags Downstream: Crossflow (MF & UF), Membrane Chromatography, Filtration Products Contract Services: Filter Validation, Process Validation, Cell Line Characterization, Molecular Biology & Protein Chemistry, Virus & Prion Safety Studies Consulting: cGMP Consulting, Drug Development Consulting, Clinical Studies (Phase II, III, IV)				

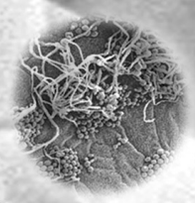
Seolin Tech					
CEO	Soon Gu Kim	TEL FAX	+82-31-391-6166 +82-31-391-6177	Homepage E-mail	www.SEOLINTECH.co.kr ST@SEOLINTECH.co.kr
Address	789-16 Dang-dong , Gunpo-si, Gyeonggi-do 435-010, Korea				
Main Technology & Products	www.AFFYMETRIX.com www.BMGLABTECH.com www.TURNERDESIGNS.com www.UVP.com www.COYLAB.com		www.HYCLONE.com www.PROMEGA.com www.CHEMICON.com www.UPSTATED.com www.DHARMACON.com		www.SARSTEDT.com www.CLPdirect.com www.MASTgrp.com www.LUCIGEN.com www.PANOMICS.com

Shinhan Scientific					
CEO	Sai-Soon KWON	TEL FAX	+82-2-3484-1323 +82-2-3484-1323	Homepage E-mail	www.shin-han.com pchbio@shin-han.com
Address	148-1 Samsung-dong, Gangnam-gu, Seoul 300-813, Korea				
Main Technology & Products	microscope				

ShinYoung Corporation					
CEO	Young Hee CHOI	TEL FAX	+82-2-575-7476 +82-2-575-7472	Homepage E-mail	www.sycos.co.kr syc@sycos.co.kr
Address	1st Fl., Gwangyeong Bldg., 97-10 Yangjae-dong, Seocho-gu, Seoul 137-891, Korea				
Main Technology & Products	Tissue culture & Molecular technology Centrifuge, PCR, Micropipette, Turntables for petri dishes, Anaerobic Jars, Colony counter, Homogenizer, Burner, Incubator				

SIWON Optical Technology					
CEO	Il-Mok KIM	TEL FAX	+82-31-479-6022 +82-31-479-6020	Homepage E-mail	http://siwon.co.kr/ optics@siwon.co.kr
Address	#519, The O Valley, 555-9 Hogye-dong, Dongan-gu, Anyang-si,,Gyeonggi-do 431-763, Korea				
Main Technology & Products	digital microscope				

Sulim					
CEO	Jung-Woo LEE	TEL FAX	+82-2-578-8974 +82-2-578-8975	Homepage E-mail	www.sulim.com slc@chol.com
Address	3F, Yongwon Bldg., 313-5 Yangjae-dong, Seocho-gu, Seoul 137-130, Korea				
Main Technology & Products	In-vivo,In-vitro image station (Kodak) PCR Machine (Techne) Power Supply (Consort)		Gel Documentation System (Kodak) Electrophoresis Gel Unit (Scie-Plas)		



Sung Woo Genetech Inc.					
CEO	Sung-Yong HONG	TEL FAX	+82-2-3401-3920 +82-2-3401-3925	Homepage E-mail	www.swlab.co.kr solee@swlab.co.kr
Address	2F Ankuk B/D, 38-3 Okum-dong, Songpa-gu, Seoul 138-855, Korea				
Main Technology & Products	* Millipore: UltraPure Water system / Millex syringe filter, Bottle top&cup filter, UF system (Amicon, TFF system) Western Blotting Membrane & Reagents * Cape Cod (U.S.A): LAL test Reagents * Welch (U.S.A): Oil-free Vacuum pump * Art moderne Labortechnik (Germany): Homogenizer * B&J: HPLC grade solution * Dr. Hielscher (Germany): Ultra Sonicator * PBI international (Italy): SAS air sampler				

Takara Korea Biomedical Inc.					
CEO	Kawakami HIROSATO	TEL FAX	+82-31-739-3320 +82-31-739-3311	Homepage E-mail	www.takara.co.kr support@takara.co.kr
Address	Biotech. Community Bio21, 66-2 Sangdaewon-dong, Jungwon-gu, Seongnam-si, Gyeonggi-do 462-120, Korea				
Main Technology & Products	1. Biological reagents & Kit: genetic engineering, cell biology, protein engineering, glycobiology etc. (TaKaRa, Clontech, Cambrex, Mirus etc.) 2. Machines: PCR machine, Real Time PCR (Cepheid Smart Cycler II, TaKaRa Perfect Real Time System TP800), Luminometer, Electrophoresis system (DNA, Protein), Power Supply, Blotter, Image Analyzer, Gel Document system, Centrifuge etc. (TaKaRa, Mupid, ATTO, KURABO, Berthold etc.)				

TECHNOMART INC.					
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Address	#307 Ace-Techno Tower II, 197-7 Guro-dong Guro-gu, Seoul 152-766, Korea				
Main Technology & Products	DNA, RNA, Protein electrophoresis system and Reagents, CO2 Incubator, ULT Freezer, Bio safety cabinet, BioMasher, Parafilm				

TechSan Microsystem					
CEO	Ui Ho SOHN	TEL FAX	+82-31-420-4714 +82-31-420-4719	Homepage E-mail	www.tsms.co.kr gmrrltk100@tsms.co.kr
Address	#720 MegaValley, 799 Kwanyang-dong, Dongan-gu, Gyeonggi-do 431-060, Korea				
Main Technology & Products	- Olympus Microsystem - It handles the Biological microscope and Metallograph. Microscope camera system and a picture dealer analysis system. It handles those Total systems.				

Young Sciences, Inc.					
CEO	KwangDuk CHUNG	TEL FAX	+82-2-2164-9700 +82-2-2164-9705	Homepage E-mail	www.youngscience.com info@youngscience.com
Address	Flat 604 Woorim e-BIZ Center, 16 Yangpyungdong 3-ga Youngdeungpo-gu, Seoul 150-103, Korea				
Main Technology & Products	Microbiological Culture Media, Cell Culture Product, Human Cell & Media, Serum, Endotoxin (LAL) Assay Kit, Mycoplasma Detection Kit, Laboratory Filter, Disinfectants, Indicators, Plasticwares, Autoclave, BSC, Clean bench				

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Others