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BIOFILM-HOST INTERACTION IN CHRONIC INFECTION

R.Ihalin

Academy Research Fellow, ASM Country Ambassador to Finland

Department of Biochemistry, FI-20014 University of Turku, Turku, Finland

Microbes growing in surface-attached communities, biofilms, are protected from various environmental and host factors, such as antibiotics, immune cells and humoral host defense factors. How then bacteria know when to switch to the biofilm mode of growth and how to regulate the composition of biofilm matrix remains partly unknown. It is known that interactions with the surface serve as signals to bacteria to start to biofilm formation. However, there are many other environmental signals which may drive bacteria into biofilm. Some opportunistic pathogens, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Neisseria meningitidis* have been shown to sense host inflammatory cytokines. My group has found that an opportunistic oral pathogen *Aggregatibacter actinomycetemcomitans*, which is associated with periodontitis, a chronic inflammation of tooth supporting tissues, is able to bind and internalize various inflammatory cytokines. The uptake of cytokines changes the composition of biofilm matrix, decreases the metabolic activity of the biofilm and may also change the gene expression of the biofilm cells. We have identified various proteins involved in the cytokine uptake and our current investigations are focused on a closer depiction of their role in sensing the inflammation as well as in manipulating the host inflammatory response. If these proteins are central in the regulation of the bacterial virulence in inflammatory environment, they may serve as potential targets for novel antimicrobials against biofilm infections.

SESSION I: ENVIRONMENTAL MICROBIOLOGY

Presenter order	**Speaker**	**Presentation**	**Country**
Invited speaker	Hanna Mazur-Marzec	Biochemical ecology of the Baltic cyanobacteria	Poland
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Speaker IV	Andrei Novikov	Chemotaxonomic lipid analysis of novel taxa of bacteria	Russia
Speaker V	Dukas Jurėnas	An acetyltransferase toxin inhibits translation by novel mechanism	Belgium
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Speaker VIII	Andrew Anak Ngadin	Characterisation of saprophytic fungi isolated from two islands in the east coast of Peninsular Malaysia	Malaysia

BIOCHEMICAL ECOLOGY OF BALTIC CYANOBACTERIA

H.Mazur-Marzec

Department of Marine Biotechnology, Institute of Oceanography, University of Gdańsk
Al. Marszałka Piłsudskiego 46, PL-81378 Gdynia, Poland

Corresponding author: biohm@ug.edu.pl

Keywords: cyanobacteria, bioactive metabolites, peptide profile, mass spectrometry

The history of cyanobacteria in the Baltic Sea is at least as old as the brackish water stage of the ecosystem. In most studies, the ecology of the N_2-fixing filamentous species of Nostocales order was investigated. Of these, the hepatotoxin-producing *Nodularia spumigena* was most wildly explored. Recently, a growing interest in the bioactive secondary metabolites produced by both the dominating and the less common Baltic cyanobacteria species has been observed. Of these metabolites, the nonribosomal peptides (NRPs) produced on large multienzyme complexes called nonribosomal peptide synthetases (NRPS) are of special interest. In one cyanobacterial strain several NRPS can be present and on each of them a number of peptide variants can be synthesised. The complete peptide profile is a unique and stable feature of individual strain, and can be used as taxonomic marker.

In the current work, the nonribosomal peptides were used to study the history of cyanobacteria in the Baltic Sea and to explore the intra- and interspecies metabolic diversity of the microorganisms in this ecosystem. For the purpose of the studies, liquid chromatography combined with tandem mass spectrometry was used. The presence of unique NRPs produced by *N. spumigena*, in deep sediment core collected in the Gulf of Gdańsk, proved the occurrence of the species in the sea more than 5 thousand years ago. The increase in the intensity of the *N. spumigena* bloom correlated with the climate warming.

Chemical analyses led to the identification of high number of peptides representing several classes of the compounds. Comparison of peptide profiles in cyanobacteria belonging to the same species revealed significant strain-specific differences and allowed for separation of sub-populations defined as chemotypes.

In a final part of the work, the ecological significance and potential application of cyanopeptides will be discussed.

TOWARDS POLYPHASIC TAXONOMY OF *Synechocystis*

O.Gavrilova, M.Cherviakova, K.Kolesnichenko, A.Pinevich

St. Petersburg State University, Department of Microbiology, Vasilyevsky Island, Liniya 16-ya, 29, 199178 St. Petersburg, Russia

Corresponding author: avanti1958@inbox.ru

Keywords: Cyanobacteria, *Synechocystis*, systematics, morphology, 16S rRNA gene, *mcy* genes

Systematics of cyanobacteria (in particular, of *Synechocystis*) is problematic not only because these microorganisms were initially, and still are among botany objects. Rather, general paucity of morphology is aggravated by the disappearance, during culturing, of diagnostically valuable characters observed in environmental samples. Correspondingly, genetic characters increasingly matter.

In this work, 40 strains were isolated in Russia, and identified as *Synechocystis* based on morphology traits: cells spherical; sheath absent; capsule and gas vesicles absent. Morphology/morphometry were analyzed via light and electron microscopy; growth on various media was evaluated; pigment composition was analyzed. Also, 800 bp-fragments of 16S rRNA gene were sequenced, and the presence of *mcy* toxin genes was questioned.

Phylogenetic analysis distinguishes *Synechocystis* from morphologically similar *Microcystis*, and shows its heterogenicity. Main cluster "*Synechocystis* sensu stricto" (25% of collection, including *Synechocystis* PCC 6803): PE-containing or lacking 2–3 μm spheres occasionally producing capsules and cell aggregates. Cluster I: PC-rich 2–2.5 μm spheres. Cluster II: PE-containing 3.5–4.5 μm spheres producing capsules and aggregates. This cluster differs from "*Synechocystis* sensu stricto" via thylakoid pattern. This trait, together with cell fission type (changing division plane) allows reattribute these strains as *Geminocystis* Korelusová, Kaštovský et Komárek 2009. Cluster III: PE-containing 2.5 μm spheres ellipsoid upon division. These strains demonstrate type III chromatic adaptation, accumulate carotenoids and synthesize a long wavelenght chlorophyll. They also differ from "*Synechocystis* sensu stricto" in thylakoid arrangement. Analogous cultured forms were not described yet. Some of strains in all clusters possessed *mcy* genes; *mcyA* was mostly abundant; its sequences were mutually similar, and differed from those in *Microcystis*. In contrast, the diversity of *mcyE* gene confirmed phylogenetic heterogenicity of *Synechocystis*.

Acknowledgements:

This work was performed with support of RFBR grant No 16-04-00174.

METHODS FOR ENVIRONMENTAL MONITORING

A.-G.Klees

[1]Merck Life Science GmbH, Lilienthalstrasse 16, D-69214 Eppelheim, Germany

Corresponding author: anne-grit.klees@merckgroup.com

Keywords: air, surface and personal monitoring, compliance with ISO 14698

Background and objectives. Microbial monitoring programs in controlled clean room production areas give information on the effectiveness of cleaning and sanitization of air, surfaces and personnel. Common monitoring programs include the use of contact plates and swabs for personnel and surface monitoring. Air monitoring is performed passively using petri dishes as well as actively using air samplers for quantitative detection of microorganisms. The ability of air samplers to detect airborne microorganisms should be compliant with ISO 14698. The impaction speed of the air sampler should be high enough to collect low particle sizes but low enough to ensure viability of collected microorganisms, expressed as physical and biological sampling efficiency respectively.

The selected culture medium has an impact on the physical and biological efficiency results which are performed on growth based methods.

Further important criteria for monitoring devices are the grade of cleanliness, which should be adapted to the clean room zone for intended use, as well as ease of use with regard safety and production efficiency.

Methods. The physical and biological efficiency of full range of tested MAS® air samplers was validated externally using a TSA + LTHTh - ICR+ culture medium.

The physical efficiency test was performed using nebulized *Bacillus arthrophaeus* spores with particle sizes down to 0.8 µm in comparison to a filtration device with a pore size of 0.8 µm. The biological efficiency test was performed with a mixture of nebulized *Staphylococcus epidermidis* and *Bacillus arthrophaeus* in comparison to a Casella Slit Sampler.

Conclusions. The results for the full range of tested MAS® air samplers are in a high range of more than 70% for both parameters tested.

As an example for adaption to the cleanliness and handling requirements the IsoBag™ allows the use of culture media in isolators without the need of time consuming decontamination steps.

ADAPTATION OF BACTERIA-BIODEGRADERS TO CRUDE OIL

O.Muter[1], A.Eglītis[1], S.L.Borissov[2] , R.Nikolajevs[3], E.Klievēns[3]

[1]Institute of Microbiology & Biotechnology, University of Latvia, Jelgavas Str., 1-534, Riga LV-1004, Latvia.

[2]INTERGEO Umwelttechnologie und abwitschaft GmbH, Robinigstraße 93, A-5020, Zalcburg, Austria.

[3]Intergeo Baltic SIA, Tvaika Str., 64, Riga, LV-1034, Latvia.

Corresponding author: olga.mutere@lu.lv

Key words: Biodegradation; hydrocarbons; nutrient formulation; emulsification; ecotoxicity.

Bioremediation is a promising technology, particularly in dealing with petroleum hydrocarbon contamination. Addition of microbial biomass with a high degradation potential to the contaminated site could notably improve the clean-up process. Efficiency of bioaugmentation on the contaminated site is dependent on many factors. In particular, the specified growth conditions for biomass production resulted in adaptive changes in microbial populations.

The aim of this study was to optimize the cultivation conditions of bacteria-biodegraders for further bioaugmentation of oil-contaminated soil. Experiments were performed with bacterial consortium consisted of *Pseudomonas* spp. and *Stenotrophomonas maltophilia* [1]. Growth kinetics and metabolic activity of bacterial consortium was evaluated by optical density and the number of colony forming units in culture. Besides, enzyme activity, respiration intensity, cell morphology etc., was tested. The OxiTop® device was used for monitoring the respiration and degrading activity of bacteria.

Biodegradation experiments were performed under laboratory conditions with soil contaminated by acid tar, with TPH concentration up to 21200 mg/kg. Soil was sampled at the historically contaminated site where the total amount of contaminated material is more than 30`000 t and which is located in average of 1.5 m depth from ground surface. No culturable microorganisms were detected in soil, which was sampled from three different depths. This fact indicated to the obligate bioaugmentation in order to start the biodegradation process. Addition of bacterial consortium and the specific nutrient formulation to soil resulted in a considerable increase of microbial respiration activity and a significant decrease of the concentration of hydrocarbons during 23 days. Further study on soil clean-up technology is necessary, with emphasis on: i) maintaining the microbial activity during a long period, when easily degradable hydrocarbons are degraded; ii) scaling-up of the process, according to the specific environmental conditions.

Acknowledgements:

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CHEMOTAXONOMIC LIPID ANALYSIS OF NOVEL TAXA OF BACTERIA

A.A.Novikov[1], D.S.Kopitsyn[1], D.A.Petrova[1]

[1]Center for Nanodiagnostics, Department of Physical and Colloid Chemistry, Gubkin University,
65-1 Leninsky prospect, 119991, Moscow, Russia.

Corresponding author: novikov.a@gubkin.ru

Keywords: fatty acids, respiratory quinones, polar lipids, extremophiles, mass spectrometry

Chemotaxonomic analysis is considered as important part of the description of novel taxa [1]. For bacterial strains, this analysis is usually restricted to the lipid analysis: gas chromatography of fatty acids derivatives, high pressure liquid chromatography of respiratory quinones, and thin-layer chromatography of polar lipids. However, traditional analytical methods can lead to the significant flaws due to the extreme variability in bacterial lipid composition.

We have analyzed the cellular fatty acids of novel and reference bacterial strains using gas chromatography with mass-selective detection. Either classical two-step derivatization [2] or direct methanolysis [3] approach was used, and the extracts were analyzed with Thermo Scientific Trace GC Ultra DSQ II system. Identification of fatty acids methyl esters was performed by comparison of their retention times with those of standards (Supelco), calculation of equivalent chain lengths (ECL), and by further comparison of ECL values with either those of fatty acids encountered earlier or calculated by rules proposed by Härtig [4]. Mass spectra of detected fatty acids were compared with database entries [5] and interpreted using guidelines by Christie [6].

Novel strains of bacteria may have very unusual fatty acids as components of their membrane or intracellular lipid bodies. Even nowadays, standard samples cannot cover the whole variety of bacterial fatty acids and their derivatives. These fatty acids can be reliably identified only by approach combining equivalent chain length and mass spectral data.

Acknowledgements: This work was supported by President of Russia (grant MK-4530.2015.4).

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AN ACETYLTRANSFERASE TOXIN INHIBITS TRANSLATION BY NOVEL MECHANISM

D.Jurėnas[1,2], A.Garcia-Pino[1], L.Van Melderen[1]

[1]Département de Biologie Moléculaire, Université Libre de Bruxelles, Rue des prof. Jeener et Brachet 12, Gosselies, Belgium.

[2]Department of Biochemistry and Molecular Biology, Vilnius University Joint Life Sciences Center, Lithuania, Saulėtekio al. 7, Vilnius, Lithuania.

Corresponding author: dukas.ju@gmail.com

Keywords: toxin-antitoxin, acetylation, translation, initiation

Background and objectives: bacterial toxin-antitoxin (TA) systems are small genetic loci coding for a toxic protein and its antidote. These systems are proposed to play an important role in stress response by sending bacteria to dormancy. Toxins of widespread type II TA systems are most commonly mRNAses, with the exception of gyrase inhibitors and several families that have more elaborate enzymatic functions. We demonstrate a novel class of type II TA toxins – AtaT, that possess acetyltransferase domain. Upon expression AtaT severly impairs translation by selectively acetylating its target. We aimed to deconstruct the mechanism of activity of this novel toxin by following *in vivo* and *in vitro* translation.

Methods: by following the transfer of isotope-labeled acetyl group we've detected the target and precise position of acetylation. We have further reconstructed translation *in vitro* to demonstrate the impairment caused by modified target. Finally we have confirmed our model by tracking the ribosome profile changes *in vivo* upon toxin expression.

Conclusions: our study has revealed that AtaT is a novel GNAT-family acetylatransferase and novel translation inhibitor that prevents formation of ribosome initiation complex *in vivo* and *in vitro*.

INVESTIGATION AND CHARACTERIZATION OF TWO CRYPTIC PLASMIDS FROM *Arthrobacter* spp.

R.Stanislauskienė[1], K.Šalkauskienė[1], V.Časaitė[1], M.Bratchikov[2], R.Rutkienė[1], R.Meškienė[1], R.Meškys[1]

[1] Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Vilnius University, Saulėtekio av. 7, LT-10221, Vilnius, Lithuania

[2] Biosta JSC, S. Žukausko 18-117, Vilnius, LT-08234, Lithuania

Corresponding author: ruta.stanislauskiene@bchi.vu.lt

Keywords: *Arthrobacter* sp., plasmid, theta replication

Background and aim: *Arthrobacter* bacteria are able to gain energy from the degradation of various natural or man-made organic compounds present in environment. Recently, these organisms have received considerable attention as they can be used in the detoxification of aromatic, *N*-heterocyclic compounds and pesticides. The enzymes participating in degradation pathways could be used for developing new processes in biotechnology. Unfortunately, the functional screens and expression of enzymes may be inefficient in *Escherichia coli* cells. The main limitations are usually related to recognition of promoters, protein maturation and cofactor requirements. Reliable hybrid shuttle or expression vectors would help to solve this problem. The replicons of small arthrobacterial plasmids are atractive targets for the construction of such hybrid vectors. Therefore, we have investigated and characterized two cryptic plasmids from *Arthrobacter* spp.

Methods: To find the small replicons in *Arthrobacter* spp. bacteria, the isolated total DNA was analyzed using electrophoresis in agarose gel. The promising plasmids were cloned into *E. coli* vectors and sequenced. The sequences were analyzed using program VectorNTI AdvancedTM 9.0 [1]. The alignments were performed with BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The copy number of plasmids in host cells was determined by qPCR. Minimal operons were identified using cloning techniques, and the shuttle vectors were constructed.

Results and conclusions: Plasmid pPRH was 5000 bp in length. It contains six putative *orfs*, two of them encoded replication proteins homologous to theta replication pAL5000 subfamily of ColE2 family. The size of plasmid pVP3 was 6135 bp. Sequence analysis revealed 9 *orfs*, none of them encodes replication proteins. It could be stated that the replication of this plasmid is initiated by the RNA and depends on the host replication proteins. Minimal replicon of plasmid pPRH was used to construct hybrid *Escherichia coli-Arthrobacter* vectors. The plasmid pRMU824, harbouring chloramphenycol resistance genes was created. In addition, the kanamycin or tetracycline resistance gene was inserted into the plasmid pRMU824 to expand the applicability of the vector. Thus, two shuttle vectors pRMU824Km and pRMU824Tc were obtained. All shuttle vectors successfully replicated in *Arthrobacter* and *Rhodococcus* spp. as well as in *E. coli*. The developed vectors were compatible with pART vectors. Hence, they might be used for genetic complimentation studies both in *Arthrobacter* and *Rhodococcus* spp.

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DISCOVERY OF A NOVEL GENE, INVOLVED IN THE CONVERSION OF 2-THIOURACIL INTO URACIL

A.Aučynaitė[1], R.Rutkienė[1], R.Meškys[1], J.Urbonavičius[1,2]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Vilnius University, Vilnius, Lithuania

[2]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania

Corresponding author: Jaunius.Urbonavicius@vgtu.lt

Keywords: modified nucleotides, nucleosides, and heterocyclic bases

Modified nucleotides are present in many RNA species in all Domains of Life. The biosynthetic pathways of such nucleotides are well studied. However, much less is known about the degradation of RNAs and the salvage of modified nucleotides, their respective nucleosides or heterocyclic bases to the metabolism.

Using an *E. coli* uracil auxotrophic strain, we screened the metagenomic libraries for genes, which would allow the conversion of 2-thiouracil to uracil and thereby lead to the growth on a defined synthetic medium. We show that a novel gene encoding previously uncharacterized Domain of Unknown Function (DUF) is responsible for such phenotype. We have purified this recombinant protein and demonstrated that it contains a Fe-S cluster. The substitution of cysteines, which have been predicted to bind such clusters, with alanines abolished the growth phenotype.

We conclude that this domain is required for conversion of 2-thiouracil into uracil *in vivo*.

Acknowledgements:

This work is supported by the Research Council of Lithuania (LMT, MIP-103/2015).

CHARACTERISATION OF SAPROPHYTIC FUNGI ISOLATED FROM TWO ISLANDS IN THE EAST COAST OF PENINSULAR MALAYSIA

A.A.Ngadin[1, 3, 4], N.M.Razali[1], A.Mohammad[2], Y.Andriani[3]

[1]School of Food Science and Technology,

[2]School of Marine Science and Environment,

[3]Institute of Marine Biotechnology,

[4]Institute of Aquatic Tropical, School Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia

Corresponding author: andrew@umt.edu.my

Keywords: Saprophytic fungi, decolourisation, *Schizophyllum commune*, antioxidant, antibacterial

The islands of east coast of Peninsular Malaysia has been explored on their flora and fauna but have not been evaluated or reported its fungal status. In this study, two efforts to identify and evaluate saprophytic fungi from two islands of Bidong and Kapas were carried out.

A total of 196 macrofungi specimens were collected and plotted using GPS for the distribution analysis. There are 81 percent of saprophytes specimens were identified from both islands. Four species of saprophytes cultures were screened for decolourisation assay and the only *Schizophyllum commune* was obtained and was proved highly potential due to its ability to decolourise of Congo red, Sudan III and Trypan blue within 10 days of incubation at 28°C. The phytochemical analysis showed the presence of proteins, glycosides, steroids, terpenoids and alkaloids as chemical constituents in the extract of *S. commune*. Results showed that extracts without treatment of Sudan III revealed higher free radical scavenging activity than the extracts with treatment of Sudan III at all concentrations. From the antibacterial tests, ethyl acetate extract without treatment at 40 mg/ml showed partially active against all the tested bacteria. Minimum inhibition concentration (MIC) of this extract was obtained at 0.1506 mg/mL against *Pseudomonas aeruginosa* and *Escherichia coli*.

The results of this study suggest that *S. commune* not only has promising potential in dyes removal from textile industry, but may lead to the isolation of novel natural products for use in medical, agriculture and industry.

SESSION II: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY

Presenter order	**Speaker**	**Presentation**	**Country**
Invited Speaker	Thomas Alter	Microbial behaviour in the food chain	Germany
Speaker I	Hermann Heipieper	Bioremediation of organic pollutants in planted systems: the rhizospheric effect	Germany
Speaker II	Tiina Alamäe	Novel prebiotic fructooligosaccharides prepared by enzymatic synthesis and degradation of levan	Estonia
Speaker III	Egils Stalidzans	Impact of number of adjustable parameters on the optimisation potential of kinetic model	Latvia
Speaker IV	Gintarė Zakarienė	Prevention of cross-contamination between *Campylobacter jejuni* contaminated poultry products and kitchen environment	Lithuania
Speaker V	Alexander Rapoport	Anhydrobiosis in yeasts: from live nature to modern biotechnologies	Latvia
Speaker VI	Kärt Kontram	Flour origin determines the evolution of bacterial communities in laboratory rye sourdoughs	Estonia
Speaker VII	Ene Viiard	Bacterial communities of fresh and fermented birch sap	Estonia

MICROBIAL BEHAVIOR IN THE FOOD CHAIN

T.Alter

Institute of Food Safety and Food Hygiene, Free University Berlin, Germany

Corresponding author: thomas.alter@fu-berlin.de

Keywords: food microbiology, stress response, stress adaptation

Foodborne bacterial pathogens encounter different stressors during food production, processing, storage and preparation. These stressors include physical treatments (e. g. heat treatment, pressure application, osmotic shock), chemical treatments such as acids or detergents, and biological stresses.

It is becoming increasingly evident that microorganisms can sense changes in their environment, and can respond and adapt to such stressors by various strategies. Such stress adaptation can aid survival of microorganisms in the food chain. In addition, stress adaptation may alter virulence properties of pathogenic microorganism and can contribute to *in vivo* survival.

New innovative food preservation techniques – focussing on minimally processed food - (e. g. mild heat treatment, modified atmosphere packaging, high hydrostatic pressure, pulsed electric fields, or the application of natural antimicrobials) are increasingly replacing traditional ways to control microbial contamination. Elucidating the molecular mechanisms underlying the stress response and stress adaptation in foodborne pathogens is essential to optimize food processing parameters and to develop efficient control measures in the food chain.

BIOREMEDIATION OF ORGANIC POLLUTANTS IN PLANTED SYSTEMS. THE RHIZOSPHERIC EFFECT

H.J.Heipieper[1], V.Lünsmann[2], U.Kappelmeyer[1], J.A.Müller[1], N.Jehmlich[2]

[1] Department of Environmental Biotechnology, Helmholtz Centre for Environmental Research-UFZ, Permoserstr. 15, 04318 Leipzig, Germany (hermann.heipieper@ufz.de)

[2] Department of Proteomics, Helmholtz-Centre for Environmental Research - UFZ, Permoserstr. 15, 04318 Leipzig, Germany

Corresponding author: hermann.heipieper@ufz.de

Keywords: constructed wetlands; degradation of organic compounds; community structure, oxygen; root exudates

Constructed wetlands are well established for the treatment of wastewater and bioremediation of gasoline contaminated aquifers. The removal of organic compounds is carried out by microorganisms inhabiting the rhizosphere. Organic plant exudates and oxygen released by the roots stimulate the microbial degradation activity. These processes are called the rhizospheric effect. Despite the good performance of constructed wetlands, the understanding of the processes in the rhizosphere is still limited. In order to gain detailed knowledge about the process of organic pollutant degradation in the rhizosphere, we applied a model system, Planted Fixed-bed Reactor (PFR) which was running with toluene as sole external carbon source continuously for 6 years. In order to characterise the bacterial community and catabolic activities within this PFR, we applied DNA-based methods in combination with stable isotope fractionation and protein stable isotope probing (protein-SIP) techniques using ^{13}C-toluene. In addition, abiotic parameters such as redox potential and oxygen concentration were monitored continuously. With these top notch approaches, the predominant pathway for toluene degradation as well as the bacterial key players carrying out this process could be identified.

The rhizospheric bacterial community was complex and numerically dominated by Rhizobiales and Burkholderiales, which contributed each about 20% to total taxon abundances. Notably, metaproteome and protein-SIP data revealed that *Burkholderiaceae* were main degraders of toluene in the wetland system. Thus, while the system was mostly hypoxic, toluene was preferentially degraded by aerobic processes. The initial degradation steps were apparently catalysed by *para* ring-hydroxylating monooxygenases which are known to be predominant under oxygen limiting conditions. A deeper insight into the molecular processes of toluene degradation was achieved by combining functional and taxonomic information. Our study is a promising proof of concept for future investigations of rhizospheric ecosystems degrading organic pollutants.

Acknowledgements:

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NOVEL PREBIOTIC FRUCTOOLIGOSACCHARIDES PREPARED BY ENZYMATIC SYNTHESIS AND DEGRADATION OF LEVAN

K.Mardo[1], T.Visnapuu[1], A.Aasamets[1], K.Kõrgesaar[1], H.Vija[2], T.Alamäe[1]

[1]Department of Genetics, Institute of Molecular and Cell Biology, University of Tartu, Riia 23, 51010 Tartu, Estonia.

[2]National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, 12618 Tallinn, Estonia.

Corresponding author: talamae@ebc.ee

Keywords: prebiotics, levansucrase, levanase

Background and objectives. Gut microbiota has many important functions for the host. It affects food digestion, vitamin synthesis, body weight, status of the immune system and even mood. Gut microbiota can be shifted by prebiotics. Plant-derived inulin-type fructooligosaccharides (FOS) are common and most used prebiotics in the world. We introduce here enzymatic synthesis of novel fructose-based candidate prebiotics.

Methods. The levansucrase and levanase enzymes applied in this work originate from bacteria. They were synthesized heterologously in *E. coli* as His-tagged proteins and purified. Levansucrase Lsc3 [1] was used to synthesize levan from sucrose. Levanase was applied to produce FOS from levan. The amount and spectrum of FOS was analysed using HPLC. Stability of levan and FOS to gastric acid and moist heat sterilisation was also assayed.

Conclusions. Levansucrase Lsc3 of *Pseudomonas syringae* pv tomato used in this work is a highly active and stable catalyst suiting perfectly for the synthesis of levan. Levan synthesis and purification was further optimised to save enzyme and materials. Levanase was studied for substrate specificity and appropriate reaction conditions. Levanase-treatment of Lsc3-derived levan and reference levans yielded mixture of FOS of varied chain length. Both, levan and levan-derived FOS resisted 2-4 h treatment in hydrochloric acid (pH 2.0) and heat sterilization in the autoclave. Therefore, these preparations are ready to be tested as novel prebiotics.

Acknowledgments:

The work was financed by ERC grant GLTMR1050P.

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IMPACT OF NUMBER OF ADJUSTABLE PARAMETERS ON THE OPTIMISATION POTENTIAL OF KINETIC MODEL

E.Stalidzans[1], A.Pentjuss[1], A.Elsts[1]

[1]Biosystems Group, Department of Computer Systems, Latvia University of Agriculture, Liela iela 2, LV-3001, Jelgava, Latvia.

Corresponding author: egils.stalidzans@gmail.com

Keywords: kinetic model, optimisation, number of adjustable parameters

Applying the approach of systems biology and synthetic biology, new designs of biotechnologically applicable microorganisms are developed in an iterative cycle of computer experiments and biological experiments. Analysis of all potential adjustable parameter combinations at computational level by optimisation of kinetic models to find a small and efficient adjustable parameter set is computationally demanding due to the combinatorial explosion of the number of parameter combinations. Therefore, experience-based subsets designs are often chosen without extensive analysis, probably leaving some promising alterations of microorganism unrevealed.

The total optimisation potential (TOP) approach [1] was used to analyse the impact of the number of adjustable parameters in a set by steady state optimisation of kinetic models using COPASI software [2]. Metabolic yield related objective function was applied and all adjustable parameters are related to the overexpression of enzymes.

The TOP value (value of objective function optimising all adjustable parameters at once) serves as an efficiency indicator of particular number of adjustable parameters in combination. The analysis of optimisation potential per number of adjustable parameters in combination suggests the reasonable number of adjustable parameters in a design as well as the fraction of unused TOP of particular design to assess its competitiveness.

Acknowledgements:

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PREVENTION OF CROSS-CONTAMINATION BETWEEN *C. jejuni* CONTAMINATED POULTRY PRODUCTS AND KITCHEN ENVIRONMENT

G.Zakariene[1], Š.Meškinis[2], S.Tamulevičius[2], T.Alter[3], M.Malakauskas[1]

[1]Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Tilzes st. 18, LT-47181, Kaunas, Lithuania.

[2]Institute of Materials Science, Kaunas University of Technology, Barsausko st. 59, LT-51423, Kaunas, Lithuania

[3]Department of Veterinary medicine, Institute of Food Safety and Hygiene, Königsweg st. 69, 14163, Berlin, Germany

Corresponding author: gintare.zakariene@lsmuni.lt

Keywords: Campylobacter, DLC nanocomposites, real time PCR

Campylobacter jejuni – the most common cause of bacterial diarrheal illness in EU with 236,851 reported human campylobacteriosis cases in 2014. Cross-contamination between *C. jejuni* contaminated poultry products and kitchen environment is one of the main causes of campylobacteriosis infection. *C. jejuni* numbers on kitchen surfaces may be reduced by various antimicrobial techniques, including silver nanoparticles. Therefore, the aim of this research was to test diamond like carbon (DLC) based silver nanocomposites for the antimicrobial activity against *C. jejuni* on silicon coupons.

C. jejuni numbers were counted by two different methods: culture-based enumeration on mCCDA plates and the combination of propidium monoazide (PMA) with quantitative real-time PCR (qPCR). Culture-based method showed the numbers of culturable *C. jejuni*, while PMA-qPCR helped to distinguish viable bacteria cells including viable-but-not-culturable cell form from dead bacteria cells.

Culture-based enumeration revealed that *C. jejuni* numbers were reduced by 3.6 $\log_{10}$CFU/ml after 15 min and 4.87 $\log_{10}$CFU/ml after 30 minutes on DLC based silver nanocomposite coated silicon coupons in comparison to control sample ($P \leq 0.05$). However, PMA-qPCR showed that viable *C. jejuni* numbers were underestimated when counted by culture-based method. Nonetheless, *C. jejuni* numbers were similar to initial bacteria count in inoculation tube, when counted by PMA-qPCR, indicating that tested nanoparticles had a bacteriostatic effect on *C. jejuni* during the first 30 min of experiment. No viable bacteria cells were found after 1 hour treatment neither by culture-based method, nor by PMA-qPCR real-time.

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ANHYDROBIOSIS IN YEASTS: FROM LIVE NATURE TO MODERN BIOTECHNOLOGIES

A.Rapoport, D.Kulikova, L.Rozenfelde

Laboratory of Cell Biology, Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas Str., 1, LV – 1004 Riga, Latvia

Corresponding author: rapoport@mail.eunet.lv

Keywords: yeasts, anhydrobiosis, biotechnology

The discovery of anhydrobiosis made by great Anthony van Leeuwenhoek in 1701 still is an unsolved problem. This state of live organisms in which their metabolism is temporarily reversibly suspended as the result of their strong dehydration is interesting as unique nature phenomenon and has important applications in various areas of humans activities. The most traditional applications of anhydrobiosis in biotechnologies are linked with the use of active dry yeasts for the production of bread, beer, wine and ethanol. Comprehensive studies of mechanisms of anhydrobiosis showed the ways for the improvement of quality of such yeasts preparations by increase of cells‘ resistance to dehydration and activation of intracellular protective reactions. The results of these studies gave possibility to propose also a number of non-conventional applications of dehydrated yeasts. For example, on the basis of new information on the changes of the state of cell wall there were proposed: efficient application of dry yeasts in biofilters, innovative non-expensive method for the production of very stable in biotechnological processes immobilized yeast preparations, new approach for the improvement of quality of yeast β-glucans used as active immunomodulators [1,2]. Yeast cells‘ response to dehydration is perfect indicator of physiological state of eukaryotic organisms and can be efficiently used as express-test of the influence of various substances including new pharmaceutical compounds upon the cells [3]. Other non-conventional applications of dehydrated yeasts proposed during last 2 decades are linked with some processes in winemaking, biotransformations, drug delivery systems, biocontrol, animal feed etc [1].

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FLOUR ORIGIN DETERMINES THE EVOLUTION OF BACTERIAL COMMUNITIES IN LABORATORY RYE SOURDOUGHS

K.Kontram[1, 2], M.Tõnutare[1, 2], E.Viiard[1]

[1]Center of Food and Fermentation Technologies, Akadeemia tee 15A, 12618, Tallinn, Estonia.

[2]Department of Food Processing, Tallinn University of Technology, Ehitajate tee 5, Tallinn, Estonia.

Corresponding author: ene@tftak.eu

Keywords: Metagenomic sequencing, sourdough, lactic acid bacteria

Microbial community of sourdoughs is determined by the composition of rye flour and environmental factors of the bakery environment. Non-sterile raw materials are used and fermentation is commonly carried out in open tanks, which allows microorganisms from the bakery air, equipment and employees to enter the sourdough [1]. In laboratory sourdough renewal experiments the environmental contamination is eliminated – fermentation is performed using sterilized equipment and the risk of human contamination is taken to the minimum. In this case microbial composition of the flour determines the outcome of sourdough fermentation. The objective of this study was to determine the effect of flour origin on the evolution of bacterial communities in rye sourdough and to obtain lactic acid bacteria strains with rapid acidifying ability essential for small bakeries.

Laboratory sourdoughs were prepared from three commercially available rye flours. The spontaneously started sourdoughs were renewed daily for two weeks following the cycle of 6 h fermentation and 18 h refrigeration. This is a common practice in many small scale bakeries. pH and total titratable acidity were measured after each fermentation step to monitor the maturation of sourdoughs. Metagenomic sequencing of 16S rRNA gene amplicons was performed to characterize the evolution of bacterial communities.

Sourdoughs made from three rye flours of different origin evolved through a previously described three-stage evolution [2], however the composition of bacterial communities varied among the sourdoughs in each stage. The mature sourdoughs were dominated by either i) *Lactobacillus brevis* and *Lactobacillus plantarum*, ii) *Pediococcus pentosaceus*, or iii) *Lactobacillus pontis* and *Lactobacillus sanfranciscensis*. All three sourdoughs reached maturity (pH below 4.3) by day three. As a result of this work several fast acidifying lactic acid bacteria strains were obtained and can be further studied in industrial sourdough renewal experiments in order to evaluate their competitiveness in the bakery environment.

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BACTERIAL COMMUNITIES OF FRESH AND FERMENTED BIRCH SAP

E.Viiard [1], R.Kuldjärv [1, 2]

[1]Center of Food and Fermentation Technologies, Akadeemia tee 15A, 12618, Tallinn, Estonia.

[2]Department of Food Processing, Tallinn University of Technology, Ehitajate tee 5, Tallinn, Estonia.

Corresponding author: ene@tftak.eu

Keywords: Metagenomic sequencing, birch sap, lactic acid bacteria

Birch sap or birch water is the liquid directly tapped from birch trees (*Betula pendula*). It is a popular drink in many Eastern European countries, especially in the Baltic region. Birch sap is collected only during a short period during spring, when the sap moves intensively. Fresh birch sap it is a clear water-like liquid, that has a slightly sweet and astringent taste and a characteristic fresh and woody aroma. The sap starts fermenting 2-3 days after collecting from trees, since it contains several fermentable sugars (glucose and fructose). Birch sap can be consumed both fresh and fermented. It is believed to have health promoting benefits. While the chemical composition of birch sap has been studied [1], the bacterial communities of both fresh sap and the species responsible for spontaneous fermentation have not been identified. The aim of the current study was to evaluate the bacterial composition of fresh and naturally fermented birch sap.

Eleven birch sap samples were subjected to metagenomic sequencing of 16S rRNA gene amplicons. In addition to freshly collected samples and traditional naturally fermented sap drinks, two commercially available birch sap drinks were analyzed.

Fresh birch sap samples were dominated by species belonging to the genera *Pseudomonas*, *Rahnella*, *Pelosnius*, and *Flavobacterium*. No human pathogens were detected in any of the samples. The fermented birch sap drinks contained several *Lactobacillus*, *Leuconostoc*, *Acetobacter*, *Gluconacetobacter*, or *Lactococcus* species, depending on added flavour and aroma components. Information regarding the bacterial composition of fermented birch sap drinks with desirable flavour profile allows producers to understand the development of these characteristics and to select starter strains for controlled production of high quality fermented birch sap products.

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SESSION III:
GENETICS, BIOCHEMISTRY AND PHYSIOLOGY OF MICROORGANISMS AND BACTERIOPHAGES

Presenter order	**Speaker**	**Presentation**	**Country**
Invited Speaker	Dennis Bamford	More order to the viral universe	Finland
Speaker I	Anne-Grit Klees	Culture media for used in environmental monitoring	Germany
Speaker II	Maia Kivisaar	Recombinational processes and evolution of *Pseudomonas putida* under stress	Estonia
Speaker III	Riho Teras	Fis affects biofilm formation of *Pseudomonas putida* through regulation of lapA and lapF expression	Estonia
Speaker IV	Hermann Heipieper	Outer membrane vesicle secretion in Gram-negative bacteria: from cell envelope stress adaptation to biofilm formation	Germany
Speaker V	Jekaterina Martynova	Effects of acetate on *Kluyveromyces marxianus* growth and metabolism	Latvia
Speaker VI	Diana Kulikova	The role of aquaporins during *Saccharomyces cerevisiae* dehydration-rehydration	Latvia
Speaker VII	Agnese Kokina	General stress resistance phenotype is not cell cycle dependent in budding yeast *Saccharomyces cerevisiae*	Latvia

CULTURE MEDIA FOR USE IN ENVIRONMENTAL MONITORING

A.-G.Klees

[1]Merck Life Science GmbH, Lilienthalstrasse 16, D-69214 Eppelheim, Germany

Corresponding author: anne-grit.klees@merckgroup.com

Keywords: neutralization of disinfectants, VHP, antibiotics

Background and objectives. Culture media for environmental monitoring in controlled clean room production areas should be able to collect a broad spectrum of typical environmental microorganisms. The culture media are usually supplemented with neutralizers to suppress antimicrobial activity present at the sampling point. Typical antimicrobial residuals may be originated from disinfectants, antibiotics or decontamination with VHP.

In addition air sampling causes dehydration stress on culture media. Tests were developed to check the suitability of culture media to provide good growth promotive properties in all these environments.

Methods. Growth promotion tests (GPT) are performed by the spread plate method using fresh cultures of spore suspensions of selected microorganisms and the colony forming units are compared to a suitable reference medium. For worst case neutralization efficiency tests GPT are performed after antimicrobial agents have been applied on the test plates and are compared to growth on untreated plates. A more practical oriented approach is the surface sampling on disinfected surfaces followed by GPT.

GPT are performed on dehydrated plates after passive or active air sampling.

Conclusions. The tested ICR media for surface and air monitoring show good growth promoting properties for a wide variety of microorganisms. TSA - ICR culture media supplemented with the neutralizers lecithin, polysorbate 80, histidine and sodium thiosulfate demonstrated sufficient neutralization efficiency for a wide number of disinfectants such as alcohols, phenols, per acetic acid and hydrogen peroxide. Whereas the inactivation of polyhexamethylene biguanides and highly concentrated quaternary ammonium compounds require a specific neutralizing mixture, so-called Neutralizer A.

Furthermore the tested TSA – ICR media are suitable for air sampling with regards to their good growth promoting properties after dehydration in passive or active air monitoring.

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RECOMBINATIONAL PROCESSES AND EVOLUTION OF *Pseudomonas putida* UNDER STRESS

M.Kivisaar

Institute of Molecular and Cell Biology, University of Tartu, 23 Riia Street 51010 Tartu, Estonia

Corresponding author: maia.kivisaar@ut.ee

Keywords: mutagenesis and recombination under stress; evolution of bacteria; pseudomonads

In natural environments the growth of bacteria is usually restricted due to a shortage of nutrients. Still genetic changes in bacterial populations can occur fast through the acquisition and incorporation of foreign DNA or through mutation. Homologous recombination has a major impact in bacterial evolution because of horizontal transfer of genes. The aim of this presentation is to give an overview of our recent studies in this field by focusing on factors affecting homologous recombination under carbon starvation [1, 2] and impact of nonhomologous end-joining (NHEJ) in the evolution of soil bacterium *Pseudomonas putida* [3].

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FIS AFFECTS BIOFILM FORMATION OF *Pseudomonas putida* THROUGH REGULATION OF *lapA* AND *lapF* EXPRESSION

H.Ainelo, A.Lahesaare, A.Teppo, R.Teras

Institute of Molecular and Cell Biology, Tartu University, Estonia

Corresponding author: riho.teras@ut.ee

The biofilm matrix of the rhizospheric bacterium *Pseudomonas putida* consists mainly of a proteinaceous component. Two largest *P. putida* proteins LapA and LapF play an important, if not the key role in *P. putida*'s biofilm development. They prevail in different developmental stages of the biofilm matrix. LapA is abundant in the initial stage of biofilm formation whereas LapF is found in the mature biofilm.

Bacteria form biofilm as a response to a number of environmental signals that are mediated by global transcription regulators and alarmones. Although the transcriptional regulation of adhesins is not exhaustively studied, some factors that can be involved in their regulation have been described. For example, RpoS, the major stress response sigma factor activates *lapF* expression, and FleQ activates *lapA* transcription.

The overexpression of *fis* increases the amount of LapA and decreases the amount of LapF in *P. putida*. Our results suggest that profusion of LapA in the Fis-overexpressed cells causes enhanced biofilm formation in mature stages of *P. putida* biofilm.

We have shown that Fis represses the transcription of *lapF* by binding to the promoter region of *lapF*. The mapped 5' end of the *lapF* mRNA and DNase I footprint analyses localized the promoter and Fis-binding site Fis-F2 to the same region. Monitoring the *lapF* promoter activity by a β-galactosidase assay revealed that Fis overexpression causes a 4-fold decrease in the transcriptional activity. Furthermore, mutations that diminished Fis binding to the Fis-F2 site abolished the repression of the *lapF* promoter. Thus, these data suggest that Fis is involved in the biofilm regulation via repression of LapF expression.

In conclusion, we have described the involvement of the global regulator Fis in biofilm formation. Higher amount of Fis increases the quantity of LapA in *P. putida*, resulting in enhanced biofilm formation. At the same time, Fis strongly reduces the amount of LapF through the repression of *lapF* transcription.

OUTER MEMBRANE VESICLE SECRETION IN GRAM-NEGATIVE BACTERIA: FROM CELL ENVELOPE STRESS ADAPTATION TO BIOFILM FORMATION

H.J.Heipieper[1]*, F.Klein[1], C.Eberlein[1], T.Baumgarten[2]

[1]Department of Environmental Biotechnology, Helmholtz Centre for Environmental Research-UFZ, Permoserstr. 15, 04318 Leipzig, Germany

[2]Center for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden

Corresponding author: hermann.heipieper@ufz.de

Keywords: Gram-negative bacteria, envelope stress adaptation, membrane vesicles, biofilms

Modifications of the cell envelope are one important mechanism of bacteria to deal with environmental stress as well as to cope with toxic hydrocarbons [5,6]. Especially the bacterial cell envelope as complex interface to the environment is very sensitive to stress. Therefore, several mechanisms have been evolved with which bacteria respond to the presence of different environmental stresses. Among these mechanisms, the release of outer membrane vesicles (OMV) in Gram-negative bacteria has gained research interest especially because of its involvement in pathogenic processes such as that of *Pseudomonas aeruginosa* biofilm formation in cystic fibrosis lungs. The role of MV formation as an adaptive response of *Pseudomonas putida* to several stresses and its correlation to biofilm formation was investigated. In the presence of long chain alcohols, high NaCl concentrations, EDTA, and after heat shock cells of this strain release MV very rapidly [4]. The formed MV show similar size and charge properties as well as comparable composition in proteins and fatty acids [3]. Strikingly, the release of MV leads to a dramatic increase in cell surface hydrophobicity as well as to a higher tendency to form biofilms [3]. Thus, cell surface stress in *P. putida* leads to an increased cell surface hydrophobicity by the secretion of MV resulting in elevated biofilm formation [1,3]. This will be discussed as a global mechanism present in all Gram-negative bacteria [1,2].

Acknowledgements:

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EFFECTS OF ACETATE ON *Kluyveromyces marxianus* GROWTH AND METABOLISM

J.Martynova, A.Kokina, J.Liepins, K.Kovtuna, A.Vigants

Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas street 1, LV-1004, Riga, Latvia

Corresponding author: jekaterina.lukjanenko@lu.lv

Keywords: *K.marxianus*, acetic acid, weak acid stress

Background and objectives. *Kluyveromyces marxianus* is non-conventional GRAS that can efficiently metabolize wide range of substrates including lactose. Due to this advantage this yeast can be used for some value biotechnological products synthesis. Acetate is a weak acid and one of the fermentation by-products with pKa 4.76. During fermentation medium pH value decreases and acetate undissociated forms concentration increases. In undissociated state acetate immediately diffuse into the cell cytosole and leave up negative effect on yeast metabolism and cell growth. Due to metabolic and physiologic diversity among the yeast species, the mechanism of cell stress adaptation in response to acetic acid is also different. This mechanism is well studied in model organism *S.cerevisiae*, but there is a little information about *K.marxianus*.

Methods. Yeast cells were cultivated in flasks, bioreactors and TECAN M200Pro 96 well reader. The yeast growth was monitored spectrophotometrically by the OD_{600}. Acetate tolerance plating on YPD and solid semisynthetic medium supplemented with acetic acid. The lactose, ethanol, and acetate concentrations were determined by HPLC.

Conclusions. Inhibition of five *K.marxianus* strains biomass growth by acetate at different pH on glucose, galactose and lactose were tested. Our results showed that an increased concentration of acetate slowed down yeast growth depending on medium pH and carbon source. During of DSM5422 cultivation in bioreactors at different pH values in all cases fermentation profiles were similar. But in the presence of sodium acetate delays in growth and metabolism were observed. Examination of the yeast cell population structure showed that populations are heterogeneous regarding tolerance to acetate. Finally, we investigated the influence of undissociated acetate on the respiration rate of DSM5422 cells from different growth phases after a 4-h starvation period. Combined effects of acetate concentration and growth phase on respiration rate were demonstrated.

THE ROLE OF AQUAPORINS DURING *Saccharomyces cerevisiae* DEHYDRATION-REHYDRATION

D.Kulikova[1], A.Liepina[1], G.Soveral[2], A.Rapoport[1]

[1]Laboratory of Cell Biology, Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas street 1 - 537, LV-1004 Riga, Latvia.

[2]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal.

Corresponding author: kulikovadiana@inbox.lv

Keywords: aquaporins, *Saccharomyces cerevisiae*, dehydration-rehydration

The discovery of aquaporins 20 years ago has changed the knowledge on water and solute movements in cells and organisms. *S. cerevisiae* possesses two paralogous genes, *AQY1* and *AQY*2, which encode orthodox aquaporins. It was shown that yeast aquaporins play important roles in establishment of freeze tolerance, during spore formation as well as in determination of cell surface properties for substrate adhesion and colony formation (1, 2, 3). At the same time nothing was known till the moment about their role at dehydration-rehydration processes and this topic was studied in the current research. The used strains were: *Saccharomyces cerevisiae* 10560-6B/pYX012 (*KanMX*) (ANT29) (the parental strain), *S. cerevisiae* 10560-6B/pYX012 (*KanMX AQY1-1*) (ANT27) (the strain overexpressing *AQY1*) and *S. cerevisiae* 10560-6B/pYX012 (*aqy1Δ*::*kanMX4 aqy2*::*HIS3)* (the strain with deletion of AQY1 and AQY2) (YSH 1172). Yeast cells from the stationary growth phase were taken for dehydration-rehydration treatments. The observed parameters were: dynamics of cells' dehydration, cells' viability, their remaining water content and the changes of plasma membrane permeability after dehydration-rehydration. The differences in the rates of water losses by yeast cells during dehydration of strains with double deletions of aquaporins genes and with their overexpression were revealed. Deletion of *AQY1* and *AQY*2 genes diminished the resistance of yeast cells against dehydration-rehydration. These changes correlated also with increase of plasma membrane permeability. All these data confirm our previous results on the importance of the state of various membrane proteins for the maintenance of viability of dehydrated yeast cells.

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GENERAL STRESS RESISTANCE PHENOTYPE IS NOT CELL CYCLE DEPENDENT IN BUDDING YEAST *Saccharomyces cerevisiae*

A.Kokina[1], I.Vamža[1], D.Lubenets[2], J.Liepins[1]

[1]Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas 1, LV-1004, Riga, Latvia

[2]Institute of Cell and Molecular Biology, University of Tartu, Riia 23, 51010, Tartu, Estonia.

Corresponding author: agnese.kokina@lu.lv

Background and objectives. Adenine auxotrophic strains are typical workhorses used in budding yeast, *Saccharomyces cerevisiae* based laboratory research. If non-defined media is used, adenine might get depleted and auxotrophic starvation set in. Our previous results show, that adenine depletion significantly increase yeast cell size, desiccation tolerance and decrease chronological life span (Kokina et al., 2014).

Here we demonstrate more phenotypic effects elicited by purine starvation (either starvation for adenine and guanine or adenine alone). The auxotrophic starvation impact on impact cell cycle, various stress (heat, weak acid, oxidative) tolerance were assessed.

Methods. *S. cerevisiae* cultivation and media shift experiments was done in synthetic broth [2] with appropriate auxotrophic supplements [3]. Yeast growth was monitored by Beckman-Coulter Z2 Particle Counter, cell cycle was determined by BD FACSAria instrument. Sublethal stress tolerance were determined by CFU counting (spot tests).

Conclusions. Purine starvation effectively transfer cells to G1 phase, in fact, adenine starvation alone starts cell cycle arrest in G1. In contrast to widely accepted idea of G1 arrest as sufficient prerequisite to attain general stress resistance, we demonstrate, that depending on carbon source and auxotrophic starvation, yeast stress resistance varies depending if cells are starved for adenine specifically or all purines (adenine and guanine). Adenine or purine starvation affects yeast cells differently and point to different involvement of PKA and G-protein coupled signalisation on stress resistance. Results hint to mechanisms how stress is perceived in the cells and gives insights in role of auxotrophic starvation in general and cross stress resistance.

Acknowledgements:

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SESSION IV: OMIC APPROACHES AND BIOINFORMATICS IN MICROBIOLOGY

Presenter order	**Speaker**	**Presentation**	**Country**
Invited Speaker	Jean Armengaud	Amazing perspectives of microbial proteogenomics and metaproteomics	France
Speaker I	Thomas Kuri	Standardizing microbiomics – removing bias in collection, purification and analyses	Germany
Speaker II	Mikk Espenberg	Metagenomic analysis of nitrogen cycling potential in natural and drained tropical peatlands of French Guiana	Estonia
Speaker III	Denis Kainov	Multi-omics studies towards novel modulators of *Influenza A* virus-host interaction	Finland
Speaker IV	Rita Demidenko	Genetic analysis solutions in microbiology and infectious disease research	Lithuania
Speaker V	Lidija Truncaitė	Genomic comparison of *Felixo1virus* bacteriophages	Lithuania
Speaker VI	Eugenijus Šimoliūnas	Investigation of tail-forming proteins of *Escherichia* phage vB_EcoM_NBD2	Lithuania

AMAZING PERSPECTIVES OF MICROBIAL PROTEOGENOMICS AND METAPROTEOMICS

J.Armengaud

CEA, Laboratory "Innovative technologies for Detection and Diagnostics", France

Next-generation proteomics based on the use of high-throughput and high resolution tandem mass spectrometers opens a new era where the whole proteome content of any microorganism can be quickly assessed. Nucleic acid sequencing information and protein quantitative data can be incorporated at any stage for improving the global picture of the molecular players of the microorganism of interest. Proteogenomics strategies help to reveal unannotated genes, precise their structures, the post-translational modifications of proteins and their intimate regulations. Proteogenomics is also helpful when working with clinical or environmental novel isolates. The use of draft genomes and novel database search strategies such as those used in pan-proteomics will be illustrated. These approaches have outstanding perspectives as soon as complex mixtures of microorganisms have to be analyzed. Metaproteomics is about to move up to a new dimension with the help of improved mass analyzers and new bioinformatics tools.

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STANDARDIZING MICROBIOMICS – REMOVING BIAS IN COLLECTION, PURIFICATION AND ANALYSES

T.Kuri[1], R.Kemp[2], S.Tang[2], E.Becket[2], L.Jia[2], M.Van Eden[2], S.Forman[2], E.Kircher[2], J.Sherman[2], L.Basillio[2], M.Thai[2], S.Wong[2], and M.Mager[2]

[1]Zymo Research Europe GmbH, Mülhauserstr.9, 79110 Freiburg, Germany

[2]Zymo Research Corp., Irvine, CA, USA

Corresponding author: tkuri@zymoresearch.de

The rapid growth of Microbiomics has increased the demand for standard methods to improve the reproducibility and quality of the data being generated. Therefore, there is a need for standard reference materials for the development, evaluation, calibration, and validation of complex microbiomics workflows encompassing sample collection, sample preparation, and analyses.

Standardization of the following methods would greatly improve the quality of data generated: (1) sample collection tools that can reliably provide a molecular snapshot at the time of collection by stabilizing the nucleic acids at ambient temperature and rendering the sample noninfectious for safe transport; (2) purification methodologies that take into consideration the biases associated with differential lysis efficiency of the organism being processed (e.g. gram-negative/positive bacteria, fungus, viruses, and associated spores); (3) analytical pipelines that reduce bias due to library preparation methods, PCR, and bioinformatics (e.g. GC content, log difference organism abundances, detection limit, and ability to distinguish closely related organisms).

To address these fundamental challenges, the scientists at Zymo Research have created reference materials for the development of the most accurate and unbiased workflows from sample collection to analyses.

METAGENOMIC ANALYSIS OF NITROGEN CYCLING POTENTIAL IN NATURAL AND DRAINED TROPICAL PEATLANDS OF FRENCH GUIANA

M.Espenberg[1], J.Truu[1], M.Truu[1], K.Kasak[1], T.Ligi[1], K.Oopkaup[1], J.Järveoja[1], M.Maddison[1], Ü.Mander[1]

[1] Department of Geography, Institute of Ecology and Earth Sciences, University of Tartu, 46 Vanemuise Street, 51014 Tartu, Estonia

Corresponding author: mikk.espenberg@ut.ee

Keywords: tropical peatland; nitrogen cycling potential; shotgun metagenomics; qPCR

Anthropogenic actions are transforming biogeochemical cycles in the biosphere substantially. It has long been known that drainage of peatlands result in a loss of carbon stock but a comprehensive study of the nitrogen cycling potential in the tropical peatland is lacking. This study examined physiochemical conditions and prokaryotic community structure (the bacterial and archaeal 16S rRNA genes and key functional genes involved in nitrogen cycling (*nirS*, *nirK*, *nosZI*, *nosZII*, bacterial and archaeal *amoA*, *nifH*, *nrfA*, ANAMMOX bacteria specific 16S rRNA genes)), and evaluated the effect of drainage on these factors, in the 10 cm thick residual peat layer of tropical peatland located in French Guiana.

The sampling was carried out in October 2013 at two sites (undisturbed and drainage influenced) of the northern part of French Guiana. Quantitative PCR was applied to evaluate the community sizes by quantifying the abundance of different genes. Edge principal component analysis was used to detect important differences between natural and drained metagenomic samples that contain closely related taxa.

Absolute abundance of the bacterial 16S rRNA genes and *nirK*, *nosZI*, *nosZII*, *nifH*, *nrfA* functional genes was statistically significantly higher in undisturbed than in drained tropical peatlands, however, the effect was opposite in case of abundance of archaeal 16S rRNA genes, *nirS* and archaeal *amoA* genes. *nrfA* genes and bacterial *amoA* genes were only identified in natural and drained tropical peatlands, respectively. Proportion of *nosZI* and *nifH* genes in the bacterial community was statistically significantly higher and proportion of *nirS* and *nirK* genes in the bacterial community was statistically significantly lower in natural tropical peatland compared to drained conditions. In cases of *nirS*, *nirK*, *nosZ*, archaeal *amoA*, *nifH* genes, edge principal component analysis indicated a significantly different nitrogen-transforming community was present in undisturbed tropical peatlands compared to those that were drained.

MULTI-OMICS STUDIES TOWARDS NOVEL MODULATORS OF *Influenza A* VIRUS-HOST INTERACTION

S.Söderholm[1,2], Y.Fu[3], L.Gaelings[3], S.Belanov[3], L.Yetukuri[3], M.Berlinkov[4], A.Cheltsov[5], T.Aittokallio[3,6], T.Nyman[2], S.Matikainen[3], D.Kainov[3]

[1]Institute of Biotechnology, University of Helsinki, Finland

[2]Finnish Institute of Occupational Health, Helsinki, Finland

[3]Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Finland

[4]Institute of Mathematics and Computer Science, Ural Federal University, Russia

[5]Q-Mol L.L.C., San Diego, CA, USA

[6]Department of Mathematics and Statistics, University of Turku, Finland

Corresponding author: denis.kainov@helsinki.fi

Keywords: Influenza virus; omics; virus-host interaction; inhibitors

Background and objectives. Human influenza A viruses (IAVs) cause global pandemics and epidemics. These viruses evolve rapidly, making current treatment options ineffective. Our aim was to identify novel modulators of IAV-host interactions.

Methods. We re-analyzed our recent transcriptomics, metabolomics, proteomics, phosphoproteomics, and genomics/virtual ligand screening data.

Conclusions. We identified 713 potential modulators targeting 200 cellular and 2 viral proteins. Anti-influenza activity for 48 of them has been reported previously, whereas the antiviral efficacy for 665 of them remains unknown. Studying anti-influenza efficacy, immuno-modulating properties and potential resistance of these compounds or their combinations may lead to the discovery of novel modulators of IAV-host interactions, which might be more effective than the currently available anti-influenza therapeutics.

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GENETIC ANALYSIS SOLUTIONS IN MICROBIOLOGY AND INFECTIOUS DISEASE RESEARCH

R.Demidenko[1]

[1]UAB "Linea libera", Akademijos str. 2, LT-08412 Vilnius, Lithuania.

Corresponding author: r.demidenko@linealibera.lt

Key words: new generation genetic analysis

Genomics research plays a role across the full range of clinical & translational research applications: discovery of unknowns, metagenomics research, outbreak investigation, identification, variant association studies etc.

Well known Thermo Fisher Scientific brands Applied Biosytems together with Ion Torrent sequencing has been critical in facilitating rapid research results from archived samples for disease surveillance, outbreak investigation, and disease etiology determination. Downstream of whole genome microbial sequencing, data analysis methods include de novo and reference-guided assembly and typing of microbial strains.

The topic will cover most of next generation genetic analysis methods - next generation sequencing, mid-density arrays and standard qPCR, Sanger sequencing - and solutions in microbiology & infectious disease research.

GENOMIC COMPARISON OF *Felixo1virus* BACTERIOPHAGES

L.Alijošius, E.Šimoliūnas, L.Kalinienė, R.Meškys, L.Truncaitė

Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, JGMC, Vilnius University, Saulėtekio av. 7, LT-10257 Vilnius, Lithuania.

Corresponding author: lidija.truncaite@bchi.vu.lt

Keywords: Bacteriophages; *Felixo1virus* genus; comparative analysis.

Extensive sequencing of bacteriophage (phage) genomes and metagenomic samples revealed enormous diversity of the phage-derived sequences. This unexploited material meets further requirement for investigation of their functions. Availability of genomic sequences of the related phages offers an opportunity to use comparative analysis for this purpose particularly.

Salmonella virus FelixO1, which has been known since 1943 [1, 2] is a prototype phage of the genus *Felixo1virus*. During the last few years, a dozen of *FelixO1* relatives, including a large group of phages infecting *E. coli* or *Salmonella* strains, have been isolated and sequenced. In this study, we performed the genomic comparison of two *Felixo1virus* phages vB_EcoM_VpaE1 (VpaE1) and vB_EcoM_Alf5 (Alf5) that both were isolated in Lithuania, and showed specificity towards *E. coli* B or K12 strains, respectively.

Neighbor-joining tree based on the whole genome sequence alignment positioned both phages close to each other within the clade of viruses infecting *E. coli* and/or *Salmonella* strains. Bioinformatics analysis revealed similar organisation of both genomes with the absence of 7 VpaE1 ORFs from Alf5 genome and 9 Alf5 ORFs from that of VpaE1. Differing ORFs encoded 7 putative endonucleases and 9 hypothetical proteins of unknown function. At the amino acid level, most of the shared ORFs showed more than 95 % identity, with the exception of two putative tail-fiber proteins gp74 and gp75 exhibiting 87 and 74 % identity, respectively. These results suggest that both intracellular and tail fiber proteins contribute to the host-range of the investigated phages.

Acknowledgements:

This research was funded by a grant (No. MIP-002/2014) from the Research Council of Lithuania.

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INVESTIGATION OF TAIL-FORMING PROTEINS OF *Escherichia* PHAGE vB_EcoM_NBD2

E.Šimoliūnas[1], V.Časaitė[1], L.Kalinienė[1], R.Rutkienė[1], L.Truncaitė[1], M.Vilkaitytė[1], K.Goda[2], R.Meškys[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, the Joint Life Sciences Centre, Vilnius University, Saulėtekio av. 7, LT-10257 Vilnius, Lithuania

[2]Department of Eukaryote Gene Engineering, Institute of Biotechnology, the Joint Life Sciences Centre, Vilnius University, Saulėtekio av. 7, LT-10257 Vilnius, Lithuania

Corresponding author: eugenijus.simoliunas@bchi.vu.lt

Keywords: bacteriophage, self-assembling proteins, TEM

According to the literature, a number of phage structural proteins self-assemble into well-defined and highly stable structures. Such stuctures have been used to fabricate various nanomaterials, as well as hybrid nanomaterials, applicable in bionanomedicine, bionanotechnology, etc.

In this study, we present the investigation of biosynthesis of the recombinant major tail protein from *Escherichia* phage vB_EcoM_NBD2 (NBD2), and demonstrate its propensity to form self-assembled structures. Based on bioinformatics analysis, the major tail protein of NBD2 is encoded by ORF039, and shares 52% amino acid sequence identity with a putative major tail protein from *Salmonella* phage FSL SP-126. NBD2 ORF039 has been cloned into a pET21a cloning vector and expressed in *E. coli* BL21-DE3 cells. Both cell-free extracts and the samples obtained after protein purification have been analyzed by TEM, which revealed that, even in the absence of other viral proteins, the recombinant gp039 self-assembles into long tubes. The approximate width of the tubular structures was similar to that of the tail of NBD2 (~12 nm), and the length varied from ~10 to ~700 nm. Our results also indicate that the N-terminus hexahistidine tag has no impact on the self-assembly of the recombinant gp039, since the tubular structures formed by the recombinant gp039 have been visually similar to those formed by a native protein.

SESSION V: MEDICAL AND HEALTH ASPECTS OF MICROBIOLOGY

Presenter order	**Speaker**	**Presentation**	**Country**
Invited Speaker	Hanne Ingmer	Bacterial resistance to antibiotics	Denmark
Speaker I	Karlis Shvirksts	FTIR spectroscopy and Raman microsscopy of cancer cells grown under hypoxic conditions	Latvia
Speaker II	Eglė Lastauskienė	Membrane permeabilisation and growth inhibition of the skin infections causative microorganisms by using electro-magnetoporation	Lithuania
Speaker III	Jelena Beljantseva	Biochemical characterization of the small alarmone synthetase of *Enterococcus faecalis*	Estonia
Speaker IV	Miglė Janulaitienė	Distribution of *Gardnerella vaginalis* subtypes in characterized vaginal samples of Lithuanian women	Lithuania
Speaker V	Joana Šalomskienė	Antimicrobial compounds produced by selected lactic acid bacteria	Lithuania

BACTERIAL RESISTANCE TO ANTIBIOTICS: EXAMPLES FROM *Staphyloccoccus aureus*

H.Ingmer

University of Copenhagen, Denmark

Antibiotic resistance in the human pathogen *Staphylococcus aureus* is very common and it is an increasing problem. As an opportunistic pathogen *S. aureus* is know to give both hospital- and community associated infections and in recent years attention has extended to the livestock associated, methicillin resistant *S. aureus* clones that are found in pigs and other farm animals but still colonize and infect humans. *S. aureus* is known to harbor different types of mobile genetic elements and although they have been implicated in the transmission of antibiotic resistance it has been unclear how resistance genes can spread so quickly between *S. aureus* strains. We have recently shown that bacteriophages integrated in the bacterial chromosome, the prophages, are important and very efficient tools for the hosting bacterium to acquire antibiotic resistance genes from phage susceptible cells sharing the same environment. By this novel mechanism it appears the prophages are released by a subpopulation of propage-hosting cells to attack phage susceptible cells nearby and from these cells antibiotic resistance genes are transferred back to the prophage containing population. The efficiency with which this process occurs may explain why resistance genes can be transferred so effectively between staphylococcal isolates.

FTIR SPECTROSCOPY AND RAMAN MICROSCOPY OF CANCER CELLS GROWN UNDER HYPOXIC CONDITIONS

K.Shvirksts[1], E.Zandberga[2], M.Staemmler[3], P.Lasch[3], A.Line[2], M.Grube[1]

[1]Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas str 1, Riga, LV1004, Latvia

[2] Latvian Biomedical Research and Study centre, Ratsupites str 1, LV1067, Riga, Latvia

[3] Robert Koch Institute, Seestraße 10, 13353 Berlin, Germany

Corresponding author: kshvirksts@gmail.com

Keywords: FTIR spectroscopy, Raman microscopy, cancer cells, hypoxia

Fourier transform infrared (FT-IR) spectroscopy and Raman microscopy could be useful tools for assessment of different therapy effects on cancer cells. The response of cells to various growth factors *via* the biochemical composition is well known and has been studied by FT-IR spectroscopy and Raman microscopy. The aim of this study was to investigate the composition changes in cancer cells as response to the growth environment change.

Human colorectal cancer cell lines SW480 and SW620 derived from primary and metastatic tumour from a single patient were cultured under hypoxic or normoxic conditions. Cells were grown on CaF_2 windows, washed with PBS and fixed with 37% formaldehyde. Single cell Raman spectra were acquired using WITec alpha-R300 Confocal Microscope (Ulm, Germany). FT-IR spectra of ~200,000 cells were acquired with HTS-XT microplate reader (Etlingen, Germany). Data analyses were performed using WITec Project 2.10 and Opus 6.5. Quantitative analysis of macromolecular composition was carried out as in Ref. [1].

Both methods showed similar results in terms of total macromolecular composition changes in cells. Main differences being increased lipid content for cells grown in hypoxic conditions – in case of SW480 lipids content increased from 4% of dry weight (dw) to 6% and in case of SW620 increase was from 3% to 5% dw. Main difference between cell lines under normoxy showed total nucleic acids – 9% for SW480 and 12% dw for SW620 cells. Raman microscopy showed no significant component spatial distribution within single cell, suggesting low cancer cell inner structure organization. FT-IR spectroscopy was shown to be useful for fast cell screening, whereas Raman microscopy was more informative for analysis of single cell changes.

Acknowledgements: This study was supported by the LCS Joint Project No. 625/2014.

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ELECTROPORATION MEDIATED BIOCONTROL OF THE SKIN COLONIZING YEASTS

E.Lastauskienė[1]*, V.Novickij[2], A.Grainys[2], A.Zinkevičienė[3], I.Girkontaitė[3], L.Kalėdienė[1], J.Novickij[2]

[1]Vilnius University, Department of Biotechnology and Microbiology, Saulėtekio7, LT-03101, Vilnius, Lithuania

[2]Vilnius Gediminas Technical University, Institute of High Magnetic Fields, Saulėtekio al. 11, LT-10223, Vilnius, Lithuania

[3]State Research Institute Centre for Innovative Medicine, Žygimantų str. 9, LT-01102, Vilnius, Lithuania

Corresponding author: egle.lastauskiene@gf.vu.lt

Keywords: Electroporation, membrane permeabilisation, Candida, skin infections.

More than 20 types of *Candida* yeasts can cause infections in humans. *Candida lusitaniae* is infrequent cause of the infections, but has unique ability to develop resistance to antifungal drugs. Recently, electroporation, as an effective method for the biocontrol of the skin microflora, was proposed. For this purpose, various invasive electrodes can be used. The efficient treatment of *Candida* caused infections is difficult due to the structure and metabolisms of this unicellular microorganism that is similar to the eukaryotic host. Typically, in vitro electroporation is performed in electroporation cuvettes and the concentration of the microorganisms in the range of 10^6 to 10^9 is used. In our research we showed that monopolar, bipolar symmetric and bipolar asymmetric pulses can effectively be used for the inactivation of *C. lusitaniae*, while bipolar pulses were slightly more effective for permeabilization. It was shown that inactivation dynamics are electric field strength dependent.

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BIOCHEMICAL CHARACTERIZATION OF THE SMALL ALARMONE SYNTHETASE OF *Enterococcus faecalis*

J.Beljantseva[1], P.Kudrin[1], T.Tenson[1], V.Hauryliuk[1,2], J.A.Lemos[3]

[1]University of Tartu, Institute of Technology, Nooruse 1, 50411 Tartu, Estonia

[2]Department of Molecular Biology, Umeå University, Building 6K, 6L University Hospital Area, SE-901 87 Umeå, Sweden

[3]Center for Oral Biology and Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, New York, 14642

Corresponding author: jelena.beljantseva@gmail.com

Keywords: Stringent response, (p)ppGpp, RelA/SpoT homolog (RSH) protein family

One of the main mechanisms of bacterial adaptation to stress is the stringent response, which is mediated by alarmone molecules guanosine pentaphosphate and tetraphosphate (collectively (p)ppGpp). These nucleotides are key regulators of bacterial growth, stress adaptation, pathogenicity and antibiotic tolerance (*1*)

The intracellular levels of the ppGpp are controlled by members of the RelA/SpoT homolog (RSH) protein family. This family is divided in two categories, "long" multidomain RSH-s and "short" single-domain RSHs. In *Enterococcus faecalis* ppGpp metabolism is mediated by bifunctional synthetase/hydrolase Rel and the small alarmone synthetase (SAS) RelQ. Although Rel is the main activator of the stringent response, there is emerging evidence that SAS-s can make crucial and distinct contributions to bacterial physiology – SAS-s respond to different internal or external cues to activate stringent response; SASs constitutive synthetase activity during balanced growth ensures persistent basal (p)ppGpp production (*2*,*3*). These findings and SAS-s distinct domain structure suggest that SAS-s activate stringent response in a different manner than long RSH-s.

In orded to investigate the mechanism of action of SAS-s we preformed biochemical characterization of the *E. faecalis* SAS RelQ using *in vitro* stringent response mimicing system. Our results showed that RelQ produces ppGpp more efficiently than pppGpp without the need for ribosomes, tRNA or mRNA. In fact ppGpp production by RelQ is inhibited by single stranded mRNA in a sequence-specific manner. Moreover in addition to ppGpp synthesis from GDP and ATP, RelQ can efficiently utilize GMP and produce pGpp which can not be observed in the case of RSH from *E. coli* RelA. Thus pGpp might provide unique regulatory properties.

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DISTRIBUTION OF *Gardnerella vaginalis* SUBTYPES IN CHARACTERIZED VAGINAL SAMPLES OF LITHUANIAN WOMEN

M.Janulaitienė[1,2], V.Paliulytė[3,4,6], Š.Grincevičienė[1], J.Zakarevičienė[3,4,6], A.Vladišauskienė[4,6], A.Marcinkutė[5,6], M.Plečkaitytė[1]

[1]Institute of Biotechnology of Vilnius University, Saulėtekio g. 7, LT-10257 Vilnius, Lithuania.

[2]National Public Health Surveillance Laboratory, Žolyno g. 36, LT-10210 Vilnius, Lithuania.

[3]Clinic of Obstetrics & Gynaecology, Faculty of Medicine, Vilnius University, Čiurlionio 21/27, LT-03101 Vilnius, Lithuania.

[4]Centre of Obstetrics & Gynaecology, Vilnius University Hospital Santariškių Klinikos, Santariškių g. 2, LT-08661 Vilnius, Lithuania.

[5]Departament of Gynaecology, Vilnius City Clinical Hospital, Antakalnio g. 57, LT-10207 Vilnius, Lithuania.

[6]Antiaging Clinic, Sakalų g. 22, LT-08108 Vilnius, Lithuania.

Corresponding author: migle.j@gmail.com

Keywords: bacterial vaginosis; vaginal microflora; *Gardnerella vaginalis* subtyping; clade.

Bacteria *Gardnerella vaginalis* remains ambiguous inhabitant of the vaginal microflora considered to be implicated in the development of bacterial vaginosis (BV). *G.vaginalis* was subtyped to four different clades reflecting gene possession differences among strains [1]. Elucidation of clade-specific association with the disorder may help to understand the pathogenesis of BV.

One-hundred nine vaginal samples after laboratory examination for the assessment of the Nugent score (microscopy of Gram-stained smear), detection by PCR of 8 anaerobic bacteria and 4 *Lactobacillus* species, *Candida* both by culture and PCR and 7 STI by qPCR were defined as positive for BV, partial BV and non-BV. *G. vaginalis* was detected and subtyped by clade-specific PCR [1]. *G. vaginalis* was found in 87% (46/53) of non-BV samples. Multiple *G. vaginalis* clades were found in 82% (23/28) of BV samples and in 52% of non-BV samples. Clades 1 and 2 have positive association with BV rather than partial BV. Clade 4 was most frequently identified in vaginal samples and not connected with the disorder. Clade 3 was the less frequently found clade (13%) equally distributed among BV and non-BV samples.

Our findings contradicts findings in US where demonstrated that frequently detected clade 3 is positively linked with BV, meanwhile there was no correlation between clade 2 and high Nugent scores 7-10 [1]. Independent *G. vaginalis* isolates of clade 1, 2 and 4 were obtained from 33 consecutive vaginal samples. *G. vaginalis* isolates of three vaginal samples did not belong to any clade demonstrating that more than four *G. vaginalis* clades exist. It has yet to be determined whether clinically significant phenotyping characteristics are differentially distributed among clades.

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ANTIMICROBIAL COMPOUNDS PRODUCED BY SELECTED LACTIC ACID BACTERIA

J.Šalomskienė[1], J.Žeimė[1], A.Abraitienė[2], D.Jonkuvienė[1], I.Mačionienė[1], L.Vaičiulytė-Funk[1]

[1]Food Institute of Kaunas University of Technology, Radvilėnų 19, LT 50270, Kaunas, Lithuania

[2]Vilnius Un*i*versity, Institute of Biotechnology, V. A. Graičiūno 8, Vilnius, Lithuania

Corresponding author: joana.salomskiene@ktu.lt

Keywords: lactic acid bacteria, strains, antimicrobial compounds.

The aim of this research was to find out the differences in production and combined activity of antimicrobial compounds produced by the selected strains of lactic acid bacteria (LAB).

12 LAB strains with the strongest antibacterial and fungicidal properties [*Lactococcus lactis* (3), *Lactobacillus helveticus* (4), *Lactobacillus reuteri* (2), *Enterococcus faecium* (2) and *Streptococcus thermophilus*(1)] from the KTU Food Institute‘s collection were selected and their dependence to 4 genera: *Lactococcus*, *Lactobacillus*, *Enterococcus* ir *Streptococcus* was confirmed by the 16 S rDNA sequence analysis.

Production of high amounts of lactic acid (5,6 – 19,9 g/l) by tested LAB strains highly correlated with titratable acidity. Other metabolites (citric acid, ethanol, hydrogen peroxide, benzoic and sorbic acid) were found in significantly lower amounts. Inter-specific differentiation of LAB played a crucial role in production of lactic acid, while amount of produced ethanol, citric, benzoic and sorbic acids was highly strain-dependent. Thus, inter-specific differentiation among the tested *Lactococcus* and *Lactobacillus* strains was important in determining their potential in antibacterial activity, while antifungal activity was more strain-dependent. The significant intra-specific genotypic differentiation found between *E. faecium* strains was an exceptional and caused large differences in production and efficiency of antagonistic compounds. 5 strains (*E. faecium* 59-30, *E. faecium* 41-2, *S. thermophilus* 43, *L. reuteri* 3 and *L. reuteri 7*) produced substances of protein origin. The presence of A and P enterocin coding genes were revealed in both *E. faecium* strains. Proteins were produced by both *Lb. reuteri* strains. The identification of produced antimicrobial compounds provided valuable information about the differences in the potential of their practical application. The selected strains could be used as one or few strain starters in the manufacture of fermented products.

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ABSTRACTS FOR POSTER PRESENTATIONS

1. ANTIMICROBIAL RESISTANCE OF *C. jejuni* STRAINS ASSIGNED TO NOVEL MLST SEQUENCE TYPES

J.Aksomaitienė[1], S.Ramonaitė[1], A.Novoslavskij[1], M.Malakauskas[1]

[1]Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės 18, LT-47181 Kaunas, Lithuania.

Corresponding author: jurgita.aksomaitiene@lsmuni.lt

Keywords: *Campylobacter*, antimicrobial resistance, MLST, Novel, ST

Background and objectives: *Campylobacter* are among the most frequently reported foodborne diseases worldwide. *Campylobacter jejuni* is leading causes of gastroenteritis in humans, however most *Campylobacter* infections are self-limiting and antimicrobial treatment is necessary only in severe prolonged cases. Antimicrobial resistance of *Campylobacter* has increased dramatically in many countries, and such strains continue to exist with high-level resistance.

The aim of this study was to evaluate the antimicrobials resistance of *C. jejuni* strains isolated from children clinical cases and broiler meat and attributed to novel Multilocus sequence typing (MLST) genotypes.

Methods: In total 199 *C. jejuni* isolates including isolates from children (n=101) and broiler products (n=98) were genotyped by MLST and tested against phenotypic resistance to ciprofloxacin, tetracycline, gentamycin, ceftriaxone and erythromycin by agar dilution method.

Conclusions: MLST revealed 45 known and 23 novel sequence types (ST) out of 199 tested *C. jejuni* strains. ST-5 was dominant among children clinical cases. ST-464 and novel ST-6410 were dominant in broiler products. In total 94 % of *C. jejuni* strains were resistance to ciprofloxacin, 59.8 % to ceftriaxone, 42.7 % to tetracycline respectively. Whereas, only two strains were resistant to erythromycin. All strains were sensitive to gentamycin. The study revealed that 97 % of C. jejuni strains were resistant to at least one of 5 tested antibiotics. Interestingly, the strains assigned to novel ST‘s were confirmed with significantly higher multidrug resistance. Strains (69.8 %) assigned to novel ST displayed especially high minimum inhibitory concentrations in range of 32-256µl to tetracycline.

Aknowledgements:

Additional study on detection of point mutations related to elevated antimicrobial resistance of *C.jejuni* strains is in progress. (Study was funded by the Research Council of Lithuania; MIP-041/2015).

2. TRANSESTERIFICATION OF VEGETABLE OIL BY IMMOBILIZED *Aspergillus niger* MYCELIUM

N.R.Almyasheva[1], D.S.Kopitsyn[1], D.A.Beskorovaynaya[1], A.A.Novikov[1]

[1]Center for Nanodiagnostics, Department of Physical and Colloid Chemistry, Gubkin University, 65-1 Leninsky prospect, 119991, Moscow, Russia.

Corresponding author: novikov.a@gubkin.ru

Keywords: whole-cell biocatalyst, biodiesel fuel, transesterification, immobillization

Biodiesel comprising of fatty acid alkyl esters is a promising alternative fuel. The biodiesel fuel can be produced under milder conditions and with fewer steps using biocatalysts instead of conventional alkali catalyst. In order to increase the biocatalyst stability and to ensure its easy separation from the biodiesel fuel, it is required to immobilize the biocatalyst on a suitable carrier [1]. The most promising approach is immobilization of living microorganism used as lipase producer [2].

Tributyrine and triglycerides standards were from Sigma-Aldrich. Methanol was HPLC grade purchased from Vekton, Russia. Vegetable oil was food grade purchased in Moscow, Russia. *Aspergillus niger* DSM823 strain was from DSMZ. Activity of biocatalysts was assayed by the hydrolysis of tributyrine with analysis of butyric acid by GC. Methanolysis of triglycerides was controlled by HPLC with ELSD detection. The immobilization of microorganisms was controlled by SEM.

The highest yield of biodiesel fuel (fatty acid methyl esters) was observed for the DSM823 mycelium immobilized on polyurethane foam in packed bed bioreactor. This suggests the whole-cell biocatalysis in continiuous mode bioreactors is the most promising approach in biodiesel fuel production.

Acknowledgements:

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3. *Zymomonas mobilis* STRAINS OVEREXPRESSING TYPE II NAD(P)H DEHYDROGENASE: IMPLICATIONS FOR RESPIRATORY CHAIN ENGINEERING

E.Balodite[1], I.Strazdina[1], N.Galinina[1], U.Kalnenieks[1]

[1]Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas street 1, LV-1004, Riga, Latvia

Corresponding author: ebalodite@gmail.com

Keywords: NADH dehydrogenase, respiratory chain, *Zymomonas mobilis*

Zymomonas mobilis is an ethanologenic bacterium with desirable charasteristics for metabolic engineering and industrial use. It possesses a constitutive respiratory chain in which NAD(P)H type II dehydrogenase (Ndh) is the sole functional respiratory dehydrogenase [*1*]. It provides NAD(P)+ regeneration, contributing to the rapid catabolism of this bacterium. Increase in Ndh activity is necessary to improve the applicability of *Zymomonas mobilis* in biosynthesis, such as acetaldehyde generation. In the present study our aim was overexpression of the *Z.mobilis ndh*, to obtain strains with higher respiratory chain activity.

Zymomonas mobilis strains Zm6 (ATCC29191) and a strain with an Ndh-deficient background (strain Zm6-*ndh*, derived from Zm6) Zm6-*ndh* were transformed by electroporation with plasmid pBBR1MCS2 containing *ndh* under its own promoter, yielding strains Zm6 pBBR1MCS::*ndh* and Zm6-*ndh* pBBR1MCS::*ndh*. Both mutant and parent strains were cultivated under aerobic conditions. Eight hours after inoculation acetaldehyde concentrations were assayed using the HPCL. The cells were harvested and disintegrated by ultrasonication. Ndh oxidase and dehydrogenase activities were measured in cell disintegrates.

Comparison between parent and mutant strains confirmed overexpression of functional Ndh. Reconstitution of Ndh in Zm6-*ndh* restored the NAD(P)H activity, surpassing that of the wild type. Both strains bearing pBBR1MCS::*ndh* were similar in the measured parameters. Acetaldehyde concentration in supernatants of strains bearing pBBR1MCS::*ndh* was approximately two times higher than in the parent strains Zm6 and Zm4. Oxidase activity was increased 1,7 times, while the dehydrogenase (NADH:CoQ_1 oxidoreductase) activity rose 2,7 times, indicating that at high level of *ndh* expression other components of the respiratory chain might be limiting.

Acknowledgements:

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4. SORPTION OF OIL BY CARRIER FOR MICROORGANISMS IMMOBILIZATION

A.Berzins[1]

[1]Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas iela 1, LV-1004 Riga, Latvia.

Corresponding author: andrejs54@inbox.lv

Key words: porous ceramic, immobilization support, motor oil, sorption

The impacts of oil spills and their effects had taken on much more importance and became a major form of pollution. There are different methods for oil spill removal. One of mainly used are sorption method using wide range of organic (natural and artificial) and inorganic sorbents. Mostly they have high oleophilic and hydrophobic properties [1]. Used sorbent need to process for reuse the sorbent or utilization after sorption. Another way to remove oil pollution is the remediation of environment by microorganisms immobilized on insoluble support or microbial purification of polluted sorbent for reuse.

The sorption of oil on porous ceramic granules for use as the immobilized microorganisms support was investigated. Motor oil was used in the oil sorption study. The ceramic granules in the water was drenched and then its to water polluted with oil were displaced to investigate the oil sorption on wet granules.

Using as sorbent dry porous ceramic pellets for oil spilled water treatment the sorption of water and oil occurs at the same time. With increasing the amount of spilled oil sorption quantity of oil increased and water decreased respectively. Reaching spill approximately 90 g oil per m^2 the sorption of water did not decrease but the oil sorption capacity continued to grow.

As the microorganisms will be immobilized before oil spill processing the surface of granules was wetted. The sorption of oil on wetted porous ceramic granules decreased approximately 10 times compared to dry one.

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5. RESPONSE OF EXTENDED-SPECTRUM ß-LACTAMASE PRODUCING *Escherichia coli* TO HUMAN SERUM

E.Bozcal Dagdeviren[1]

[1] Department of Biology, Basic and Industrial Microbiology Section, Faculty of Science, Istanbul University, 34134, Istanbul, TURKEY

Corresponding author: elif.bozcal@istanbul.edu.tr

Background and Objectives: Extended-spectrum β-lactamases (ESBLs) are enzymes that hydrolyze most penicillins and cephalosporins, including oxyimino-β-lactam compounds (1). By far clinically the most important group of ESBLs are CTX-M enzymes. ESBL producing *Escherichia coli* (*E. coli*) O25:H4, mainly have CTX-M is globally disseminated pathogen should be taken into consideration and might lead to bloodstream infections (2). While most bacteria are unable to resist to strong bactericidal effects of human serum comprising complement cascade, several pathogens have enhanced mechanisms that successfully survive in human serum (3). Since ESBL producing bacteria are main problem for the human health, it's significant to study response of ESBL producing *E.coli* to human sera. The start point of this study was to investigate how different concentration of β-lactam antibiotics affects growth phase and serum survival of ESBL producing *E. coli* O25:H4.

Methods: Commercially available CTX-M positive *E. coli* O25:H4 isolated from human was used in this study. To disrupt the classical and alternative pathways of the complement cascade, serum was heat inactivated (HIS). Serum survival assay was carried out with normal human serum (NHS) and HIS. The Minimum Inhibitory Concentration test was performed with cefotaxime and ceftazidime. Antibiotic sensitivity together with NHS and HIS were tested with different time and temperature intervals.

Conclusions: According to the our results, CTX-M producing *E.coli* cells were monitored to see cells weather are eternally serum resistant and/or to what extent survival in the human sera in correlated with antibiotic concentration. Most of the stationary-phase *E. coli* cells survived serum treatment during the first hour, when the cells were in lag phase. When ESBL producing cells were incubated until first hour, cell growth was detected as indicated by the increased CFU counts in HIS. Our preliminary results indicated that antibiotic concentration is significant for the antibiotic therapy against to multidrug resistant bacteria.

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6. NUCLEOCAPSID-LIKE PARTICLES OF HUMAN PARAINFLUENZA VIRUSES 2 AND 4 PRODUCED IN YEAST *Saccharomyces cerevisiae*

A.Bulavaitė[1], R.Lasickienė[1], A.Vaitiekaitė[1], P.L.Tamošiūnas[1], K.Sasnauskas[1], A.Žvirblienė[1]

[1]Institute of Biotechnology, Vilnius University, Saulėtekio 7, LT-10257 Vilnius, Lithuania.

Corresponding author: aiste.bulavaite@bti.vu.lt

Key words: HPIV2, HPIV4, nucleocapsid-like particles, yeast-expressed, immunodiagnostics

Background. Human parainfluenza viruses (HPIV1–4) cause respiratory tract disease. HPIV4 is not assessed by most serological kits. Recombinant viral proteins can be more convenient for use in diagnostics than the whole virus. Our aims were to produce HPIV2 and HPIV4 (genus *Rubulavirus*) nucleocapsid (N) proteins in yeast, explore their antigenic structure and applicability in seroepidemiology.

Methods. PCR mutagenesis was used to construct sequence variants. N protein-encoding genes were cloned into *Saccharomyces cerevisiae* expression vector under galactose-inducible promoter. N proteins were purified by density gradient ultracentrifugation and observed by electron microscopy. Protein 3D-structures were modelled by ModBase. Mice were immunized with yeast-expressed HPIV2 and HPIV4 N protein nucleocapsid-like particles (NLPs). Monoclonal antibodies (MAbs) were developed and tested in immunofluorescence assay with HPIV(1–4)-infected cells. Epitope locations were mapped using *E. coli*-produced Nus-tagged N protein fragments and synthetic peptides. N proteins were used as coating and antibody-depleting antigens in ELISA with sera of 154 patients with respiratory tract infections.

Conclusions. HPIV2 and HPIV4 N protein NLPs were effectively produced in yeast, whereas Asp332 in place of Val332 or, respectively, of Leu332 abolished NLP formation. Eighteen of the generated MAbs were HPIV2 N-specific (most epitopes in C-terminal part); three were HPIV4 N-specific. Cross-reactive MAb 18D1 recognized 443–450 aa of HPIV2 N and a conformational epitope comprising 433–480 aa of HPIV4 N. HPIV2 N ELISA was 82% sensitive, 76% specific compared to the whole-virus kit. N protein ELISA categorized 73 patients as HPIV2 seropositive, 72 as HPIV4 seropositive. After depletion of cross-reactive antibodies using heterologous N protein, 60 patients were HPIV2 seropositive (81% sensitivity, 99% specificity compared to non-competitive assay) and 61 HPIV4 seropositive (82% sensitivity, 98% specificity). In summary, yeast-produced NLPs and specific MAbs are prospective tools for investigating HPIV2 and HPIV4 infection.

7. ANTIFUNGAL EFFECT OF ANTIMICROBIAL PEPTIDES DERIVED FROM LACTIC ACID BACTERIA

I.Burneikienė, L.Kalėdienė

Department of Microbiology & Biotechnology, Joint Life Sciences Center, Vilnius University, Saulėtekio avenue 7 LT-10257 Vilnius, Lithuania

Corresponding author: burneikiene.inga@gmail.com

Keywords: antifungal, antimicrobial peptide, lactic acid bacteria

Background and objectives: Every year food industry and agricultures receive a lot of loss of their production because of food spoilage and plants diseases caused by molds, yeast and bacteria. Chemical treatment are mainly used to control that type of damages, but more environmental friendly solutions are looked forward. Lactic acid bacteria (LAB) and their supernatants are useful tools for inhibiting bacteria and fungi. LAB have a status Generally Recognized As Safe (GRAS), and it makes them and their products even more attractive to apply. In our study we used lactic acid bacteria cultures isolated from different sources: sour cow and gout milk, pickled vegetables, bread sourdough.

Methods: We applied microbiological analysis to determine the influence of antifungal activity of LAB supernatants on *Botrytis sp.* and *Fusarium sp.* molds' mycelium growth and a viability of the fungal spores. LAB cultures were grown in MRS broth at 37 ºC for 48 hours and molds were grown on ME agar at 26 ºC for 48 hours.

Conclusions: Accordingly to other authors' papers, our results suggest that antifungal peptides derived from lactic acid bacteria reduce *Botrytis sp.* and *Fusarium sp.* molds' mycelium growth and have negative effect on viability of the fungal spores. Similar effect of LAB was shown on *Aspergillus sp., Rhipus sp., Mucor sp.* and *Penicillium sp.* by Gupta and Srivastava [1]. *Botrytis sp.* mold was more sensitive for treatment by LAB supernatant.

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8. A GALLERY OF CURIOSITIES: THE EXTRACHROMOSOMAL REPLICONS OF THE GENUS *Paracoccus*

J.Czarnecki[1], M.Szuplewska[1], A.Pawłot[1], R.Lasek[1], E.Prochwicz[1], M.Puzyna[1], A.Brozio[1], K.Kotecka[1], P.Dymek[1], Ł.Dziewit[1], D.Bartosik[1]

[1]Department of Bacterial Genetics, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland.

Corresponding author: jczarnecki@biol.uw.edu.pl

Keywords: *Paracoccus* spp., plasmid, chromid, essential genes

Background and objectives. The genomes of *Alphaproteobacteria* are known to contain numerous extrachromosomal replicons (ERs), which constitute an important source of exogenous genetic information. ERs very often carry genes enabling better adaptation of their host cells to various environmental conditions. In our study we analyzed the genomes of 18 *Paracoccus* strains (*Alphaproteobacteria*) to learn more about genetic load, diversity and evolution of ERs of this ubiquitous group of bacteria.

Methods. Genomic nucleotide sequences of 14 *Paracoccus* strains were obtained (Illumina). The nucleotide sequences of all identified ERs were annotated (Artemis, GenDB) and compared with the genomic sequences available in the GenBank database (NCBI). The specific role of selected ERs was analyzed using target-oriented replicon curing technique and phenotypic analyses.

Conclusions. The genomes of *Paracoccus* spp. vary widely in the number of ERs and their genetic load. Based on their size and properties, these replicons were divided into three groups: (i) small cryptic plasmids, (ii) large plasmids and (iii) chromids. The two first groups are not evolutionary conserved in the genus. They have replication systems typical for small plasmids or RepABC-type replication-partitioning modules and do not contain genes essential for growth. The group of chromids is much more conserved. Majority of the analyzed strains had two types of such replicons, whose replication initiation was dependent on DnaA-like (usually the largest ER) or RepB (usually the second largest ER) proteins. The chromids of *Paracoccus* spp. share long evolutionary history with co-residing chromosomes and they carry some housekeeping genes of chromosomal origin. Some chromids cannot be removed from bacterial cells (the DnaA-like chromids), and some can be cured but their loss results in reduction of evolutionary fitness of their host (the RepB chromids).

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9. EFFECT OF PHARMACEUTICALS ON BACTERIAL ACTIVITY IN WASTEWATERS

M.Dāvids[1], T.Selga[2], V.Bartkevics[3], O.Muter[1]

[1]Institute of Microbiology & Biotechnology, University of Latvia, Jelgavas Str., 1-534, Riga LV-1004, Latvia.

[2]Faculty of Medicine, University of Latvia, Jelgavas Str., 1-525, Riga, LV-1004, Latvia

[3]Faculty of Chemistry, University of Latvia, Jelgavas Str., 1-634, Riga, LV-1004, Latvia

Corresponding author: olga.mutere@lu.lv

Key words: activated sludge; pharmaceuticals; biodegradation; confocal laser microscopy; enzyme activity.

An issue of water pollution by pharmaceuticals attracts a great attention worldwide. In this respect microorganisms associated with the cometabolic and metabolic degradation of pharmaceuticals in wastewater treatment process are intensively studied. The biodegradation rate of organic micropollutants increased in the presence of carboxylic acids, compounds containing hydroxyl groups, and carbonyl groups, but decreased in the presence of ethers, substances with formula including halogens, aliphatic ethers, methyl groups and ring structures [1]. Besides, the efficiency of biodegradation is dependent on temperature, pH, redox conditions, availability of nutrients, as well as sludge retention time and sludge characteristics [2].

This study was aimed at comparing the bacterial activity in wastewaters spiked with different concentrations of pharmaceuticals. Experiments were performed with wastewaters sampled at the municipal WWTP. Bacterial consortium was isolated from the activated sludge (AS). The growth kinetics, enzyme activity, morphology of microorganisms was evaluated after exposing them by different groups of pharmaceuticals, added as a mixture or single contaminant.

Special emphasis in our study was put on ibuprofen. Incubation of AS amended by ibuprofen in a high-loading dosage (in the range from 10 mg L^{-1} to 500 mg L^{-1}) did not reveal any inhibition of the respiration activity as confirmed by the OxiTop device. Further investigation of AS will be focused on microbial succession in response to the presence of pharmaceuticals, as well as biodegrading activity of microorganisms, which were previously obtained by selective pressure of pharmaceuticals.

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10. DIVERSITY OF MOULDS IN SPICES AND HERBS AND ANTIFUNGAL ACTIVITY OF SPICE AND HERB ETHANOLIC EXTRACTS AGAINST *Aspergillus glaucus, A. versicolor, alternaria alternata* AND *Lichtheimia corymbifera*

B.Fogele, M.Rozenfelde, O.Valciņa, A.Bērziņš

Institute of Food Safety, Animal Health and Environment "BIOR", Lejupes street 3, Riga, Latvia, LV – 1076

Corresponding author: baiba.fogele@bior.lv

Keywords: spices, herbs, moulds, antifungal activity

Despite the use of various preservation methods food poisoning is still a problem among consumers and the food industry (Baljeet *et al.* 2015). Lately more attention is turn to antimicrobial properties of spices and herbs and their importance in cooking, industrial food production and storage. Extracts from spices and herbs consist mainly of essential oils, which possess not only concentrated organoleptic characteristics, but also high antimicrobial activity (Vidanagamage *et al.* 2016). Aim of this study was to determine fungal contamination in spices and herbs as well as verify whether these spices and herbs exhibit antifungal activity against *Aspergillus glaucus*, *A. versicolor*, *Alternaria alternata* and *Lichtheimia corymbifera*.

Overall, 70 samples of 13 different types of herbs and 11 different spice types were tested for the presence and diversity of moulds. Samples were obtained from supermarket chains and local markets. Presence of moulds were tested by ISO standard. Mould species were determined by Matrix Assisted Laser Desorption/Ionisation method. Agar diffusion method was used to determine the antifungal activity of the 11 different spice and herb extracts (5%, 10%, 15%, 20%) against *Aspergillus glaucus*, *A. versicolor*, *Alternaria alternata* and *Lichtheimia corymbifera*.

Results revealed that moulds were found in 55/70 (79%) samples. Number of moulds varied between 1 $\log_{10}$ CFU/g to 4,93 $\log_{10}$ CFU/g. The highest number of moulds were found in black, ground pepper 4,78 $\log_{10}$ CFU/g. In all samples were detected 21 different mould species. Ten mould species were found in one of the dill and basil samples. Also the highest average number of species (7.75 ± 2.06, n=4) was in sample of basil. The most common species were toxin-producing fungi *A. glaucus* (28%), *A. niger* (27%), *A. flavus* (18%), *A. versicolor* (18%) and others (9%). All concentrations of clove extracts 100% inhibited mould growth, while caraway, sage, thyme, ginger and black pepper extracts showed antifungal activity only at the highest concentrations of the extract (15%, 20%). Inhibitory effect of extracts observed mostly against *L. corymbifera*

(63%). Regardless from place of purchase the results demonstrated strong potential of clove as a natural preservative in food products.

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11. SYNTHETIC HOMODIMER SynKer-TT FOR EFFICIENT BIODEGRADATION OF PROTEINACEOUS MATERIALS

A.Gegeckas, A.Šimkutė, R.Gudiukaitė, D.J.Čitavičius

Department of Microbiology and Biotechnology, Faculty of Natural sciences, Vilnius University, Saulėtekio 7, LT-10257 Vilnius, Lithuania.

Corresponding author: gegeckas.audrius@gmail.com

Keywords: *Geobacillus*; keratinolytic peptidase; keratin; biodegradation

Background and objectives: Conventional chemical and thermal keratin waste decomposition methods are fully explored and not enough effective for biotechnology perspectives [1]. However, traditional keratin-rich waste decomposition methods could be replaced by environmentally-friendly and economical microbial waste biodegradation methods without energy wastage and essential amino acids and nutrition elements loss [2]. Unfortunately, most naturally produced enzymes are not effective or suitable for biotechnology processes. For decades, protein engineering has been utilized to modify natural enzymes to meet the needs of different industrial biotechnology. It is therefore a powerful tool in synthetic biology through the altering of enzyme properties to suit the requirements of any hydrolysis process. Enzyme modification or construction of chimeric proteins open up new possibilities for industrial application [3,4].

Methods: Bioinformatic methods were used for analysing GEOker gene. SynKer-TT was constructed by protein engineering joining two protein domains by a protein linker ELGGS. Construct was cloned into pET-28c(+) vector and expressed in *Escherichia coli* BL21(DE3). Obtained hydrolysis products by artificial protein were investigated by thin-layer chromatography and compared with commercial peptides generated by non-biological treatment and peptides obtained from enzymatic biodegradation by natural GEOker monomer.

Conclusions: We conclude that SynKer-TT is powerful biocatalyst for efficient keratinous by-product biodegradation and can replace conventional non-biological hydrolysis processes. High-value bioactive peptides obtained from keratin waste biodegradation by SynKer-TT are suitable for industrial applications in white and red biotechnology.

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12. GTC1 LIPASE OF THE URINARY TRACT INFECTIONS-CAUSING *Staphylococcus saprophyticus*

A.Gricajeva, G.Kareivaitė, L.Kalėdienė

Department of Microbiology and Biotechnology, Faculty of Natural Sciences, Vilnius University, Sauletekio avenue 7, LT-10257, Vilnius, Lithuania

Corresponding author: alisa.gricajeva@gmail.com

Keywords: lipases, *Staphylococcus*, hydrolysis, fatty acids

Background: *Staphylococcus saprophyticus* is causative agent of 10-20% of uncomplicated urinary tract infections. In human, *S. saprophyticus* is found in the normal flora (female genital tract and perineum). It has been also isolated from various other sources like food and soil, human and animal intestinal tracts [1]. *Staphilococcal* lipases are important not only for being a main protein of lipid metabolism and their significance in pathogenic processes, but also for the potential possibility of their applications in various biotechnology processes. So far, only one surface-associated lipase (Ssp) [2, 3] was characterized for the *Staphylococcus saprophyticus* species. In the present study, four more lipolytic proteins-coding genes were detected in the chromosomal DNA of the bacterium. One of them, named GTC1 lipase, was subjected for a detailed study.

Methods: Phylogenetic affinity of the studied bacterial strain was approved by the sequencing and phylogenetic tree construction on the basis of partial 16S rDNA gene sequences comparing with those of typical strains of the genus. MALDI-TOF mass spectrometry studies and further protein purification from the extracellular medium of the *Staphylococcus saprophyticus* were done. GTC1 lipase was purified in two steps: ammonium sulphate precipitation and protein elution from the SDS-PAGE gel. GTC1 lipase thermostability, stability in various chemical substances and substrate specifity were determined. Using thin layer chromatography ability of GTC1 lipase to hydrolyze different natural oils with releasing different fatty acids was studied as well.

Conclusions: Mature GTC1 lipase had a molecular mass of 50 kDa. Enzyme was active in a broad range of temperatures with optimum being reached at 35^{o}C, pH 8. GTC1 lipase was active not only at lower temperatures but also was highly thermostable and was activated by Ca^{2+} ions and methanol. Lipase was specific toward $C_{16:0}$ *p*-NPP substrate and hydrolyzed natural fatty substrates releasing fatty acids which can have beneficial effect on the human health. Detection of GTC1 lipase widens current knowledge about *S. saprophyticus* secreted lipases.

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13. FTIR SPECTROSCOPY STUDIES OF BREAST CANCER CELL RESPONSE TO INCUBATION WITH BSA OR AU-BSA NANOCLUSTERS

M.Grube[1], K.Shvirksts[1], D.Dapkute[2], M.Matulionyte[2], R.Rotomskis[2]

[1]Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas str 1, Riga, LV-1004, Latvia

[2]Laboratory of Biomedical Physics, National Cancer Institute, Santariskiu str 1, Vilnius, LT-08660, Lithuania

Corresponding author: kshvirksts@gmail.com

Keywords: FT-IR spectroscopy, gold nanoclusters, cancer cells, BSA

Fourier transform infrared (FT-IR) spectroscopy could be useful tool for assessment of different therapy effects on cancer cells. The response of cells to various growth factors *via* the biochemical composition is well known and has been studied by FT-IR spectroscopy. Lately gold nanoclusters (Au NCs) have emerged as promising photoluminescent agents for biosensing, bioimaging, and targeted therapy. Despite propitious prospects, interaction of NCs with cells remains poorly understood. In this study the effect of Au NCs to macromolecular composition of breast cancer cells was investigated by FT-IR spectroscopy.

MCF-7 and MDA-MB-231 breast cancer cells were incubated for 24h with BSA or BSA-encapsulated photoluminescent gold nanoclusters (Au-BSA NCs) [1]. FT-IR spectra of ~200'000 cells were acquired by HTS-XT microplate reader (Etlingen, Germany). Quantitative analysis of macromolecular composition was carried out as in Ref. [2].

MCF-7 cells showed little response to BSA or Au-BSA NCs. Nucleic acid amount decreased by 1.44% dry weight (dw) in case of BSA, and 1.69% in case of Au-BSA NCs, whereas lipid content increased from 6.11% (control) to 6.69% for BSA, and 8.17% dw for Au-BSA NCs incubated cells. MDA-MB-231 cells were more sensitive. Protein amount decreased from 65.40% in control to 61.16% in BSA, and 61.31% in Au-BSA NCs incubated cells. MDA-MB-231 cells had twofold increase in lipid content from 4.83 to 10.77% dw for BSA and 10.57% for Au-BSA NCs incubated cells. Results show minor difference in macromolecular composition between BSA and Au-BSA NCs incubated cells suggesting Au-BSA NCs have no significant effect on cells and are relatively safe to use.

Acknowledgements: This study was supported by the Lithuania-Latvia-Taiwan Joint Project No. LV-LT-TW/2015/7.

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14. SCREENING OF *Aspergillus spp.* TO PRODUCE HYDROLYTIC ENYZMES

Z.Gülsünoğlu[1], F.Karbancıoğlu Güler[1], M.Kılıç Akyılmaz[1], K.Raes[2]

[1]Department of Food Engineering, Faculty of Chemical and Metallurgical, Istanbul Technical University, Maslak Campus, 34469, Istanbul, Turkey.

[2]Department of Industrial Biological Sciences, Faculty of Bioscience Engineering, Ghent University, Graaf Karel de Goedelaan 5, 8500, Kortrijk, Belgium.

Corresponding author: gulsunoglu@itu.edu.tr

Keywords: *Aspergillus*, hydrolytic enzymes

Enzymes are bioactive components that are used for several applications in the food industry. Recent researches have been focused on production of new enzymes to use in commercial applications with desirable biochemical and physico-chemical characteristics and low production cost. Microbial enzymes have gained great importance in the development of industrial bioprocesses. In this research, *Aspergillus spp.* were isolated from foods and screened their ability to produce industrially important enzymes including cellulase, tannase, pectinase, polygalacturonase and xylanase.

To determine the enzyme production ability of *Aspergillus* strains, different fruits and vegetables such as carrot, apple, fig, grapes (dry and fresh), corn, apricot (dry and fresh), garlic, onion, and dates were used as a source for isolation of *Aspergillus* strains. The black colonies were collected for molecular and morphological identification. Totally six molds were chosen from grape and date for further screening and enzyme activity. The hydrolytic enzyme production ability of these molds were determined with screening methods using specific carbon sources for each enzyme both on solid and in liquid media.

According to the screening results, each *Aspergillus* strain had ability to produce cellulase, tannase, and pectinase enzymes, however xylanase enzyme production was not observed in plates and also in liquid media. One of *Aspergillus* strains had the ability to produce a wide spectrum enzymes with activity of 35±15.0 U/g dry biomass, 262±110.0 U/g dry biomass, 117±28.7 U/g dry biomass and 90±43.3 U/g dry biomass for cellulase, tannase, pectinase and polygalacturonase enzymes, respectively. Overall, these results showed that newly isolated *Aspergillus* strains had a potential for hydrolytic enzyme production.

15. WHEAT STRAW BIODEGRADATION BY NEW BIOFERTILISER AND ITS SEPARATE COMPONENTS

D.Jonkuvienė[1,2], S.Šliachtič[1]

[1]Laboratory of Microbiology, UAB BIOENERGY LT, Staniūnų str. 83, LT-36151, Panevėžys, Lithuania

[2]Food institute, Kaunas University of Technology, Taikos av. 92, LT-51180, Kaunas, Lithuania

Corresponding author: dovile.jonkuviene@yahoo.com

Microorganisms play an important role in the recycling of agricultural wastes. The use of suitable biofertiliser can generate bioconversion of agroresidues to compost. This enhance soil fertility and soil health which can lead to increased agricultural productivity, improved soil biodiversity, reduced ecological risks and lead to a healthier environment. In this study, the ability of new biofertiliser RUINEX and effect of its separate constituent microorganisms on wheat straw degradation *in vitro* was evaluated.

Bacillus subtilis, *Bacillus megaterium*, *Azotobacter vinelandii*, Trichoderma sp. and Lactobacillus sp. were fermented separately in bioreactors still high biomass/spores were obtained. The composition of new biofertiliser RUINEX was obtained by mixing all fermented bacteria and fungus at a certain ratio. The effect of biofertiliser and its separate constituent microorganisms on wheat straw degradation was determined by spraying tested material on straws and weighing it after 14 and 28 days. Tested samples were stored at 15 and 25^{o}C.

The significant straws degradation was observed after storage for 14 days and confirmed by decreased biomass. It was found that separate microorganism did not significantly affect the degradation of straws, except Trichoderma sp., which statistically significant reduced biomass of tested material. The enhanced effect of biodegradation were appreciate by using Trichoderma sp. with *A. vinelandii*, meanwhile full composition of biofertiliser had the highest ability to degradate the composition of straws.

After corresponding author provide 3-5 keywords or phrases. Do not insert a blank line after the corresponding author.

The role of new biofertiliser RUINEX in wheat straw degradation was quite evident and this eco-friendly biofertiliser can be applied for fast biodegradation of straws.

16. THE STUDY OF THE GROWTH OF STARTER CULTURES IN MILK USING ISOTHERMAL MICROCALORIMETRY

N.Kabanova[1], I.Stulova[1] and R.Vilu[1,2]

[1]Competence Centre of Food and Fermentation Technologies, Akadeemia tee 15b, Tallinn 12816, Estonia.

[2]Tallinn University of Technology, Department of Chemistry, Akadeemia tee 15, Tallinn 12816, Estonia.

Corresponding author: natalja@tftak.eu

Keywords: microcalorimetry; bacterial growth; starter culture; milk fermentation; concentrated milk

Growth of different starter cultures in milk, 2x concentrated milk and the mixture of these milks in case of different rennet concentrations was studied using thermal activity monitor TAM III in parallel to iCinac pH controlling system. Milk concentrated by reverse osmosis has a great developmental potential for innovative fermented dairy products. Utilization of concentrated milk allows to reduce production time, increase the product yields and minimize the amount of produced whey.

The comparative description of the growth of starter cultures in milk and concentrated milk was carried out. The growth of two quark starter cultures containing two different *Lactococcus lactis* subspecies and three different *Lactococcus lactis* species + *Leuconostoc mesenteroides* were studied in different milks. Further, the effect of the temperature (30°C and 42°C) on the growth of the mixture of mesophilic and thermophilic starter culture bacteria was investigated. The results showed that microcalorimetric curves provide remarkably more information regarding the growth and metabolic peculiarities of starter cultures in comparison to standard pH curves that are commonly used for the description of starter culture activity in dairies. The growth of bacteria in concentrated milk is faster resulting in higher produced biomass amounts in comparison to ordinary milk due to higher concentrations of nutrients available. The pH in the beginning of the fermentation was lower and at the end of fermentation was higher in concentrated milk due to higher buffering capacity in comparison to ordinary milk. The addition of rennet has a minor negative effect on bacterial growth both in milk and in concentrated milk.

The results of this work are the basis for further research and understanding of the peculiarities of bacterial growth in concentrated milk for the development and production optimization of innovative dairy products.

17. APOPTOTIC ACTIVITY OF *Lactobacillus plantarum* DGK-17 FERMENTED SOYBEAN SEED EXTRACT IN HUMAN COLON CANCER HCT-116 CELLS

S.C.Kang, I.Khan

Department of Biotechnology, Daegu University, Kyoungsan, Kyoungbook 712-714, Republic of Korea.

Corresponding author: sckang@daegu.ac.kr, Ph: +82-53-850-6553; Fax: +82-53-850-6559

Keywords: FSS, apoptosis, ROS, DGK-17

Fermented food has been always possesses upper hand as compared to normal food, due to presence of antibacterial, antioxidant and anticancer properties. Soybean has been consumed widely in in Korea, which has high nutritional values. Here, *Lactobacillus plantarum* DGK-17 which was previously isolated from Kimchi found to be fermenting soybean seed powder which shown to have anticancer potential.

We found that fermented soybean supernatant (FSS) found to be causing apoptotic cell death. It causes the morphological changes and reduces cancer cell colony formation in a dose-dependent manner. FSS found to be possessing IC_{50} value at 100 µg/µl. FSS treatment causes reduction in cell growth in dose dependent manner *via* releasing lactate dehydrogenase enzyme. FSS treatment causes HCT-116 apoptotic cell death as confirmed by presence of fragmented nuclei, oxidative burst and reduced mitochondrial membrane potential ($\Delta\Psi m$).

Therefore, this study reveals the apoptotic role of DGK-17 fermented soybean seed extract on human colon cancer HCT-116 cells.

18. PREVALENCE OF VIRULENCE GENES OF *Legionella* STRAINS ISOLATED FROM ENVIRONMENTAL WATER SOURCES OF PUBLIC FACILITIES

T.Kazanova[1,2], L.Kaledienė[2]

[1]National Public Health Surveillance Laboratory, Žolyno 36 LT- 10210 Vilnius, Lithuania.

[2]Department of Microbiology and Biotechnology, Faculty of Natural sciences, Vilnius University, Čiurlionio 21/27, LT-03101 Vilnius, Lithuania.

Corresponding author: tanchora2001@mail.ru

Keywords: *Legionella*, Legionnaires‘ desease, virulence genes.

The genus *Legionella* comprises over 60 species and 71 distinct serogroups. Up to now, only 20 species have been associated with human disease, and *Legionella pneumophila* appears responsible for more than 90% of reported cases of Legionnaires‘ desease**.** Legionella are ubiquitous in aquatic natural and antropogenic freshwater environment Gram-negative resides in biofilms bacteria. It’s able to multiply intracellularly in fresh water protozoa. This natural host cells provide nutrients, protect the bacteria from adverse conditions and serve as a vehicle for the colonization of new habitats. Over evolutionary time the protozoa-Legionella interaction may have generated a pool of virulence traits which preadapted this pathogen for human infection. Legionnaires’ disease is notifiable in all EU and EEA countries but is thought to be underreported for two main reasons. Firstly, it is underdiagnosed by clinicians who only rarely test patients for LD before empirically prescribing broad-spectrum antibiotics that are likely to cover Legionella spp. Secondly, some health professionals fail to notify cases to health authorities.

Despite the fact that in Lithuania from environmental samples are isolated a significant number of Legionella bacteria the disease statistics, compared to other European countries, remains to be low.

Using cultural microbiological methods from environmental water samples were determined 217 isolates of *Legionella*. The isolates using biochemical methods were serotyped and by PCR method were examined their virulence with genes: *lvh; mip; rtxA;pilB;pilD, dotA, hsp60.* In comparison of virulence were checked six clinical isolates, three of them belonged to *L.pneumophila sg.2-14* and the other three - *L. pneumophila sg.1*.

The results allow to conclude that the environmental isolates of *Legionella* have a very high virulence, since the main virulent genes - *lvh* and *rtxA* resolution seeks almost 100%. It remains to answer the question why in Lithuania is recorded a low number of Legionnaires disease cases if environmental samples dominated by virulent strains of *Legionella.*

19. IDENTIFICATION OF *Pseudomonas* spp. IN LITHUANIAN WHEAT

J.Kelpšienė[1], S.Supronienė[1], D.Burokienė[2]

[1]Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Instituto al. 1, LT–58344 Akademija, Kėdainiai dist., Lithuania.

[2]Institute of Botany at the Nature Research Centre, Žaliųjų Ežerų Str. 49, LT–08406 Vilnius, Lithuania.

Corresponding author: jurgita.kelpsiene@lzi.lt

Key words: *Pseudomonas syringae*, bacteria, cereal diseases

Pseudomonas syringae pathovars are known to cause bacterial diseases of cereals in nearly all temperate and subtropical cereal-growing regions. *P. syringae* has not been studied in cereal crops in Lithuania as being of low importance in comparison to fungal diseases. However, leaf blights and glume discolorations not typical for fungal diseases, were found rather frequently in the fields. The aim of this study was to identify the occurrence of *P. syringae* in cereals grown in Lithuania.

Winter wheat, winter triticale, winter rye, spring wheat and spring barley fields in various regions in the Lithuania were surveyed in the summer of 2015 and 2016. Leaf, stems, heads or mature seeds of symptomless plants were collected. Pure cultures of the selected *P. syringae*-like isolates were recovered from accumulative media onto selective [1, 2]. The isolates were tested for production of blue-green fluorescent pigment on King's medium B and used for LOPAT test (levan production from sucrose, oxidase reaction, pectolytic activity on potato slices, the presence of arginine dihydrolase, hypersensitivity to tobacco leaves) [1, 2].

The disease symptoms on cereal plants matched previous descriptions of bacterial diseases caused by *P. syringae* [1]. Twelve isolates out of 534 were identified as *Pseudomonas syringae* according to the above mentioned features [1, 2]. During the pathogenicity tests to host plant seedlings (winter wheat, spring wheat and winter rye) only half of them caused symptoms of leaf blight, therefore, bacterial strains were considered as pathogenic *P. syringae*.

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20. MOLECULAR MECHANISMS OF PHENOTYPIC HETEROGENEITY THAT INFLUENCE SERUM TOLERANCE OF *Escherichia coli*

I.Kerkez, M.Putrinš, T.Tenson

Institute of Technology, University of Tartu, Nooruse St.1, Tartu, Estonia

Corresponding author: kerkez-ivana@hotmail.com

Keywords: phenotypic heterogeneity, UPEC, serum killing, rcsB, ettA

Bacterial heterogeneity, which improves the survival of bacterial populations under variable environmental and stress conditions, may result from both phenotypic and genotypic variations. An example of phenotypic variation is the phenomenon of bacterial persistence, where subpopulation of non-growing cells survives antibiotic treatment. Our work-group has previously shown that heterogeneity among uropahogenic *Escherichia coli* (UPEC) strain CFT073 affects not only the outcome of antibiotic treatment but also bacterial tolerance to immune system mediated killing [1].

In order to find out molecular mechanisms responsible for population heterogeneity we used several different genetic mutants of UPEC strain CFT073. Experiments were performed in active or heat-inactivated human serum. Survival of cells was determined by counting colony forming units. Cell divisions were monitored with flow cytometry using fluorescent reporter proteins TIMER and GFP, which serves as cell-division reporter.

Two mutants showed higher resistance to serum treatment compared with wild type strain: *rcsB* mutant, deficient in cell envelope stress response, and *ettA* mutant, lacking a factor that modulates translation elongation. Single cell analysis using the TIMER and GFP reporters, enabled us to distinguish three different subpopulations in CFT073 strains. We noticed that non-dividing cells with lower expression of fluorescent proteins were surviving most efficiently in human serum. The abundance of subpopulations was altered in *rcsB* and *ettA* mutants differently: *ettA* has nondividing population larger and *rcsB* knock out cells resume growth slower. Therefore our data suggest that the survival of UPEC cells in human can be affected by different mechanisms.

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21. ISOLATION AND CHARACTERIZATION OF A LYTIC BACTERIOPHAGE FROM WASTEWATER INFECTING *Enterobacter* spp.

M.Khorshidtalab[1], I.Durukan[1], S.S.Nas[2], E.F.Tufekçi[1], M.Abdurahman[1], A.O.Kiliç[1]

[1]Department of Medical Microbiology, Faculty of Medicine, Karadeniz Technical University, 61080 Trabzon, Turkey.
[2]Department of Medicine, Faculty of Medicine, Karadeniz Technical University, 61080 Trabzon, Turkey.

Corresponding author: Inci Durukan, idurukan87@hotmail.com

Keywords: Bacteriophage, *Enterobacter*, transmission electron microscope, wastewater treatment

Background: *Enterobacter* is an important opportunistic pathogen causing mostly urinary and respiratory tract infections. In recent years, as a result of the insufficiency of antibiotics in persistent bacterial infections, bacteriophage therapy has been the focus of many studies to combat the pathogenic bacteria. In this study, we described the isolation and characterization of a broad host range lytic bacteriophage, ϕssn-021, from *Enterobacter spp.*

Methods: Wastewater treatment samples obtained from sewage treatment plants, Trabzon, Turkey. Samples were inoculated on agar plates for bacterial isolation. Filtered samples were used as phage sources to infect the isolated bacteria using standard plaque assay methods. Isolated phage were propagated by liquid enrichment technique and its host range was determined by double-agar layer method. Transmission electron microscopy was used for the phage morphology. Molecular characterization of ϕssn-021 was carried out by digesting the phage DNA with *Ava* I, *BstE* II, *EcoR* I, *Hind* III, *Nco* I and *Pst* I restriction enzymes.

Conclusions: Among the isolated bacteria, ϕssn-021 infected two *Enterobacter spp.*, identified as *E. asburiae* and *E. cloacae* using MALDI-TOF system. In addition, ϕssn-021 was able to infect *Escherichia coli* C600 strain. Morphologically, ϕssn-021 is a member of the *Myoviridae* family phages with a contractile tail. Restriction analysis indicated that bacteriophage ϕssn-021 had a dsDNA with an approximate genome size of 60 kb.

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22. PREVALENCE OF ENTEROPATHOGENIC *Yersinia* IN PIG TONGUES AT RETAIL MARKETS IN LITHUANIA

A.Kiaušaitė[1], A.Novoslavskij[1], M.Malakauskas[1]

[1]Department of Food Safety and Quality, Veterinary Faculty, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės 18, Kaunas, LT-47181, Lithuania.

Corresponding author: Agne.Kiausaite@lsmuni.lt

Keywords: *Y. enterocolitica*, prevalence, pig tongue, multiplex PCR.

Yersiniosis is one of three most prevalent foodborne zoonoses in humans in Lithuania and the incidence of 6.69 per 100 000 population was one of the highest among EU member states in 2014. Pigs are considered the major reservoir for human enteropathogenic *Yersinia* and contaminated pork and pork products, including pig tongues, are known as a main sources of human infection. The objective of the study was to determine the prevalence of enteropathogenic *Yersinia* in pig tongues at retail market in Lithuania.

Altogether, 58 pig tongue samples collected from 10 markets in Lithuania in a period of 2015-1016 were tested for prevalence of human pathogenic *Yersinia*. Detection of *Yersinia* spp. was done using cold enrichment method: samples were stored for 21 day at 4^{o}C in PMB broth (Phosphate-buffered saline supplemented 1% mannitol and 0-15% bile salts) with subsequent plating on a cefsulodin-irgasan-novobiocin (CIN) selective agar. CIN-positive *Yersinia* spp. isolates were confirmed using multiplex PCR.

Yersinia spp. was found in 79% of sampled pig tongues. All obtained isolates were confirmed as *Y. enterocolitica* and no other *Yersinia* species were detected. Based on multiplex PCR results human pathogenic *Y. enterocolitica* serotype O:3 and serotype other than O:3 (O:8; O:9; O:5,27) was found in 10% and 7% of sampled pig tongues, respectively. Additionally, CIN-positive bacteria isolates which did not harbour *ail*, *virF* and *rfb* virulence genes were detected in 62% of tested pig tongues and were assigned to human non-pathogenic *Y.enterocolitica* biotype 1A. The study revealed a high prevalence (17%) of enteropathogenic *Y. enterocolitica* in pig tongues at retail market in Lithuania. These findings indicate a risk of human yersiniosis due to consumption of improperly heat treated pig tongues and also suggest that enteropathogenic *Y. enterocolitica* contaminated pig tongues could be one of the main sources of human yersiniosis in Lithuania.

23. THYMOL DISINTEGRATES *E.coli* MEMBRANE INTEGRITY *IN VITRO* AND REDUCES THE OXIDATIVE STRESS CONDITION OF INFECTED MACROPHAGES IN *EX VIVO* MODEL

S.H.Kim[1], A.Bahuguna[2], S.C.Kang[2]

[1]Department of Biology Education, Daegu University, Kyoungsan, Kyoungbook 712-714, Republic of Korea.

[2]Department of Biotechnology, Daegu University, Kyoungsan, Kyoungbook 712-714, Republic of Korea.

Corresponding author: sangkim@daegu.ac.kr

Keywords: Thymol, β-lactamases, Macrophages

Escherichia coli that harbor a plasmid extended spectrum β-lactamases (ESBL) is considered as severe threat to human health as it encodes an enzyme β-lactamases capable of hydrolyzing β-lactam ring, and thus conferring resistance toward, most β-lactam antibiotics. Plant essential oil and their major components have been used to treat several diseases since a long time and have been reported to inhibit the drug resistance mechanism of bacteria that is the major concern.

In view of the above, present study was designed to introduce thymol as an antibacterial against ESBL producing *E. coli*. Thymol demonstrated a potent antimicrobial activity at 750 µg/ml by altering permeability and destruction of bacterial cell membrane as evident by uptake of crystal violet, release of intracellular content 260 nm absorbing material and proteins in time dependent manner. Concentration of cellular protein and 260 nm release matter was observed 297 ± 4.3 µg/ml, 0.67 ± 0.07 as compared to 67 ± 2.1 µg/ml and 0.15 ± 0.02 in the control untreated cells after 150 min respectively. More over in the *ex-vivo* study, thymol treated macrophages showed reduced NO level in compared to untreated macrophages suggesting a positive role of thymol in the recovery of macrophages from oxidative stress.

The findings of this study suggest thymol as an alternative antimicrobial for combating ESBL producing *E.coli* infection.

24. SURFACE-ENHANCED RAMAN SPECTRA OF BACTERIA OBTAINED WITH ALUMOSILICATE SUBSTRATES

D.S.Kopitsyn[1], E.A.Bochkova[1,2], M.V.Gorbachevskiy[1], A.A.Novikov[1]

[1]Center for Nanodiagnostics, Department of Physical and Colloid Chemistry, Gubkin University, 65-1 Leninsky prospect, 119991, Moscow, Russia.

[2]Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, 33-2 Leninskiy Prospect, 119071, Moscow, Russia.

Corresponding author: novikov.a@gubkin.ru

Keywords: surface-enhanced Raman spectroscopy, bacteria, spores, fluorescence background subtraction

Immunochemical assays, while extremely popular in clinical practice, demand the supply of antibodies, which make the rapid detection of multiple pathogens very complex and expensive. Modern genomic methods of bacteria identification can provide the reliable identification of vast majority of known bacteria and their spores, but suffer from the high analysis cost. Thus, rapid detection of bacterial pathogens has to be the antibody-independent chemical assay. Raman spectroscopy of bacteria provides the fingerprint-quality spectra that can be used for the identification of bacterial genera [1,2].

We used cheap portable Raman spectrometer BWS-415 (BWTEK, Germany) for Raman spectra acquisition. We obtained SERS spectra of Gram(+) and Gram(-) bacteria either on reference aluminum foil substrates or on aluminosilicate substrates based on halloysite ceramic nanotubes (Applied Minerals, Inc., NY, USA). Spectra processing was performed by GNU/Octave subroutines, first by fluorescence background subtraction [3], and then by filtering, normalizing and automated peak recognition.

Aluminosilicate filtering materials could be exploited for the accumulation of biomaterial form air or water for the subsequent SERS analysis. Spectra of bacterial active cells and spores obtained on the Raman peak-free substrates could be processed automatically, thus opening the possibility of automated rapid detection of airborne pathogens.

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25. ANTIBIOFILM ACTIVITY OF SELECTED PLANT SECONDARY METABOLITES AGAINST METHICILLIN-RESISTANT *Staphylococcus aureus*

B.Kot, D.Lohinau, M.Piechota

Department of Microbiology, Institute of Biology, Siedlce University of Natural Sciences and Humanities, 12 Bolesława Prusa Str., 08-110 Siedlce, Poland

Corresponding author: barbara.kot@uph.edu.pl

Keywords: MRSA, antibiofilm activity, phytochemicals

Background and objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of serious infections. Biofilm formation is an important virulence factor impeding eradication of bacteria. Many plant metabolites are well known for their antimicrobial activities. The aim of this study was to investigate the antibiofilm activity of selected plant metabolites on MRSA.

Methods: The adhesive properties of 38 MRSA strains from different clinical materials were determined with using a microtiter plate assay. To determine whether the plant metabolites had effect on biofilm control, microtiter plates with 24 h aged biofilms were exposed to 0,1% of plant metabolites. In the present study, the strains that formed moderate or strong biofilm were used. The results are presented as percentages of biofim reduction.

Conclusions: The percentage of strains which formed moderate and strong biofilm were 24% and 8%, respectively.

The thyme essential oil and *trans*-cinnamaldehyde showed a higher potential to reduce the mass of biofilms formed by MRSA, however biofilm removal was not complete. Depending on the strains, the thyme oil promoted reduction about of 54% to 89% biofilm mass after 48 h of treatment, while *trans*-cinnamaldehyde reduced mass of biofilms from 45% to 82% after 48 h. Percentage of biofilm biomass reduction treated with *p*-coumaric acid after 48 h was from 11,6% to 37,7%. In the case of many strains reduction of biofilm mass in the presence of ferulic and caffeic acid, geranium, lavender and tea tree essential oil at 0,1% concentration was not observed.

26. A CURIOUS CASE OF 'TWIN' PLASMID-BORNE RESTRICTION-MODIFICATION SYSTEMS OF AN ARCTIC *Psychrobacter* SP. STRAIN

R.Lasek[1], D.Bartosik[1]

[1]Department of Bacterial Genetics, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland

Corresponding author: r.lasek@biol.uw.edu.pl

Keywords: restriction-modification systems, transcriptional regulation, *Psychrobacter* sp.

Background and objectives: Given their biological role, bacterial restriction-modification (R-M) systems have to accommodate a number of functions: (i) the maintenance of the restriction activity against the exogenous genetic material invading the cell, (ii) the inhibition of the potential "autotoxic" behaviour of restriction endonucleases, and (iii) the maintenance of an adequate level of the host's DNA methylation. For this reason they tend to be controlled by fine-tuned regulatory mechanisms [1]. Here we present the preliminary results of the project aimed at the elucidation of the regulatory mechanisms of the two highly related type II R-M systems identified in plasmid pP62BP1 of an Arctic strain *Psychrobacter* sp. DAB_AL62B [2].

Methods: The employed bioinformatic methods included similarity searches, multiple sequence alignments and identification of promoter sequences and protein conserved domains. The pP62BP1-encoded genes for restriction endonucleases (REases) and methyltransferases (MTases) were cloned and expressed in *Escherichia coli*. Promoter strength was examined in β-galactosidase activity assays with/without co-expression of potential regulators.

Conclusions: The activity of the pP62BP1-encoded R-M systems was found to be under control of a complex regulatory network. It encompasses (i) the function of MTases encoded therein (methyltransferase-DNA interactions in regulatory sequences and cytosine methylation in C<u>C</u>NGG sequences, influencing the activity of the component gene promoters) as well as (ii) the effect of a potential regulatory RNA transcribed from P_{REV} promoters which are located within the genes encoding REases. As far as we know, a regulatory mechanism of such a level of complexity has not been described for any other R-M system to date.

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27. THE INFLUENCE OF PRESERVATIVES ON THE GROWTH OF YEASTS AND FUNGI IN MILK PRODUCTS

I.Mačionienė

Food Institute of Kaunas University of Technology, Radvilėnų pl. 19, LT 50254, Kaunas, Lithuania

Corresponding author: irena.macioniene@ktu.lt

Keywords: milk products, preservatives, yeasts, fungi.

The aim of this study was an investigation the influence of preservatives permitted by EU Regulation for milk products on the growth of yeasts and fungi.

For experiments were used: preservatives (sorbic acid, 0.1 %, 0.2 %; potassium sorbate, (0.1 %, 0.2 %; calcium propionate, 0.2 %, 0.3 %; preparation „Delvocid“ 0.1 %, 0.2 %); 5 yeast strains (*Saccharomyces cerevisiae, Candida parapsilosis, Debaryomyces hansenii, Kluyveromyces marxianus, Trichosporon cutaneums*) isolated from milk products and 4 fungi strains (*Aspergillus brasiliensis*, *Mucor racemosus*, *Penicillium verrucosum*, *Penicilium granulatum*) found in the food production area. The agar well diffusion method was used for detection of impact of preservatives on the growth of yeasts and fungi. Yeasts and fungi strains were prepared for analysis culturing on Sabouraud glucose agar at 25 ºC temperature for 3-5 days, then their suspensions (10^6 cfu/ml) were prepared.

It has been determined that sorbic acid (0.1 %, 0.2 %) affects the growth of yeasts and fungi and does not depend on the pH of the medium. Activity of potassium sorbate (0.1 %, 0.2 %) was found to depend on the pH of the medium: the preservative was active only at pH 4.0. The yeasts *Debaryomyces hansenii, Trichosporon cutaneum* and fungi *Penicillium verrucosum* were mostly sensitive to these preservatives. It has been determined that preservatives such as „Delvocid“ (0.1 %, 0.2 %) and calcium propionate (0.2 %, 0.3 %) are active in respect of yeasts and fungi and could be used for treating cheese surface in dairies.

28. DESIGN AND ANALYSIS OF NEW FUSED LIPOLYTIC BIOCATALYST LIPGD95-GD66

V.Malūnavičius, R.Gudiukaitė, A.Gegeckas, D.Čitavičius*

Department of Microbiology and Biotechnology, Faculty of Natural sciences, Vilnius University, Saulėtekio 7, LT-10257 Vilnius, Lithuania.

***During this work, Prof. Dr. D. J. Čitavičius passed away. We remember him always with great respect and admiration.**

Corresponding author: vilius.malunavicius@gf.vu.lt; renata.gudiukaite@gmail.com

Keywords: Bacterial enzymes, *Geobacillus* lipases, Protein engineering, Fusion enzymes

Background and objectives: Lipases are among the most versatile, stable and widely used enzymes in the world. These enzymes have a broad range of catalyzed reactions. For these reasons lipases can be used as eco-friendly biocatalysts instead of chemical and toxic substances in various industrial fields. Protein engineering (e.g construction of chimeric enzymes via gene fusion) is a powerful tool for creating new lipases with modified physicochemical properties. Lipolytic enzymes from *Geobacillus* bacteria are target of these experiments. Therefore the main objective of this study was construction, expression, purification and physicochemical analysis of new lipolytic chimeric biocatalyst composed of 2 different *Geobacillus* lipases.

Methods: GD-95 [1] and GD-66 lipase genes were amplified using PCR with primers, containing sequence of linker (GGS) and *Sac*I restriction sites (both these structures are important for lipase genes fusion). Recombinant plasmid pET-21c(+) with *lip*GD95-GD66 gene was transformed into *E. coli* BL21(DE3) cells and recombinant protein LipGD95-GD66 expressed and purified using affinity chromatography under denaturing conditions. For protein analysis SDS-PAGE and zymography were carried out. Lipase activity was measured spectrophotometrically with *p*-NP dodecanoate used as substrate at pH 9, 55 ^{o}C. This method was also applied for physicochemical analysis.

Conclusions: In this work, a novel chimeric lipase LipGD95-GD66 was successfully constructed, cloned, expressed and purified. The LipGD95-GD66 lipase is more active and stable at higher temperatures. This lipase is also more effective at higher pH values than both GD-95 and GD-66 lipases, hydrolyzes medium chain length substrates better than its precursor - GD-66 lipase and has twice the lipolytic activity when compared to GD-66 lipase. These results suggest that protein engineering can be powerful tool to modify the properties of native proteins and create enzymes with improved characteristics.

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29. STUDIES OF *Lactococcus lactis* INFECTION BY PHAGE C2

V.Marozas, I.Žievytė, R.Daugelavičius

Department of Biochemistry, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania

Corresponding author: v.marozas@gmf.vdu.lt

Keywords: *Lactococcus lactis*, lactic acid bacteria, phages.

Background and objectives. *Lactococcus lactis* is one of the most commonly used in the dairy industry lactic acid bacteria [1]. It is used for the production of cheese, sour cream, yogurt, buttermilk, and other dairy products. *L. lactis* is quite frequently infected by bacteriophages belonging to the Siphoviridae family. These bacterial viruses are divided into three main groups: 936, c2, and P335. The phage infection inhibits fermentation of lactose to lactic acid. Therefore, quality of the products changes, in some cases the production is stopped. It is ordinary in the dairy industry to inactivate bacteriophages by heat [2], but higher the temperature is, more taste and presentation of the final product is affected. It is known [3], that the supplement of a medium with divalent cations, such as calcium, magnesium, or manganese, is required for the productive infection. Usually *L. lactis* starter cultures are freeze-dried or spray-dried.

Methods. We applied microbiological and spectral analysis to determine the influence of heat, concentration of divalent cations in the medium and drying on the infectivity of bacteriophages c2.

Conclusions. After 15 min of incubation at 90 °C, the infectivity of phage c2 decreased by more than 7 orders of magnitude and after 45 min of incubation – this phage was completely inactivated. The pasteurization temperature 63°C is too low to damage this phage and stop the infection. The reproduction of phage c2 does not occur if the growth medium is not supplemented with divalent cations. The yield of this phage infection is the highest in the presence of calcium and strontium. In 20 weaks *L. lactis* cell viability decreased 6 orders of magnitude, while phage c2 infectivity decreased 4 orders of magnitude.

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30. PHOTOSENSITIZED DAMAGE TO BACTERIAL AND TUMOR CELLS MEDIATED BY NOVEL TRICARBOCYANINE INDOLENINE DYE TICS

A.Mlynska[1], S.Kavaliauskienė[2], P.Juzėnas[2], D.Kaškelytė[3], R.Gadonas[3], M.Samtsov[4], A.Lugovsky[4], E.Bakienė[1]

[1]Department of Biochemistry, Faculty of Natural sciences, Vilnius University, Sauletekio av. 9, LT-10223 Vilnius, Lithuania.

[2]Department of Radiation Biology, Institute for Caner Research, Oslo University Hospital, Montebello, 0379 Oslo, Norway.

[3]Department of Quantum Electronics, Laser Research Center, Vilnius University, Sauletekio av. 10, LT-10223 Vilnius, Lithuania.

[4]Department of Spectroscopy, Research Institute of Applied Physical Problems, Belarusian State Univeristy, Kurchatov st. 7, 220108, Minsk, Belarus.

Corresponding author: agata.mlynska@gmail.com

Keywords: photodynamic therapy, photosensitizer, anitbacterial treatment, anticancer treatment

Photodynamic therapy (PDT) is defined as an oxygen-dependent photochemical reaction that occurs upon light–mediated activation of a photosensitizing compound, which leads to the generation of cytotoxic reactive oxygen species which results in the eradication of target cells. It has proved to be an effective treatment of malignant tumors and represents a promising alternative to the use of antibiotics for a selective combat against resistant bacteria *[1]*. In this work, we investigated the phototoxic activity of a novel tricarbocyanine indolenine compund covalently bound with glucose (TICS No. 150). This dye emerged as a promising agent for photodynamic therapy against tumor cells *[2]*. We aimed to examine the photodynamic effect of TICS No. 150 in bacterial and cancer cells and investigate its mechanism of action.

To address these questions we performed a series of experiments on bacterial *Salmonella enterica* and eukaryotic prostate cancer cells, which were incubated with photosensitizer TICS No. 150 and irradiated with 720 nm laser diode light.

We found out that TICS No. 150 is phototoxic to *Salmonella enterica* cells in a time- and dose-dependent manner. Mutant bacteria with shorter lipopolysaccharide in outer membrane are more sensitive to TICS No. 150 photodynamic effect. TICS No. 150 remains non-toxic until it enters the cell. The sensitivity of *Salmonella enterica* cells can be enhanced by permeabilizing the outer cell membrane. Moreover, TICS No.150 also showed considerable *in vitro* phototoxicity to prostate carcinoma cells. TICS No.150 acts mainly by generating singlet oxygen, which causes cell damage and induces apoptosis.

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31. ASSESSMENT OF ANTIBIOTIC RESISTANCE OF NISIN PRODUCING *L. lactis* STRAINS

K.Mulkyte[1], M.Malakauskas[1], N.Kasnauskyte[1], L.Serniene[1], G.Golz[2], T.Alter[2]

[1]Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Tilzes 18, LT-4718, Kaunas, Lithuania.

[2]Department of Veterinary Medicine, Institute of Food Safety and Hygiene, Free University of Berlin, Königsweg 69, 14163 Berlin, Germany.

Corresponding author: kristina.mulkyte@lsmuni.lt

Keywords: nisin, antibiotic resistance, *Lactococcus lactis*, tetracyclin.

The ability of *Lactococcus lactis* strains to produce antimicrobial compounds such as bacteriocins provides a possibility to use them in the food industry as protective or starter cultures. Strains that are intended to be used in food industry have to be sensitive to antibiotic. Antibiotic resistance can be harmful to human's health because antibiotic resistant strains are capable of transferring antibiotic resistance genes to pathogenic bacteria, which can contaminate raw food products.

The aim of the study was to assess antibiotic resistance of 12 nisin producing *Lactococcus lactis* strains isolated from raw cow, goat milk and fermented wheat and buckwheat samples. Antibiotic susceptibility was evaluated using MIC Test Strips. The antibiotics tested were chloramphenicol, clindamycin, streptomycin, gentamicin, tetracycline, erythromycin and ampicillin. Minimum Inhibitory Concentrations (MIC) were determined from the MIC reading scale and expressed in µg/mL.

Three nisin producing *L. lactis* strains showed antibiotic resistance above the breakpoint provided by EFSA (2012)[1] to tetracyclin. The breakpoint for *L. lactis* suggested by EFSA is 4 µg/mL whereas strain isolated from raw cow milk with nisin A coding gene had minimum inhibitory concentration of 6 µg/mL, strains isolated from wheat and buckwheat samples harbouring novel nisin variant (GLc03 (KF146295))[2] coding gene had minimum inhibitory concentrations of 128 µg/mL and 16 µg/mL respectively. All strains were sensitive to other antibiotics tested.

Nine strains were sensitive to antibiotics and are further tested in order to apply them in food production.

Acknowledgements: This research was funded by a grant (No. MIP-63/2015) from the Research Council of Lithuania.

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32. ROLE OF THE STRINGENT RESPONSE IN ANTIBIOTIC TOLERANCE OF *Escherichia coli*

S.Oliveira[1], V.Varik[1, 2, 3], V.Hauryliuk[1, 2, 3], T.Tenson[1]

[1] University of Tartu, Institute of Technology, Nooruse 1, 50411 Tartu, Estonia

[2] Department of Molecular Biology, Umeå University, Building 6K, 6L University Hospital Area, SE-901 87 Umeå, Sweden

[3] Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå University, Building 6K and 6L, University Hospital Area, SE-901 87 Umeå, Sweden

Corresponding author: sofiaoliveira@ua.pt

Keywords: stringent response, antibiotic tolerance, bacteria.

The stringent response is a bacterial adaptation system mediated by accumulation of two guanine nucleotides ppGpp and pppGpp, collectively known as (p)ppGpp [1]. The response monitors several environmental stress inputs, such as nutrient limitation and heat shock, and remodels bacterial physiology in order to overcome the challenges [1]. In *Echerichia coli* (p)ppGpp levels are controlled by two enzymes – RelA and SpoT, the namesakes of RelA SpoT Homologue (RSH) protein family [2]. The stringent response is therefore associated with virulence, antibiotic resistance and was recently suggested to be the driving force behind the formation of so-called persister cells – antibiotic-tolerant phenotypic variants in antibiotic-sensitive population [3]. Since drug resistance and tolerance constitute a significant public health threat, understanding the connection amongst (p)ppGpp, antibiotic treatment and persistence is of great importance.

In the study we looked at the role of presence and absence of accumulation of (p)ppGpp in antibiotic tolerance, especially in case of the β-lactam ampicillin. We followed the killing of *E. coli* by ampicillin and norfloxacin (to elucidate bactericidal effects specific to ampicillin) in *E. coli* wild-type and Δ*relA* (lacking the gene) strains under various conditions and have dissected RelA-dependent and RelA-independent aspects of ampicillin tolerance.

We conclude that, first, while elevated (p)ppGpp does drive bacterial tolerance against ampicillin, the effect is specific to protection to this antibiotic as opposed to fluoroquinolone norfloxacin and, second, that challenging bacteria by antibiotics at sub-inhibitory concentrations can cause RelA-independent ampicillin tolerance.

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33. MICROBIAL COMMUNITY ABUNDANCE AND DIVERSITY DYNAMICS IN EXPERIMENTAL DOMESTIC GREYWATER TREATMENT SYSTEM

K.Oopkaup[1], I.Krustok[2], K.Kasak[1], M.Truu[1], Ü.Mander[1], J.Truu[1]

[1]Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, Tartu, 51014 Estonia.

[2]Department of environmental management, Ministry of the Environment, Narva St. 7a, Tallinn 15172, Estonia

Corresponding author: kristjan.oopkaup@ut.ee

Keywords: greywater; biological treatment; microbial community abundance; 16S rRNA sequencing

Background and objectives:

Domestic wastewater is mainly divided into greywater and blackwater. Greywater (wastewater from dishwashing, baths, showers, sinks, and laundries) constitutes 50–80% of the total household wastewater (1). Cleaned greywater can be reused in many fields of application where water quality is not a priority, such as toilet flushing, garden watering, irrigation etc. If such reuse possibilities could be applied, a large quantity of fresh water could be saved. Biological treatment systems are well suited for greywater treatment. Although many experiments have been assessing greywater treatment efficiency in biological systems (2), few studies are concerning microbiological aspects of these treatment systems.

Objectives of this study were: 1) to assess the microbial biomass and community structure of the greywater treatment filters; 2) to evaluate the dynamics in bacterial community diversity; 3) to correlate the results with system's treatment efficiency.

Methods and conclusions:

The present study was carried out in a newly established experimental greywater treatment system that treated the greywater of a 5-member family over ten-month long study period. The highly loaded filter system demonstrated good performance in COD, BOD, TSS and TN removal (3).

The studied system comprised of three parallel vertical flow filters (VF) containing lightweight aggregates (LWA) size of 4-10 mm which were followed by horizontal flow filters (HF) containing LWA which was made basic with quicklime. Samples of wastewater and filter material for microbiological analyses were taken over ten-month long period from November to August. The bacterial community abundance and structure was assessed by 16S rRNA gene copy numbers and sequencing respectively.

The average reduction of BOD, COD and TSS was about 90%, while the removal of TN and TP had lower efficiency, 62% and 32% respectively. Rapid increase in bacterial community abundance occurred during the first 100 days in both filters. The most abundant bacterial taxa were Gammaproteobacteria and Bacteroidetes in VF and Betaproteobacteria in HF. Positioning of samples in an ordination showed clear bacterial community succession in time up to day 160, after which the system stabilized. Temporal dynamics of community beta diversity components indicated that the change between samples was initially more affected by richness difference and later by species replacement in both filters. Bacterial community diversity was most affected by influent organic carbon and nitrogen compounds in VF and ammonia and phosphate in HF.

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34. 2-PHENYLETHANOL PRODUCTION BY *Kluyveromyces marxianus* ON GLUCOSE, LACTOSE AND GALACTOSE SUBSTRATES

A.Patetko, R.Scherbaka, A.Vigants

Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas street 1, LV-1004, Riga, Latvia

Corresponding author: bioarturs@gmail.com

Key words: *K.marxianus*, 2-phenylethanol, lactose, glucose, galactose

Background and objectives: 2-Phenylethanol is a compound with a rose-like flavour that is used as aromatic essence in food, pharmaceutics and perfumery. The interest of microbial synthesis of 2-phenylethanol has highly grown because of its natural and non-chemical production. One of the potential 2-phenylethanol producers are lactose utilizing non-conventional yeasts *Kluyveromyces marxianus*. The usage of lactose as a carbon source for 2-phenylethanol biosynthesis has a particular interest because lactose (whey permeate) is a by-product of dairy industry but most of the recent studies in this field have been focused on glucose substrates. The aim of the present study was to compare 2-phenylethanol biosynthesis in batch culture on glucose, lactose and galactose medium by several *K.marxianus* strains.

Methods: Yeast cells were cultivated in batch culture. The yeast growth was monitored spectrophotometrically by the OD600. The carbon source and 2-phenylethanol concentrations were determined by HPLC.

Conclusions: The strains DSM4906, DSM5422, CBS712, DSM5418 and CBS6556 were used. The highest 2-phenylethanol yield was obtained by DSM5422 and DSM5418. It was found that in the case of lactose 2-phenylethanol the production was lower by 15% comparing with glucose. Interesting results were shown on galactose substrates where in one of the strains 2-phenylethanol production was higher than on glucose or lactose substrates. The influence of fermentation conditions (substrate and phenylalanine concentrations, temperature, shaking speed) on 2-phenylethanol biosynthesis was investigated by the multifactorial experimental design and the interaction between factors was evaluated. There was a positive correlation between the yield of 2-phenylethanol and the shaking speed (better aeration). The positive effect on higher substrate concentration was more pronounced at higher shaking speeds. The optimal temperature for 2-phenylethanol production was in the range of 30-35 °C.

35. CHARACTERIZATION OF THE HETEROPOLYSACCHARIDE UTILIZATION *YteRPL47* OPERON FROM *Geobacillus thermodenitrificans* DSM 101594

R.Petkauskaite, N.Kuisiene

Department of Microbiology and Biotechnology, Faculty of Natural sciences, Vilnius University, Sauletekio ave. 7, LT-10257 Vilnius, Lithuania.

Corresponding author: raimonda.petkauskaite@gf.vu.lt

Geobacillus thermodenitrificans DSM 101594 was isolated from soil as a moderate thermophile that displays an ability to grow using pectic-polysaccharides as a result of secretion of highly active pectate lyase homologous to pectate lyase PL47 from *Bacillus* sp. TS 47. The annotation of recently sequenced genome [1] of *G. thermodenitrificans* DSM 101594 confirmed an adaptational trait of this bacterium to utilize polysaccharides and outlined annotated genome as a valuable target for mining of thermoactive and/or thermostable polysaccharide-degrading enzymes of potential biotechnological importance. However a further detailed analysis of the genomic organization of *G. thermodenitrificans* DSM 101594 encoded polysaccharide degrading enzymes is necessary to reveal the regulation of *G. thermodenitrificans* DSM 101594 polysaccharide degrading enzymes production in order to fully describe polysaccharide utilization potential of *G. thermodenitrificans* DSM 101594.

Identification of polysaccharide utilization operons in *G. thermodenitrificans* DSM 101594 genome was performed *in silico*. Regulation of transcription of operons was predicted and architectures of identified operons were described. Identified operons were compared with polysaccharide utilization operons characterized in genomes of bacilli and geobacilli.

The genomic organization of the majority of polysaccharide degrading enzyme genes encoded by *G. thermodenitrificans* DSM 101594 is similar or identical to that previously identified in the genomes of bacilli and geobacilli. However, surprisingly *YteRPL47* operon was composed of the genes of unsaturated glucuronyl hydrolase YteR and pectate lyase PL47, respectively, that have been previously characterized in bacilli. It should be noted that the gene of unsaturated glucuronyl hydrolase has been never annotated in the genome of geobacilli. These findings suggest that geobacilli depolymerize heteropolysaccharides in the same manner as mesophilic bacilli.

Acknowledgements: Study was funded by "TermozymOS" project by the Lithuanian Science Council (no. SVE-08/2011).

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36. APPLICATION OF THE YEAST EXPRESSION SYSTEM FOR GENERATION OF CAPSID PROTEINS OF HUMAN BOCAVIRUSES

R.Petraitytė-Burneikienė[1], P.L.Tamošiūnas[1], A.Bulavaitė[1], R.Lasickienė[1], R.Firantienė[2], R.Ėmužytė[2], A.Žvirblienė[1], K.Sasnauskas[1]

[1] Institute of Biotechnology, Vilnius University, Saulėtekio al. 7, LT-10257 Vilnius, Lithuania

[2] Clinic of Children's Diseases, Faculty of Medicine, Vilnius University, Santariškių 4, LT-08406 Vilnius, Lithuania

Corresponding author: rasa.burneikiene@bti.vu.lt

Keywords: Human Bocavirus, capsid proteins, virus-like particles, monoclonal antibodies, ELISA

Background and objectives. Human bocavirus 1 (HBoV1), first described in 2005, was considered a causative agent of previously unexplained respiratory tract diseases. Recently, 3 new members of genus *Bocavirus,* HBoV2-4 were described. Recombinant viral antigens have been proven useful for serologic diagnosis of viral infections. Production of HBoV1-4 antigens in yeast expression system has not yet been reported. In the current study, the capsid proteins VP2 of HBoV1 - 4 were expressed in yeast *S. cerevisiae*. Electron microscopy demonstrated that all purified recombinant proteins self-assembled into virus-like particles (VLPs) exhibiting the typical icosahedral appearance of parvoviruses with a diameter of approximately 20 nm. HBoV1 - 4 VP2 VLPs were stable in yeast and were easily purified by cesium chloride gradient ultracentrifugation. Twelve monoclonal antibodies of IgG1 subtype were generated by immunization of mouse with recombinant VP2 VLPs. Recombinant HBoV1-4 VP2 VLPs and VP2-specific MAbs were employed to develop serological assays to detect virus-specific IgG antibodies in human serum specimens.

Methods. Cloning of HBoV VP2 proteins coding sequences into yeast vectors and purification of virus-like particles from yeast. Electron microscopy. SDS-PAGE and Western blotting analysis. Indirect ELISA. IgG capture ELISA. Generation of monoclonal antibodies.

Conclusions. Therefore, Yeast expression system proved to be simple, efficient and cost-effective, suitable for high-level production of HBoV1- 4 VP2 as VLPs, that resemble native virus in regards of morphology and antigenicity. The results of the analysis of human serum specimens by VP2-based immunoassays confirm that yeast-derived VLPs represent a useful diagnostic tool for studying the seroprevalence of human bocaviruses infection.

37. *IN VITRO* DESIGN OF VARIANTS OF ANCIENT-LIKE MALTASE PROTEIN OF AN EARLIER-DIVERGED YEAST *Ogataea polymorpha*

K.Põšnograjeva, K.Viigand, K.Mardo, T.Alamäe

Institute of Molecular and Cell Biology, University of Tartu, Riia St. 23, Tartu, Estonia

Corresponding author: kristina.poshnograjeva@gmail.com

Keywords: *Ogataea polymorpha*, alpha-glucosidases, protein mutagenesis, protein evolution

Background and objectives. *Saccharomyces cerevisiae* produces two types of α-glucosidases: maltases and isomaltases, which have different substrate specificity. These enzymes are hypothesized to have evolved from a promiscuous α-glucosidase ancMALS through duplication and subsequent mutation of the genes [1]. A methylotrophic yeast *Ogataea polymorpha* diverged earlier from the main evolution line of yeasts leading to *Saccharomyces* species and possesses a promiscuous MAL1 enzyme. The MAL1 can use both maltose- and isomaltose-type sugars being thus similar to a hypothetical ancestor of maltases and isomaltases of *Saccharomyces* and other 'modern' yeasts. Our objective is to reveal the determinants of substrate specificity of α- glucosidases by mutational study of the *Op* maltase.

Methods. The *Ogataea polymorpha MAL1* gene has been cloned earlier. Mutations of choice were introduced into *MAL1* by PCR using mutagenic primers. Plasmids containing mutated *MAL1* gene were electroporated into *E. coli* BL21(DE3) for heterologous expression of the protein. The maltase variants were purified from *E. coli* lysates using Ni^{2+} - affinity chromatography. The substrate specificity and kinetic parameters of the mutated *Op* maltase mutants were determined and compared to wild-type maltase of *Op* and maltase and isomaltase of *S. cerevisiae*.

Conclusions. The *Op* MAL1 enzyme can be mutated to change its substrate selection pattern. For example, the Thr200Val substitution of the *Op* MAL1 strongly decreased cleavage of maltose-like substrates by the enzyme making it more similar to isomaltases.

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38. ANTIBACTERIAL ACTIVITY OF MICROORGANISMS ISOLATED FROM KRUBERA-VORONJA CAVE

R.Pranckute, A.Klusaite, D.Bukelskis, N.Kuisiene

Department of Microbiology and Biotechnology, Life Sciences Center, Vilnius University, Sauletekio avenue 7, LT-10257 Vilnius, Lithuania.

Corresponding author: nomeda.kuisiene@gf.vu.lt

Keywords: cave microorganisms; Krubera-Voronja cave; antibacterial; bacteriocin; volatile compound.

Background and objectives. Caves are considered to be an under-exploited environment to screen for novel antimicrobials. It is known that a depth of caves correlates with the production of antimicrobial substances, *i.e.*, the deeper the cave, the more possibilities to identify novel antimicrobial substances producing microorganisms. Krubera-Voronja cave is the deepest known cave in the world. Therefore, the chances to discover novel antimicrobials producing bacteria in this cave are high. The aim of our study was to evaluate and characterize antibacterial activity of microorganisms isolated from this deep cave.

Methods. Tryptic Soy agar, Hickey-Tresner agar, Actinomycetes Isolation agar, Starch Casein Nitrate agar and Difco™ ISP medium 4 were used for isolation of microorganisms from Krubera-Voronja cave samples. Evaluation of antimicrobial activity was performed by agar-well diffusion method. Antibacterial compounds were extracted by organic solvents as well as by salting-out with ammonium sulphate. Thin-layer chromatography, bioautography and gas chromatography-mass spectrometry were used for analysis of volatile antibacterial compounds. Zymogram analysis was performed to study proteinaceous antibacterial compounds. Phylogenetic analysis of 16S rRNA gene was carried out in order to identify active isolates.

Conclusions. In total, 874 heterotrophic cultures were isolated from water and sediment samples collected in Krubera-Voronja cave. 13.96 % of all isolates demonstrated antibacterial activity against Gram-positive and Gram-negative test microorganisms. Six active strains were chosen for more detailed analysis. Five strains were assigned to phylum *Firmicutes*, and one strain – to phylum *Actinobacteria*. Antimicrobial activity of two strains was found to depend on volatile and semivolatile compounds, and that of other four strains – on proteinaceous compounds.

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39. ENZYMATIC HYDROLYSIS OF LIGNO(HEMI)CELLULOSE AFTER PRETREATMENT WITH LACCASE

L.Rozenfelde[1], M.Puke[2], I.Kruma[2], N.Vedernikov[2], N.Matjuskova[3], A.Rapoport[1]

[1]Laboratory of Cell Biology, Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas Str., 1, LV – 1004 Riga, Latvia

[2]Laboratory of Polysaccharides, Latvian State Institute of Wood Chemistry, Dzerbenes Str., 27, LV-1006 Riga, Latvia

[3]Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia, Jelgavas Str., 1, LV-1004 Riga, Latvia

Corresponding author: Linda.Rozenfelde@lu.lv

Keywords: bioethanol, enzymes, hydrolysis

Up to the opinion of experts the resourses of oil can be consumed within the next 30-40 years and it is supposed that about 70% of all oil stocks is already consumed. Therefore one of the most important problems now is to find the alternative to oil as a source for fuels and various chemical products. Because of that bioethanol production is supposed at the moment to be one of the most important directions in biotechnology. The most appropriate resources for such technologies are renewable substrates which have no food importance as, for example, ligno(hemi)cellulose-containing industrial and agricultural waste materials. In our work new complex technology is worked out for the simultaneous production of bioethanol and furfural. This process is based on special pretreatment stage which is worked out by Latvian researchers (Patent Publication in China CN 201210283058.2). C5-compounds of ligno(hemi)cellulose are used for the production of furfural, but C6-compounds - for the microbiological production of bioethanol by yeast. For the bioethanol production stage enzymatic hydrolysis of ligno(hemi)cellulose residues after obtaining from it of furfural should be performed. In the present study enzymatic hydrolysis was carried out by different ways. Commercial cellulases were used separately or in the combination with laccase. In the last case the efficiency of application of 2 kinds of laccase was checked. These enzymes were: commercial laccase from *Rhus vernificera* and laccase-containing enzymes complex which can be obtained after cultivation of medicinal mushroom *Lentinula edodes*. In these experiments medium for the growth of medicinal mushroom was supplemented with lignin obtained at the last stage of our new waste-less technology for the production of furfural, bioethanol and other valuable compounds. Enzymatic hydrolysis results indicated increase in glucose yield after application of both enzymes.

40. BIOCHEMICAL CHARACTERIZATION OF INDOLE DEGRADATION IN *Acinetobacter* sp. O153

M.Sadauskas, J.Vaitekūnas, R.Gasparavičiūtė, R.Meškys

Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Vilnius University, Sauletekio av. 7, LT-10257 Vilnius, Lithuania.

Corresponding author: mikas.sadauskas@bchi.vu.lt

Keywords: indole degradation, recombinant protein expression, biodegradation.

Background and objectives. Indole is an *N*-heterocyclic aromatic compound derived from L-tryptophan and is widely found in natural environment. Due to its hydrophobicity indole can cross cell membrane and regulate gene expression [1] with a particular role in biofilm formation and quorum sensing signaling [2]. However, at high concentrations (varying for different organisms) indole exhibits toxic activity by inhibiting cell division [3]. Several groups of bacteria have developed enzymatic systems to prevent the toxic effect of indole. One common detoxification strategy is an oxidation of indole to insoluble and non-toxic indigo and indigoids [4]. While indole degradation has been reported for a number of microorganisms [5,6], very limited information about genes and proteins involved in this process is available. In this study, we aimed to analyze genetic and biochemical background of possible indole catabolism in indole-degrading *Acinetobacter* sp. O153.

Methods. Genomic library of indole-oxidizing *Acinetobacter* sp. O153 was constructed and screened for pigment production by oxidation of various indole derivatives. Inserted DNA fragments from positive hits were sequenced and analyzed for possible degradation-related operons. Genes of interest were cloned into pET-28c(+) for expression in *E. coli* with *N*-terminal His-tag and resulting recombinant proteins were purified with Ni^{2+} affinity chromatography, tested for activity with indole or other substrates and reaction products were analyzed by HPLC-MS or spectrophotometrically.

Conclusions. An operon composed of five genes and responsible for indole catabolism in *Acinetobacter* sp. O153 was identified. A catabolic cascade was reconstituted *in vitro* with recombinant proteins encoded by genes from the operon and function of each protein was identified. Indole degradation started with oxidation, leading to formation of indoxyl and 2,3-dihydroxyindoline. The latter intermediate was then oxidized to 3-hydroxy-2-oxoindoline, which was finally hydrolyzed by a single enzyme to anthranilic acid. Indole degradation then proceeded through a well-documented oxidation of anthranilate.

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41. POTENTIOMETRIC ANALYSIS OF THE EFFLUX PUMP INHIBITORS IN THE SUSPENSIONS OF *Salmonella enterica* CELLS

S.Sakalauskaitė, R.Daugelavičius

Department of Biochemistry, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania

Corresponding author: sakalauskaite.sandra90@gmail.com

Keywords: RND family pumps, efflux pump inhibitors, substrates, Salmonella enterica.

Background and objectives. Multidrug resistance (MDR) efflux pumps are active transporters, responsible for the extrusion of toxic substances and antibiotic out of the cells. The major component in the development of MDR phenotype in Gram-negative bacteria is the overexpression of Resistance - Nodulation - Division (RND) family efflux pumps, actively extruding antibacterial agents and biocides from the periplasm outside of the cell. Efflux is an important mechanism of bacterial resistance and the inhibition of efflux could be a promising strategy to overcome MDR [1]. Efflux pumps inhibitors are the compounds that interfere with the activities of efflux pumps and therefore are potentially useful for strengthening of the actions of antibiotics by allowing them to reach the threshold concentrations required for their bacteriostatic or bactericidal activities [2]. The aim of our study was to find combinations of MDR pumps inhibitors (Phe-Arg-β-naphthylamide (PAβN), 1-(1-naphthylmethyl)piperazine (NMP), chlorpromazine (CPZ)) and its substrates that increase the susceptibility of bacteria to antibiotics. Effects of the combination of MDR pumps inhibitors and substrates – tetraphenylphosphonium, tetracycline and chloramphenicol – on *Salmonella enterica* ser. Typhimurium cells were also investigated.

Methods. We applied potentiometric analysis using selective tetraphenylphosphonium (TPP^+) electrodes to study the interaction between the most popular RND family efflux pumps inhibitors.

Conclusions. CPZ does not increase the effect of Chloramphenicol and Tetracycline on *S. enetrica* cells, while PAβN and NMP increase the bactericidal effect of these antibiotics. It means that Chloramphenicol and Tetracycline are not the substrates of ABC transporters. Chloramphenicol increases the effect of tetraphenylphosphonium more that tetracycline. PAβN increase the accumulation of TPP^+ ions improving the effect on *S. enterica* cells. NMP and PAβN compete for the binding sites in cells. CPZ has the weaker effect in the presence of PAβN. NMP and CPZ act synergistically inhibiting TPP^+ efflux from *S. enterica* cells.

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42. VITALITY OF *Staphylococcus aureus* IN FROZEN BUTTER, BUTTER PRODUCT AND BLEND OF MILK AND VEGETABLE FAT

A.Šarkinas[1,2], D.Jasinauskienė[1], A.Šidlauskaitė[1]

[1]Department of Microbiology, Food institute of Kaunas University of Tecnnology, [2]Deparment of Food Science and Technology , Kaunas University of Technology, Kaunas, Lithuania

Corresponding author: antanas.sarkinas@ktu.lt

Key words: *Staphylococcus aureus*, butter, butter product, blend of milk and vegetable fat, freezing

Different compositions of butter, butter product and blend of milk and vegetable fat were contaminated with *Staphylococcus aureus* and frozen at minus 72 °C. Samples of 82 % fat content butter, 68 % fat content butter product and 62 % blend of milk and vegetable fat were used in the study.

The amount of the viable microorganisms was determined upon freezing, after 1, 3 and 12 months of storage.

After freezing and 12 months of storing of 82 % fat content butter, 68 % fat content butter product and 62 % blend of milk and vegetable fat, the initial total bacteria count $(1.5\pm0.3)\cdot10^7$ CFU/g had reduced to 2.5 %; 0.56 % and 5,0 % of the initial amount, respectively. The survival rate of *Staphylococcus aureus* was much higher. After freezing, the count of cells decreased by 3,5; 2.5 and 1,09 times in the blend of milk and vegetable fat, butter product with 68 % fat and butter with 82 % fat, respectively.

After freezing for 12 months, number of viable cells *Staphylococcus aureus* in butter, blend of milk and vegetable fat and butter product with 68 % fat decreased by 4,38; 4,21 and 2,1 times, respectively.

In assessing the impact of butter composition, the 82 % fat content butter retained the lowest number of microorganisms or only 22,8 % of the initial number. After 12 months of storage, in 68 % fat content butter product 23,7 % of the initial number was found. During the freezing and storage of fat blend enriched with vegetable oil the survival of microorganisms was 46,9 %.

43. SUSCEPTIBILITY AND MULTIDRUG-RESISTANCE OF *Arcobacter* ISOLATES WITHIN THE CZECH REPUBLIC

D.Šilha[1], L.Šilhová[1]

[1]Department of Biological and Biochemical Sciences, Faculty of chemical technology, University of Pardubice, Studentská 573, CZ-532 10 Pardubice, Czech Republic.

Corresponding author: David.Silha@upce.cz

Keywords: *Arcobacter* spp., antibiotic, antimicrobial testing, antibiogram, susceptibility

Background and objectives. *Arcobacter* spp. is Gram-negative, slender, spiral-shaped rods and belong to the family *Campylobacteraceae*. Many of these species have been isolated especially from poultry, meat, faeces, and from aborted cattle foetuses. Major route of *Arcobacter* transmission to human is consumption of contaminated foods of animal origin as well as non-treated water drinking . Resistance to commonly used antibiotics observed among *Arcobacter* spp. emphasises the importance of research in this area [1]. The aim of this study was to evaluate the antimicrobial susceptibility of *A. butzleri* and *A. cryaerophilus* isolated from meat poultry, waste water and human faeces within the Czech Republic.

Methods. In this study, 100 strains of *A. butzleri* and *A. cryaerophilus* were tested for their susceptibility to overall 18 antimicrobial agents. Susceptibility of *Arcobacter*-isolates were tested by the disk-diffusion method described earlier [2].

Conclusions. We report the antimicrobial susceptibility of *Arcobacter* isolates within the Czech Republic for the first time. It can be stated that the most of *Arcobacter*-isolates were resistant to one or more tested antimicrobial agents (99.0 %, n=99). Most of isolates were resistant to ß-lactam antibiotics and lincosasmides. Tetracycline, doxycycline and aminoglykosides were found to be highly efficient against both, *A. butzleri* and *A. cryaerophilus*. However, various *Arcobacter* isolates vary in their susceptibility to various antibiotics. The multidrug-resistance was found in case of 93.8% (n=75) of *A. butzleri* isolates and 70.0% (n=14) of *A. cryaerophilus* isolates.

Acknowledgements:

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44. MOLECULAR IDENTIFICATION OF INTESTINAL MICROBIOTA OF ROACH (*Rutilus rutilus* (L.)) IN RELATION TO RIVER WATER QUALITY AND DIET

V.Skrodenytė-Arbačiauskienė, V.Pliūraitė, A.Bartašiūtė, T.Virbickas

Department of Freshwater Biology, Nature Research Centre, Akademijos 2, LT-08412, Vilnius, Lithuania.

Corresponding author: V. Skrodenytė-Arbačiauskienė: svesta@ekoi.lt

Keywords: roach *Rutilus rutilus* (L.), intestinal microbiota, 16S rRNA gene, clone library method.

Background and objectives. Molecular techniques were applied to study the composition of allochthonous (transient) and autochthonous (adherent) microbiota in the gut of roach *Rutilus rutilus* (L.) living in the natural environment, as well as changes in microbiota composition under the impact of river pollution with nutrients and the diet. The share of food components of plant and animal origin in the diet of roach in the rivers of different water quality significantly differed. Percentage of plant material in the guts of roach ranged from 10-20% in the natural Žeimena River to 40-80% in the moderately polluted Mūša River and only to 2-4% in the heavily polluted Sidabra River. In clean and heavily polluted ecosystems, the allochthonous microbiota of roach was dominated by *Aeromonas* and *Carnobacterium*, whereas in the moderately polluted river, in addition to *Aeromonas*, we detected prevailing *Enterobacter*, *Citrobacter* and *Shewanella*. In roach allochthonous microbiota from the moderately and heavily polluted ecosystems we identified several species of bacterial fish pathogens. Phylogenetic analysis of sequences demonstrated that the roach autochthonous microbiota from clean ecosystem was dominated by bacteria closely related to the *Chryseobacterium* sp., *Shewanella haliotis*, *Halomonas* sp., *Curvibacter* sp. and *Propionibacterium acnes*. *Curvibacter* sp., *Halomonas* sp., *S. haliotis* and *P. acnes* were dominating in heavily polluted river. The dominant genus in the moderately polluted river was *Aeromonas*. The roach gut epithelium harbored also 8.5% of unidentified phylotypes which impact on their host is unclear.

Methods. Total DNA extraction, 1500 bp length 16S rRNA gene PCR, clone library method, sequencing and phylogenetic analysis.

Conclusion. Investigation demonstrated that both the composition of the diet of roach and the ecological status of the ecosystem affect the formation of gut microbiota.

45. MICROBIOLOGICAL MONITORING OF ENVIRONMENTAL SAMPLES FROM A WAREHOUSE AND VEGETABLES DURING THEIR STORAGE

I.Sumeri[1], M.Jaagura[1], M.Tamm[1]

[1]Competence Center of Food and Fermentation Technology, Akadeemia tee 15A, 12618 Tallinn, Estonia

Corresponding author: ingrid@tftak.eu

Keywords: postharvest losses, diseases of vegetables, metagenomics analysis

Recent studies show that vegetables are gaining more popularity in our menu. Vegetables are healthy, well balanced, with low calories and easily digestible. In Estonia fruits and vegetables are grown mostly environment friendly. Therefore the Estonian Horticultural Association foresees in its development plan for years 2015-2020 promotion of the production of domestic horticultural produce.

In this plan the tight cooperation between the farmers, producers and scientists is advised in order to solve different problems they face in this field.

One big problem in horticultural production is significant postharvest losses. They can be caused by a wide variety of factors, ranging from growing, handling and storage conditions. Among other causes there are various diseases of plants that destroy the crop even up to 40%. The infection of plants by fungi or bacteria can occur in the field before harvest, but also at any time after harvest of the crop. The success of preserving the harvest-fresh quality of produce demands control of each step in the system.

The aim of the current study was the microbiological screening of vegetable warehouse, where beets, potatoes and cabbages were stored in order to detect possible pathogens in environment that can affect the vegetables during storage.

The samples taken from different surrfaces in warehouse and from the vegetables were subjected to metagenomic analysis using Illumina MiSeq sequencing platform Sequence data was analyzed using BION-meta open source program or Mothur software.

The preliminary metagenomic results showed extremely wide variety of microorganisms in samples taken, which needs more comprehensive analysis to make strong conclusions about conditions in warehouse.

46. FIRST REPORT OF *Rhexocercosporidium carotae* CAUSING BLACK SPOT ON *Daucus carota* IN LITHUANIA

E.Survilienė[1], D.Burokienė[2], N.Rasiukevičiūtė[1], S.Kazlauskaitė[3]

[1]Institute of Horticulture, LRCAF, Kaunas Str. 30, Babtai, LT-54333, Kaunas dist., Lithuania.

[2]Institute of Botany at the Nature Research Centre, Žaliųjų Ežerų Str. 49, LT–08406 Vilnius, Lithuania.

[3]Aleksandras Stulginskis University, Studentų Str. 11, Akademija, LT-53361 Kaunas dist., Lithuania.

Corresponding author: daiva.burokiene@botanika.lt

Keywords: *Rhexocercosporidium carotae*, Acrothecium rot, *Daucus carota*, cold storage

Background and objectives. New disease on *Daucus carota* roots became one of the most important problems in Lithuanian carrot production during storage in refrigerated storerooms in 2005–2007 and 2012–2013. The crop yield reached up to 50–77 % in some farms in 2014–2016. The causal agent of the disease was the object of intense scientific research for some time already. Therefore, the main aim of the present study was to estimate the cause of the disease.

Methods. The post-harvest sanitary condition of the samples was assessed. Morphological, cultural, microscopic properties of the isolated fungi were described. Molecular methods are being applied for identification of the species.

Conclusions. Symptoms of the disease were specific irregular dark brown to black spots on carrots' roots. During this research, for the first time in Lithuania fungus has been isolated from severe injured carrots and identified as *Rhexocercosporidium carotae* (Årsvoll) *U. Braun* (sin. *Acrothecium carotae, Pseudocercosporidium carotae*) [1, 2]. The disease is often known as Acrotecium rot or black spots. Despite of the fact that the disease is known for a long time and the knowledge of its etiology is lacking, the protective measures against epidemics caused by these phytopathogens are being searched for.

Acknowledgments:

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47. GENERATION OF MOSAIC VIRUS-LIKE PARTICLES OF PARVOVIRUSES IN YEAST AS ANTIGENS FOR SEROLOGICAL ASSAYS

P.L.Tamošiūnas[1], K.Simutis[1], R.Petraitytė-Burneikienė[1], R.Firantienė[2], R.Ėmužytė[2], A.Žvirblienė[1], K.Sasnauskas[1]

[1] Institute of Biotechnology, Vilnius University, Saulėtekio al. 7, LT-10257 Vilnius, Lithuania

[2] Clinic of Children's Diseases, Faculty of Medicine, Vilnius University, Santariškių 4, LT-08406 Vilnius, Lithuania

Corresponding author: paulius.tamosiunas@bti.vu.lt

Keywords: human bocavirus, human parvovirus 4, capsid proteins, virus-like particles, ELISA

Background and objectives. Human bocavirus 1 (HBoV1) and human parvovirus 4 (PARV4) were identified in 2005 and are the new members of *Parvoviridae* family infecting humans. HBoV1 is reported to be among the four most frequent respiratory tract viruses. The clinical significance of PARV4 infection yet remains uncertain, but a variety of clinical associations have been reported [1]. Parvoviral capsids are composed of two structural proteins, VP1 and VP2. In native virion, VP1 contains active phospholipase A2 (PLA2) domain and was shown to harbour most of the neutralizing epitopes [2]. In the current study, different approaches to generate antigenic VP1 proteins of HBoV1 and PARV4 in yeast *S. cerevisiae* were used. Synthesis of full length VP1 proteins alone in yeast were inefficient, however, eliminating putative PLA2 domain substantially enhances expression levels. Nonetheless, yeast generated VP1 alone fails to form virus-like particles (VLPs). In cases of both HBoV1 and PARV4, co-expression of VP1 with VP2 in yeast resulted in stable ~25 nm diameter mosaic VLPs, composed of both structural proteins with ratio ~5:20, similar to that of native parvoviral virion. Recombinant mosaic VP1-VP2 VLPs of HBoV1 and PARV4 were evaluated as antigens for more sensitive serological assays to detect virus-specific IgG antibodies in human serum specimens.

Methods. Cloning of HBoV1 and PARV4 VP1 and VP2 proteins coding sequences into yeast vectors and purification of virus-like particles from yeast by CsCl ultracentrifugation. Electron microscopy. SDS-PAGE and Western blotting analysis. IgG capture ELISA.

Conclusions. Synthesis of minor structural protein VP1 of HBoV1 or PARV4 in yeast *S. cerevisiae* is efficient when PLA2 domain is eliminated or co-expressed with VP2. Recombinant mosaic VP1-VP2 VLPs of both HBoV1 and PARV4 were more reactive with seropositive human sera, thus offering more sensitive antigens for IgG serodiagnostics.

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48. THE NEW PEPTIDE-NUCLEOTIDES COMPOUNDS FROM *MCC*-LIKE OPERONS

D.Tsibuslkaya[1], O.Mokina[1,2], A.Kulikovsky[1], M.Serebryakova[1,3], K.Severinov[1,2,4] and S.Dubiley[1,2]

[1] Institute of Gene Biology, Russian Academy of Science, 34/5 Vavilova st., Moscow, Russia

[2] Skolkovo Institute of Science and Technology, Skolkovo Innovation Center, Building 3, Skolkovo, Russia

[3] Moscow State University Moscow, GSP-1, Leninskie Gory, Moscow, Russia

[4] Waksman Institute for Microbiology, The State University of NJ, 190 Frelinghuysen Road, Piscataway, New Jersey, United States

Corresponding author: 2650460@gmail.com

Microcins are a group of ribosomally synthesized peptides, produced by Enterobacteria and active against close relative species. Several microcins, one of such called microcin C (McC), have different post-translational modifications. Mature McC is an adenylate heptopeptide MRTGNAD with a phosphoramidate linkage between aspartate and adenylate. In addition, activity is improved by the attachment of an aminopropyl group to the phosphate residue. McC produced by *Escherichia coli*. is processed to non-hydrolyzed aspartate-adenylate and inhibits aspartyl-tRNA synthetase, thus it inhibits translation in sensitive cells. Genes of microcin C biosynthesis are encoded by the *mccABCDEF* operon. The most important gene involved in the process of post-translational modification is *mccB,* the product of which synthesizes the peptide-nucleotide compound [1]. In previous studies, it was found that *mcc*-like operons exist in different bacterial genomes, including *Bacillus amyloliquefacien*s, *Streptococcus equi*, *Yersinia pseudotuberculosis* [2]. Interestingly, these operons contain a longer *mccB* gene than *E.coli* has and the product of this gene has an additional C-terminal methyltransferase domain.

In our work, a variety of methods have been utilized: cloning, protein expression and purification, HPLC, Maldi-MS and MS/MS analysis and others.

Our results show that the product of these operons (*B. amyloliquefacien*s, *S. equi*, *Y. pseudotuberculosis*) is a cytidinylated peptide. We also found the *mccS* gene which is absent in the *E.coli mcc*-operon. We suggest that $MccB_{CTD}$ and MccS are involved in the process of carboxymethyl modifications of this peptide. To summarize, we described the new *mcc*-like operons that encode the synthesis of the peptide-carboxymethyl-cytidine antibiotic.

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49. ANTIBACTERIAL SCREENING OF BACTERIA ISOLATED FROM THREE CAVES IN TURKEY

E.F.Tufekci[1], N.Saglam Ertunga[2], E.Aydın[3], A.Biber[4, 5], B.Altay[5], İ.Tekkilic[5, 6], K.Tan[5], A.Reis[1], A.O.Kilic[1]

[1]Department of Medical Microbiology, Faculty of Medicine, Karadeniz Technical University, 61080 Trabzon, Turkey.

[2]Department of Chemistry, Faculty of Sciences, Karadeniz Technical University, 61080 Trabzon, Turkey.

[3]Department of Medical Physiology, Faculty of Medicine, Karadeniz Technical University, 61080 Trabzon, Turkey.

[4]Department of Biotechnology, Graduate School of Natural and Applied Sciences, Middle East Technical University, 06800 Ankara, Turkey.

[5]Cave Research Association, 06445 Ankara, Turkey.

[6]Department of Art History, Faculty of Letter, Hacettepe University, 06800 Ankara, Turkey.

Corresponding author: Enis Fuat Tufekci, eft88@hotmail.com

Keywords: 16S rRNA analysis, cave, cross-streak method

Backround and objectives: The increase of multi-drug resistance among bacteria is a major public health problem. Recent studies have focused on the discovery of new antimicrobials. In this study, we have aimed to determine antagonistic effects of various bacteria isolated from caves against representatives of indicator bacteria with cross-streak method.

Methods: Seventy-three strains of various bacteria were isolated from three caves, not open to the public. Suspension and dilution of samples were prepared in PBS and 0.1 mL of each dilutions were seeded on R2A agar, soil extract agar, plate count agar and actinomycetes isolation agar. The cultures were incubated up to 28 days at 28^{o}C. Bacteria with different colony morphologies and characteristics were isolated and were grouped by Gram staining. Antimicrobial activity screening of bacteria was done using cross-streak agar method against *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Listeria monocytogenes* ATCC 43251. Bacteria that showed antimicrobial activity were further identified by 16S rDNA sequence analysis.

Conclusions: Nine of 73 isolates showed antibacterial activity against at least one of the test organisms. Based on the 16S rDNA sequence analysis four strains were *Bacillus* spp., three *Streptomyces* spp., one *Amycolatopsis* spp., and one *Stenotrophomonas* spp. The results of the present investigation reveal that the cave bacteria can be of potential source of novel antibiotics.

50. POTENTIOMETRIC ANALYSIS OF *Saccharomyces cerevisiae* CELLS DURING DEHYDRATION/REHYDRATION

N.Venslauskaitė[1], L.Beiliūnaitė[1], D.Kulikova[2], A.Rapoport[2], R.Daugelavičius[1]

[1]Department of Biochemistry, Vytautas Magnus University, Vileikos 8, Kaunas LT-44404, Lithuania

[2]Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas street 1, Riga LV-1004, Latvia

Corresponding author: ven.neringa@gmail.com

Anhydrobiosis is one of amazing states of the cryptobiosis, when cells get into the unfriendly environment, in this case – the absence of water. To survive, the cells are adapting to changes in the environmental conditions by stopping their metabolism. The aim of this study was to determine the sequence of changes in *Saccharomyces cerevisiae* cell metabolism and energetics during the processes of dehydration/rehydration.

The intensity of cell respiration, the binding of phenyldicarbaundecaborane (PCB^-) anion to cellular membranes and the amount of cell-accumulated K^+ ions were measured by electrochemical monitoring system. Two *S. cerevisiae* strains were compared: strain 14 – semi-resistant and strain L77 – very resistant to dehydration. Mitochondrial resistance and sensitivity of plasma membrane permeability barrier to dehydration were determined. The investigated yeast strains considerably differed in these two aspects: strain 14 cells started to show damage at rather earlier stages of dehydration. We found that the main distinction of strain L-77 cells is the high intensity of respiration, especially in the presence of glucose. This substrate was able to energize L-77 cells even after 24 h of dehydration, while strain 14 cells stopped their respiration and showed no reaction to glucose after 14 h of drying. At the same time, measurements of K^+ ions indicated that permeabilization of the plasma membrane in cells of both strains occurred almost simultaneously – after 8-10 h of desiccation. The highest amount of PCB^- ions was bound to strain 14 cells after 12 h of dehydration, and the bound amount of this lipophilic anion to L-77 cells increased gradually during 22 h of drying. The effects of rehydration on physiological functions the cells also were determined.

Summarizing our results we can conclude that the main difference between *S. cerevisiae* cells of L-77 and 14 strains is the intensity of respiration and the ability to perform this process after rehydration from dehydrated state. The higher viability of strain L-77 cells could be due to altered functions and higher stability of mitochondria.

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51. CHARACTERIZATION OF LOW-TEMPERATURE *Escherichia* PHAGE VB_ECOS_NBD2

M.Vilkaitytė[1], L.Kalinienė[1], L.Truncaitė[1], A.Zajančkauskaitė[1], E.Šimoliūnas[1], K.Goda[2], R.Meškys[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, the Joint Life Sciences Centre, Vilnius University, Saulėtekio av. 7, LT-10257 Vilnius, Lithuania.

[2] Department of Eukaryote Gene Engineering, Institute of Biotechnology, the Joint Life Sciences Centre, Vilnius University, Sauletekio av. 7, LT-10257 Vilnius, Lithuania.

Corresponding author: m.vilke@gmail.com

Keywords: bacteriophage, host-range determination, low-temperature virus, TEM.

During a search for bacteriophages in Lithuanian agroecosystems, a novel *Escherichia* coli phage vB_EcoS_NBD2 (NBD2) was isolated from agricultural soil using *E. coli* NovaBlue for phage propagation. Host-range determination experiments revealed that NBD2 is capable of infecting many of the common *E. coli* K-12 laboratory strains as well as *E. coli* B derivatives. The e.o.p. (the effect of temperature on the efficiency of plating) test revealed that phage NBD2 is a low-temperature virus: the phage forms plaques on bacterial lawns in a temperature range of 10 - 34°C, and has an optimum temperature for plating ~20ºC. After 16 h of incubation at an optimum temperature, NBD2 produces circular smooth plaques of about 6 mm in diameter with a clear center and turbid edge.

Transmission electron microscopy (TEM) showed that the virion morphology of NBD2 resembles that of members of the family *Siphoviridae*. Phage NBD2 has an isometric head (~65 nm in diameter) and an apparently non-contractile flexible tail (~170 nm in length and ~12 nm in width). Although neither baseplate nor tail fibers have been clearly visible by TEM, several ORFs coding for putative tail fiber proteins have been detected during bioinformatics analysis of NBD2 genome.

Phylogenetic analysis revealed that *E. coli* phage NBD2 is distantly related to phages belonging to the subfamily Tunavirinae.

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52. BACTERIAL COMMUNITY DIVERSITY IN DYSTRIC-EPIHYPOGLEYIC ALBELUVISOLS

M.Vilkienė [1, 2], D.Ambrazaitienė [1, 2], Z.Dabkevičius [3]

[1] Lithuanian Research Centre for Agriculture and Forestry, Vezaiciai branch Gargzdai str. 1, Vezaiciai, Klaipeda distr., Lithuania.

[2] Klaipeda University, Herkus Mantas str. 84, Klaipeda.

[3] Lithuanian Research Centre for Agriculture and Forestry Instituto 1, Akademija, Kedainiai distr., Lithuania.

Corresponding author: vilkiene.monika@gmail.com

Keywords: Soil community analysis, metagenomics, 16S rRNA, long-term tillage.

Soil is a complex ecosystem that is part of our biosphere. The ability of soil to provide ecosystem services is dependent on microbial diversity. The composition, abundance, and activity of soil microbial community not only depends on climate and soil physicochemical properties, but also is closely linked to plant communities through complex interaction [1] Although the number of microbial species in soil is still being debated, the metagenomic approach to estimate microbial diversity predicted about 2000 – 18 000 bacterial genomes in 1 g of soil [2]. This study focused on metagenomic analysis of bacterial diversity in long-term conventional tillage plots of *Dystric Epihypogleyic Albeluvisols (according FAO 1989), Retisols (according WRB, 2014)* in western part of Lithuania.

Soil samples for DNA purification was taken in 2015 autumn. Genomic DNA from soil sample was extracted from approximately 200 mg field-moist soil by using the D6005 Fungal/Bacterial Miniprep set (Zymo Research®) following the manufacturer's instructions. Purified DNA samples were amplified using bacterial 16S rRNA gene. Metagenomic sequencing was made with Ilumina MiSeq platform in Base Clear Company.

In our study for first time in Lithuanian agronomy history using pyrosequencing technology was identified all soil bacteria diversity. The total number of sequenced reads for soil samples was 131 194. The 10 most abundant genus soil in sample was *Arthrobacter, Candidatus Saccharibacteria, Actinobacteria, Acidobacterium, Mycobacterium, Bacillus, Alphaproteobacteria, Longilinea, Gemmatimonas, Solirubrobacter*.

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53. INVESTIGATION OF THE HOST RECEPTORS FOR *Escherichia coli* PHAGES

A.Zajančkauskaitė, L.Alijošius, L.Kalinienė, M.Vilkaitytė, R.Meškys, L.Truncaitė

Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, JGMC, Vilnius University, Saulėtekio av. 7, LT-10257 Vilnius, Lithuania.

Corresponding author: aurelija.zajančkauskaitė@bchi.vu.lt

Key words: *Escherichia coli*, tailed bacteriophages, receptor specificity

A diversity of protein or lipopolysaccharide (LPS) structures of bacterial cell surface can serve as the receptors for *Escherichia coli* phages [1, 2]. Host-specificity of tailed bacteriophages can be determined solely by tail fiber proteins or even by the protein domains, so called adhesins. These receptor-recognizing proteins are usually specific to the particular component of certain structure and can serve as an attractive tool for studying protein-protein or protein-sugar interactions.

Tailed *E. coli* bacteriophages vB_EcoM_FV3 (later FV3), vB_EcoM_VpaE1 (VpaE1) [3, 4] and vB_EcoM_Alf5 (Alf5) have been isolated in Lithuania and sequenced. *E. coli* B and K12 strains, as well as the single-gene knockout mutants of *E. coli* K-12 strain BW25113 (Keio collection) were used for the identification of phage receptors. In addition, proteinase K and periodate tests were used to clarify whether the receptor of these phages is a protein or a carbohydrate.

Spot assays of the decimal dilutions of phage suspensions and the adsorption tests using the proteinase K- or periodate-treated *E. coli* cells indicated that the carbohydrate component of the cell surface served as a receptor for all phages tested. However, using single-gene deletion mutants for genes involved in LPS core biosynthesis we showed that all phages recognized different sugar moieties of the *E. coli* LPS. The putative tail fiber proteins encoded by the phages FV3, VpaE1 and Alf5 are under further investigation.

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