ABSTRACTS

of the

5th Central European Forum
for
Microbiology

Guest-Editors

K. MÁRIALIGETI and O. DOBAY

October 18-20, 2017
Keszthely, Hungary

Acta Microbiologica et Immunologica Hungarica, 64 (Suppl), pp. 105–192 (2017)
DOI: 10.1556/030.64.2017.102

COMPARISON OF SEQUENTIAL- AND STRUCTURAL EVOLUTION REVEALS PRINCIPLES OF GENOME EVOLUTION OF THE FISSION YEASTS

LAJOS ÁCS-SZABÓ, LÁSZLO ATTILA PAPP, MATTHIAS SIPICZKI, IDA MIKLÓS

Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen, Hungary

Eukaryotic genomes evolve through accumulation of small-scale changes like point mutations, nucleotide insertions and deletions in their sequences. On the other hand, chromosomal rearrangements (inversions, translocations, fusions and fissions) can also contribute to reshuffling of the gene order. It is widely assumed that there is correlation between nucleotide and structural evolution. However, counterexamples coming from comparative sequence analyses were also revealed. As each genome has their own uniqueness, further investigation of genome evolution can help us to better understand how the different genomes were organized and changed, when and why natural selection acted. The fission yeasts (*Schizosaccharomyces*) form an ancient clade within the Ascomycete fungi and have a distinct life history from other yeasts. It has been reported that conservation of the gene content within *Schizosaccharomyces* is significantly higher than that within *Saccharomyces* or *Kluyveromyces*. Moreover, gene order and gene structure of the fission yeasts are also more conserved than expected from levels of their amino acid divergences. These observations suggest that fission yeast amino acid sequences evolve anomalously fast or that their genome structures are unusually stable. Thus, stable genome structures of fission yeasts could be consequences of insufficient rearrangements, and because of this the perceived genome conservation is just a remnant of the ancestral gene order. According to another scenario, genetic rearrangements could occur frequently, but the ancient gene order still was maintained by natural selection. In order to gain more information on the topic of genome evolution of the fission yeasts, a comparative study of sequences was carried out. Sequences of 30 fungal species of several different clades were compared and sequence evolution and genome rearrangements were investigated. We created global and genus level phylogenetic trees using the concatenated sequences of 18 putative orthologues. In addition to that, we performed whole genome alignments among the species and estimated the minimum number of rearrangements occurred by the help of locally collinear blocks found (chromosomal fragments, which does not contain any rearrangements). Thereafter, we compared the branch lengths of the phylogenetic trees to the number of rearrangements. Our results suggest that it was not only amino acid sequences of the fission yeasts that were evolving fast but their genomes were also undergoing numerous rearrangements.
That is, observed conservation of the gene content and gene order within the fission yeasts probably does not come from incomplete genome reshuffling. We assume that stable genome structures of the *Schizosaccharomyces* species are likely to have been maintained by natural selection rather than as a result of the fact that there had been few chromosomal rearrangements during their evolution.

A MECHANISM FOR A SINGLE NUCLEOTIDE INTRON SHIFT

NORBERT ÁG[1], MICHEL FLIPPHI[1], GUSTAVO CERQUEIRA[2], CLAUDIO SCAZZOCCHIO[3], LEVENTE KARAFFA[1], ERZSÉBET FEKETE[1]

[1]Department of Biochemical Engineering, University of Debrecen, Debrecen, Hungary; [2]Broad Institute of MIT & Harvard, Cambridge MA, USA; [3]Department of Microbiology, Imperial College London, UK

Spliceosomal introns can occupy nearby rather than identical positions in orthologous genes (intron sliding or -shifting). Stwintrons are complex intervening sequences, where an "internal" intron interrupts one of the sequences essential for splicing, generating after its excision, a newly formed canonical intron defined as "external". In one experimentally demonstrated configuration, two alternatively excised internal introns, overlapping by one G, disrupt respectively the donor and the acceptor sequence of an external intron, leading to mRNAs encoding identical proteins. In a gene encoding a DHA1 antiporter in Pezizomycotina, we find a variety of predicted intron configurations interrupting the DNA stretch encoding a conserved peptidic sequence. Some sport a stwintron where the internal intron interrupts the donor of the external intron (experimentally confirmed for *Aspergillus nidulans*). In others, we found and demonstrate (for *Trichoderma reesei*) alternative, overlapping internal introns. Discordant canonical introns, one nt apart, are present in yet other species, exactly as predicted by the alternative loss of either of the internal introns at the DNA level from an alternatively spliced stwintron.
An evolutionary pathway of one nt intron shift, involving an alternatively spliced stwintron intermediate is proposed on the basis of the experimental and genomic data presented.

UNDERSTANDING THE GENOMIC CHANGES LEADING TO MORPHOLOGICAL SIMPLIFICATION IN FRUITING BODY FORMING FUNGI

ÉVA ALMÁSI[1], KRISZTINA KRIZSÁN[1], ARUN PRASANNA[1], BRIGITTA KISS[1], BÁLINT BALÁZS[2], ISTVÁN NAGY[2], LÁSZLÓ NAGY[1]

[1]Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged; [2]Seqomics Biotechology Ltd., Mórahalom, Hungary

The evolution of complex multicellular development is orchestrated by a range of genetic innovations, among which changes to the regulatory repertoire are generally considered to be the most important. Throughout evolution we can observe a definite tendency of morphological complexity being coupled with the size of the gene regulatory apparatus – mainly with the number of transcription factors. Our study focuses on placing morphologically simplified organisms in this hypothesis: Are we supposed to see a decreased number of transcription factors in simplified organisms compared to their more complex relatives? In order to find out we performed comparative genomic analysis on 41 fruiting body forming Basidiomycete fungi and comparative transcriptome analysis during the five equivalent developmental stages on four species of the above mentioned group (*Armillaria ostoyae*, *Coprinopsis cinerea*, *Schizophyllum commune*, *Auriculariopsis ampla*) – two complex and two simplified fruiting body forming ones. For the experimental verification of the role of key transcription factors during the development we are planning to use DNA-Affinity-Purification-Sequencing (DAP-Seq) to identify the target genes controlled by a given regulator. Surprisingly the morphologically simplified organisms contain a higher number of transcription factors than most of the species with a more complex morphology. Examining the age distribution of

transcription factors we discovered that genomes of simplified organisms contain the highest proportion of lineage or species specific regulator genes. Furthermore the RNA-Seq data shows that these newly evolved genes play a crucial role during fruiting body formation – so their development is less characterized by conserved regulators. Our results contribute to a more comprehensive understanding of the emergence of species with morphological simplification and suggest the decreased level of complexity as a favorable attribute. The species we observed might be interesting examples of adaptive evolutionary processes resulting in simplified morphology.

COMPREHENSIVE ANALYSIS OF HXNT, AN ENZYME OF THE NICOTINATE CATABOLIC ROUTE

JUDIT ÁMON, ESZTER BOKOR, CSABA VÁGVÖLGYI, ZSUZSANNA HAMARI

University of Szeged, Faculty of Science and Informatics, Department of Microbiology, Szeged, Hungary

Since the 1990s, the investigation of catabolic processes of aromatic- or heterocyclic rings by microbes increased significantly in order to aid and make more cost –effective of the production of drugs, pesticides and intermediate chemicals. Because nicotinic acid (niacin, vitamin B3) is a carboxylic derivative of pyrimidine, the research topic of nicotinic acid degradation became popular. In the last few years four different nicotinic acid catabolic routes were described in prokaryotes, but in eukaryotes the catabolic process remained completely unknown. The investigation of the first eukaryotic nicotinic acid catabolic pathway established by our research group had already revealed the genetic background and regulation of the pathway in the model organism *Aspergillus nidulans*. We have identified eleven co-regulated *hxn* genes organized in three gene clusters. The first cluster, NDC1 (Nicotinic acid Degradation Cluster 1) comprised of 6 genes, *hxnS, hxnR, hxnP, hxnT, hxnY* and *hxnZ*. The *hxnR* codes the pathway-specific transcription factor, *hxnP* and *hxnZ* gene products are transporters and *hxnS* encodes nicotinate hydroxylase, which makes the first step of the catabolic process by hydroxylating nicotinate to 6-hydroxynicotinic acid. The *hxnY* and *hxnT* genes code for an α-ketoglutarate dependent dioxygenase and a flavin oxidoreductase. In this work we present the study of HxnT, which shows 34 % similarity to the *Candida albicans* Q5A0J5 protein, a potential NADH-dependent flavin oxidoreductase. The nearest structural analogue of HxnT is the 12-oxophytodienoate reductase 3 (OPR) from tomato. We show the modelled structure of HxnT superposed with that of OPR and the strict conservation of the FMN binding domain.

We experimentally prove that HxnT bears with NADH dehydrogenase activity, which is consistent with the predicted enzymatic properties of HxnT.

Support: National RDI Office (NKFI-K16 119516); GINOP-2.3.2-15-2016-00035.

NICHE DIFFERENTIATION OF BACTERIAL COMMUNITIES IN THE MOLNÁR JÁNOS HYPOGENIC CAVE (BUDA THERMAL KARST SYSTEM, HUNGARY)

DÓRA ANDA[1], JUDIT MAKK[1], ATTILA SZABÓ[1], LAURA JURECSKA[1], GERGELY KRETT[1], JUDIT MÁDL-SZŐNYI[2], KÁROLY MÁRIALIGETI[1], ANDREA K. BORSODI[1]

[1]Department of Microbiology; [2]Department of Physical an Applieg Geology, Faculty of Science, ELTE Eötvös Loránd University, Budapest, Hungary

The Molnár János Cave (MJC) is part of the northern Rose Hill discharge area of Buda Thermal Karst System (Hungary) and, is the largest active thermal water cave in Europe. Discharges of two types of water are characteristic for this area with different temperatures, therefore, mixed corrosion proved to be the dominant cave forming process. This study looked at different ecological niches (warm, cold and mixed water, sediment and reddish-brown biofilm on the subaqueous cave wall) within the phreatic zone of the cave system.The aim of this study was to gain detailed information about the taxonomic diversity of microbial communities using next generation sequencing (NGS). Samples for the microbiological examinations were collected from the MJC in January 2014. Proteobacteria, Acidobacteria, Chlorobi, Chloroflexi and Nitrospirae were the most abundant phyla. Unlike the water samples, relative abundance of Planctomycetes and Chloroflexi was also high in the sediment and biofilm samples. Dominance of ammonia-oxidizing bacteria (*Nitrosococcus*) and nitrite-oxidizing bacteria (Nitropspirae) were revealed from the cold water sample. Members of the phylum Nitrospirae were also abundant in the cave sediment sample. The mixed and warm water samples were predominated by the genus *Sulfuricurvum* related sequences belonging to the class Epsilonproteobacteria but this class was almost completely absent in other samples. It seems that water chemical characteristics were critical for niche differentiation among major bacterial groups participating in the nitrogen and sulfur cycle of MJC.

This research was supported by the Hungarian Scientific Research Fund (OTKA) Grant NK101356.

APPLICATIONS OF NETWORK BIOLOGY TO FUNGAL BIOTECHNOLOGY

MIKAEL RØRDAM ANDERSEN

Technical University of Denmark, Department of Biotechnology and Biomedicine, Lyngby, DK

In every aspect of biology, networks appear as self-organizing structures governing interactions from the molecular scale over organisms to entire ecosystems. As such, networks have a large potential for both understanding and engineering biological systems.

This talk will illustrate aspects of network biology and how this different perspective. on biotechnology can be applied to understand topics such as gene order on chromosomes, species evolution, cellular (primary) metabolism, and genome diversity at the species and genus level. In particular aspects relevant to cell factory-based production of small molecules will be explored.

UNUSUAL HIV-JCV CO-INFECTION

ÁKOS VINCE ANDREJKOVITS[1], CARMEN CHIRIAC[2], ANDREA INCZE[2], ADINA STOIAN[3], ANCA GEORGESCU[2], ERZSÉBET IRINGÓ ZAHARIA KÉZDI[2]

[1]1st Infectious Disease Clinic, Mures County, Targu Mures; [2]Department of Infectious Diseases, University of Medicine and Pharmacy Tîrgu-Mureş; [3]Department of Pathophysiology, University of Medicine and Pharmacy Tîrgu-Mureş, Romania

The polyoma virus, named JC virus (JCV) has a worldwide distribution, with a seroprevalence of 39% to 69% among non HIV-infected adults. Progressive multifocal leukoencephalopathy (PML) is

an opportunistic infection of the central nervous system (CNS) due to JCV. PML is rare in other immunocompromised conditions than HIV, but in this category of patients appears usually when the CD4 T-lymphocyte (CD4) cell counts are below 200 or 100 cells/mm^3. PML very rare occurs in patients treated effectively with ART. A 27-years-old male patient, diagnosed with HIV infection in 2003 (in category C3 AIDS), in the first few years of infection was not adherent to the antiretroviral therapy (ARVT). He had an ischemic stroke attack in the teritory of right arteria cerebri media (CD4 cell: 24/mm³, viral load: 99944 copies/ml), with good neurological recuperation, after this episode he became adherent to the ARVT. A progressive neurologic deterioration starts from 2015, with pronounced muscle weakness on the left side, balance disorders, aggravated in time to truncal ataxia, with positive Romberg test, left hemiparesthesia and hemiparesis grade 3, dysarthria, osteotendinous reflexes with brisk response on the left side, pathological Babinski sign bilateral, he became bradilalic and bradipsihic. From 2012 has undetectable viral load, with progressive increase in CD4 cell count, without immunreconstruction syndrome. Brain and cervical MRI was effectuated in 2016, which described cortical atrophy, with supratentorial localization, white matter hiperintensities with periventricular and fronto-parietal localizations. Cerebrospinal fluid (CSF) examination: glucose level: 61.41 mg/dL, Pandy reaction was negative, protein level: 38.9 mg/dL, smear with Gram stain: without inflammatory cells or bacteria, JCV DNA from CSF was positive (CD4 cell: 877/mm³, viral load: <20 copies/ml). Other tests as cytomegalovirus, toxoplasma – acute phase antibodies were negative, treponema pallidum: negative, respective serologic tests for hepatitis B and C were negative. He received a treatment Mega HAART with enhanced CNS Penetration Effectiveness (CPE) score. A biologically and immunologically well controlled patient, in category C3 of HIV infection, developed PML an unexpected disease. With multidisciplinary approach the patient's condition became neurologically stable, but with an uncertain prognosis. The diagnostic pitfall was to accept or not the JC virus presence as a trigger for neurologic damage. According to the imagistic findings and to the laboratory results this was the best option at that moment. We need for the best management of the patient to repeat the CSF examination.

MICROBIAL MAT FORMING EXTREMOPHILIC BACTERIA IN A HIGH-ALTITUDE SALINE LAKE (LAGUNA VERDE, CHILE)

JÚLIA MARGIT ASZALÓS[1], BALÁZS NAGY[2], ATTILA SZABÓ[1], LAURA JURECSKA[1], ANDREA K. BORSODI[1]

[1]Department of Microbiology; [2]Department of Physical Geography, Faculty of Science, ELTE Eötvös Loránd University, Budapest, Hungary

Laguna Verde (4350 meters above sea level) is a high-altitude saline lake located in the Puna de Atacama plateau, Dry-Andes that harbors microbial mats formed by hitherto unknown microbial communities. The plateau is a mountain desert characterized by aridity, great daily temperature range, intense solar radiation and volcanic features, therefore considered an extreme environment. Surrounded by hot springs and lagoons with water temperature as high as 34.5°C and slightly alkaline pH (8.8), Laguna Verde is one of the most exciting examples of high-altitude saline lakes on a volcanic field. Although a preliminary study revealed the presence of polyextremophilic bacteria in lakes of the Puna de Atacama plateau, due to the remoteness of this site, our knowledge about these bacterial communities is still sparse. Samples of microbial mats formed on the rock bed of a warm lagoon in the littoral zone of the lake were taken in 2016 February. 16S rRNA gene based molecular methods were applied to assess the structure and composition of mat forming bacterial communities. UPGMA clustering of denaturing gradient gel electrophoresis molecular fingerprints showed explicit differences amongst samples taken from various layers of the microbial mats. To further analyze this

phenomenon a selected set of samples were chosen for a more in depth analysis using high-throughput 16S rRNA gene amplicon sequencing (IonTorrent).

TRANSMITTED DRUG RESISTANCE IN NEWLY DIAGNOSED, TREATMENT-NAIVE, HIV TYPE 1-INFECTED PATIENTS IN HUNGARY

ÉVA ÁY[1], MÁRIA MEZEI[1], ÁGNES POCSKAY[1], ANITA KOROKNAI[1], ZOLTÁN GYŐRI[1], MÁRTA MARSCHALKÓ[2], BÉLA TÓTH[2], SAROLTA KÁRPÁTI[2], BOTOND LAKATOS[3], JÁNOS SZLÁVIK[3], MÁRIA TAKÁCS[1], JÁNOS MINÁROVITS[4]

[1]National Public Health Institute; [2]Semmelweis University; [3]Center for HIV, St István and St László Hospital, Budapest; [4]University of Szeged, Szeged, Hungary

Widespread accessibility of antiretroviral therapy has caused emergence of transmitted drug resistance (TDR) among HIV-positive, treatment-naive individuals. The aim of this study was to monitor the presence of transmitted drug resistant HIV-1 strains in newly diagnosed, treatment-naive patients in Hungary. 82 HIV-infected patients diagnosed between 2013 and 2016 were included into the study. Antiretroviral drug resistance was determined by sequencing the HIV protease and partial reverse transcriptase coding regions. The majority of patients (95,1%) belongs to the MSM (men who have sex with men) group. The sequences were analysed using the Stanford HIV Drug Resistance Database algorithm to determine surveillance drug resistance mutations and susceptibility to antiretroviral inhibitors. Phylogenetic analysis was used to confirm the detected subtypes of HIV-1 and to identify transmission clusters among patients. Genotyping of pol sequences revealed that the majority of patients carried subtype B of HIV-1 strains (95,1%), while subtype F, A and CRF02_AG was also detected. The overall prevalence of transmitted drug resistance was 9,76% (8/82; 95% CI: 5,03-18,09%). Nucleoside reverse transcriptase inhibitor (NRTI) associated resistance mutations, M41L and T215E were the most frequent indicators of TDR (6/82; 7,32%; 95% CI: 3,40-15,06%), followed by resistance mutations associated with non-nucleoside reverse transcriptase inhibitors (NNRTIs) (2/82; 2,44%; 95% CI: 0,67-8,46%) and protease inhibitors (PIs) (1/82, 1,22%; 95% CI: 0,22-6,59%). The prevalence of TDR in Hungary is comparable to the data reported in other studies on subtype B HIV-1- infected MSM groups in Europe.

CHANGES IN THE DOMINANT CARBAPENEMASE GENE IN *ACINETOBACTER BAUMANNII*

BENCE BALÁZS, JULIANNA MÓZES, IDAN BLUM, ANDRÁS SZILÁGYI, GÁBOR KARDOS

Department of Medical Microbiology, University of Debrecen, Debrecen, Hungary

Earlier work from our group has reported a high prevalence rate of *blaoxa23*-like carbapenemase gene in *Acinetobacter baumannii* clinical isolates in 2010-2011 together with high prevalence of aminoglycoside resistance genes *aac(6')*-Ib and *aph(3')*-VIa and appearance of the *armA* aminoglycoside resistance methylase gene. Carbapenem resistance appeared to be linked to carbapenem, especially meropenem consumption. Recently we observed appearance of a new resistance phenotype, characterised by tobramycin and amikacin susceptibility. The investigates the dynamics of carbapenem resistance in more recent *Acinetobacter baumannii* isolates and its

relationship with antibiotic consumption with the aim of examination of the observed strain switch. Isolates were identified using Microflex MALDI-TOF and the presence of *blaOXA-51*-like genes. Disk diffusion susceptibility tests were carried out using the EUCAST guidelines. Carbapenem resistance genes *blaOXA-23*-like and *blaOXA-24*-like earlier shown to appear, were sought for using PCR assays. Prevalences were compared using Fisher exact test or chi square test as appropriate. From 2012, 2013, 2014, 2016 and 2017 (January-April) 74, 118, 128, 136, and 65 *A. baumannii* isolates were collected and tested, respectively, to be compared to the 160 isolates tested in the above mentioned earlier work for the presence of carbapenem-hydrolysing oxacillinase genes (*blaOXA-23*-like, *blaOXA-24*-like). Monthly antibiotic consumption was measured in DDD/100 beddays between 2010 and 2016. Carbapenem consumption in the observation period increased markedly (yearly means 2.3 and 4.0 DDD/100 beddays in 2010 and 2016, respectively). Colistin consumption increased even more steeply, from 0.3 to 1.6 DDD/100 beddays. Aminoglycoside consumption, in contrast, decreased from 2.2 to 1.7 DDD/100 beddays. The prevalence of *blaoxa-23*-like genes steadily increased between 2010/2011 and 2014 (78.1%, 125/160 vs. 87.8% 65/74 vs. 88.8% 105/118 vs. 93.8% 120/128 in 2010/11, 2012, 2013 and 2014, respectively; p=0.01-p<0.001 between years). In 2016, a significant drop have been found in the prevalence of *blaoxa-23*-like (28,7% 39/136, p<0.001 in comparison with 2014), which decreased further in 2017 (18.5% 12/65). The *blaoxa-24/40*-like gene behaved differently, occurring infrequently (1,3%, 2/160 in 2010-11 vs. 0.0% 0/128 in 2014), however, its prevalence increased significantly in 2016 and 2017 (75.0% 102/136 vs. 92.3% 60/65, p<0.001 in all comparisons). In conclusion, prevalences of OXA-carbapenemase genes switched between 2014 and 2016, suggesting a strain switch in *Acinetobacter baumannii*. The driving force for this may be increasing colistin consumption or decreasing aminoglycoside usage; however, further experiments are needed to uncover the exact reasons.

G. Kardos was supported by János Bolyai Resarch Scholarship of the Hungarian Academy of Sciences.

HUMAN PAPILLOMAVIRUSES: FROM INFECTIOUS ENTRY TO MALIGNANCY

LAWRENCE BANKS

ICGEB, Trieste, Italy

Human Papillomaviruses (HPV) are major causes of human cancers, being responsible for over 5% of the global cancer burden. Cervical cancer is by far the most important, and this is caused by infection with a small subset of so-called high risk HPV types, of which HPV-16 and HPV-18 are the most important. HPVs are believed to gain entry into the basal keratinocyte through microtraumas in the skin, and following a complex route of endocytic transport, the viral genome eventually gains entry into the nucleus and a new productive life cycle initiates. Normally these infections are self limiting, but in rare cases these can remain persistent for many years, and ultimately progress towards the development of malignancy. These tumours are caused by the combined action of two viral oncoproteins, E6 and E7, and their continued expression is required for maintenance of the tumour phenotype. In this presentation we will review how the incoming virus redirects components of the cellular endocytic transport machinery to attain a successful virus infection. We will also highlight aspects of E6 function which play a major role in the ability of these viruses to cause cancer.

ANTIBACTERIAL ACTIVITY OF HYDRAZONE-BASED TRANSITION METAL COMPLEXES

BERTA BARTA HOLLÓ[1], JÓZSEF MAGYARI[1], ANNAMÁRIA KINCSES[2], MÁRIÓ GAJDÁCS[2], MÁRTA NOVÉ[2], BORISZ VARGA[2], ÁKOS CSONKA[2], GABRIELLA SPENGLER[2], KATALIN MÉSZÁROS SZÉCSÉNYI[1]

[1]Department of Chemistry, Biochemistry and Environmental Protection,University of Novi Sad, Novi Sad, Serbia; [2]Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Hungary

The treatment of infectious diseases remains an important and challenging problem, and the emergence of multidrug resistant bacterial pathogens revealed a pressing need for new classes of antimicrobial agents. Biological metal ions play key roles in the biological functions of enzymes and in the structural organisation of various organisms. Pyridazine-based metal complexes have received substantial attention as potential pharmacological agents, however there is very little known about their characteristics as antimicrobials. The aim of our study was the evaluation of the antibacterial activity of novel hydrazone-type ligands with N-donor atoms and their coordination compounds with various transition metals [Co(III)/Co(II), Ni(II), Cu(II), Zn(II)]. Sixteen compounds were investigated in our study. The antibacterial activity of the tested compounds was assessed against various Gram-positive and Gram-negative reference strains using the broth microdilution method, according to CLSI laboratory standards. An efflux pump expressing strain, and its corresponding pump mutant was also included in our experiments, to determine the influence of these pumps on the MICs of the derivatives. The hydrazone-type ligands did not present any antibacterial activity, and the minimal inhibitory concentrations of the tested metal-containing complexes were >20 μg/ml against all Gram-negative bacteria. The Co(III)-containing coordination compounds exerted potent antibacterial activity against Gram-positive reference strains.

The presence or absence of the efflux pump did not alter the MICs of the tested compounds. Since the ligands themselves did not have antibacterial properties, the level of activity should be modulated by the nature of the metal ions incorporated inside the complex. While their mode of action is unclear at this time, the Co(III)-containing hydrazone-based metal complex showed promising antimicrobial properties against important Gram-positive pathogens. Based on our results, their antimicrobial activities should be further elucidated using alternative model systems.

This study was supported by the project GINOP-2.3.2-15-2016-00038. This study was also supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

AN APPROACH FOR COMPLETE ENVIRONMENTAL REHABILITATION OF A HUNGARIAN RAILWAY STATION AREA POLLUTED WITH USED LUBRICATING OILS (ULOS): A CASE STUDY

ATTILA BODOR[1, 2], PÉTER PETROVSZKI[1], NAILA BOUNEDJOUM[1], ÁGNES ERDEINÉ KIS[1, 2], KRISZTIÁN LACZI[1], GÁBOR RÁKHELY[1, 2], KATALIN PEREI[1, 2]

[1]Department of Biotechnology; [2]Institute of Environmental and Technological Sciences, University of Szeged; [3]Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Pollution of soils and waters by oil-related compounds can occur in natural environments and also in built-up or industrial areas due to transportation, accidents or improper storage. Lubricating oils (LOs) with varying composition are mostly produced for reducing friction in engines of motorized vechicles such as cars, motorcycles or locomotives, therefore, used lubricating oils (ULOs) containing long chain hydrocarbons, additives and heavy metals are considered as widespread, hazardous

pollutants and hence potential targets of different rehabilitation processes. Several physicochemical and biological waste management techniques are available for neutralizing oil-related pollutants in the environment. Bioremediation, which involves the use of plants and/or microorganisms, is one of the most promising approach, since it is an environmentally friendly and cost effective technology. For bioremediation of a railway station area contaminated with ULOs near Szeged, the most effective hydrocarbon-degrading bacterial strains from our departmental strain collection were tested for biodegradation of fresh lubricating oil in liquid minimal salt medium. Then, they were further used in small-scale biodegradation experiments performed in the ULO-contaminated soil to model and monitor a bioremediation process. Although the optimal conditions of biodegradation are still unveiled and require further development, our results represent a targeted tool for bioconversion of these common petroleum-related wastes and on the other hand, brings us closer to our final goal aiming the complete bioremediation of a ULO-polluted railway station area.

Supported by the European Union and Hungarian State (grant agreement no. EFOP-3.6.2-16-2017-00010) and by the Norway Grant (grant agreement no. HU09-0044-A1-2013).

FUNCTIONAL ROLE OF HXN GENES IN THE NICOTINATE CATABOLISM OF *ASPERGILLUS NIDULANS*

ESZTER BOKOR, JUDIT ÁMON, CSABA VÁGVÖLGYI, ZSUZSANNA HAMARI

University of Szeged, Faculty of Science and Informatics, Department of Microbiology, Szeged, Hungary

Many microorganisms are able to utilize nicotinic acid as sole nitrogen-source, however the degradation of nicotinic acid was studied only in a limited number of prokaryotes. In eukaryotes the nicotinic acid degradation process is completely unknown, only the first step of the nicotinic acid catabolism of *Aspergillus nidulans* had been thoroughly studied which is carried out by the Purine hydroxylase II (coded by *hxnS*). As a result of our previous studies we identified the genetic background of the nicotinate catabolic pathway of *A. nidulans*. We found a set of co-regulated genes organized in three clusters (Nicotinic acid Degradation Cluster 1, 2 and 3 - NDC1, NDC2 and NDC3 - comprised of *hxnP/S/T/Y/R/Z*, *hxnX/W/V* and *hxnM/N*, respectively). Through systematic deletion of the *hxn* genes, the role of the *hxn* gene products could be assessed. Here we present the result of growth tests, which led us to reveal that HxnS, HxnT and HxnY are involved in the initial steps of the degradation and that the pathway splits up to alternative routes right after the first step where nicotinic acid is converted to 6-hydroxynicotinic acid. Furthermore, we revealed that the alternative routes meet at one point from where the HxnV, HxnX, HxnW and HxnM further carry on the process. Based on these results we improved our concept of the nicotinic acid degradation pathway.

Supported by the National RDI Office (NKFI-K16 119516) and by GINOP-2.3.2-15-2016-00035.

POULTRY MANURE FOR EFFICIENT BIOGAS PRODUCTION AS MONO-, AND CO-SUBSTRATE FOR BIOGAS AND BIOHYDROGEN PRODUCTION

TAMÁS BÖJTI[1], KORNÉL L. KOVÁCS[1, 2, 3], BALÁZS KAKUK[1], ROLAND WIRTH[1], GÁBOR RÁKHELY[1, 2], ZOLTÁN BAGI[1]

[1]Department of Biotechnology, University of Szeged; [2]Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences; [3]Department of Oral Biology and Experimental Dental Research, University of Szeged, Szeged, Hungary

Biogas technologies from by-products offer several benefits: the substrates are available in large quantities, the generated energy carrier is environmentally friendly, easy to transport and store. Poultry manure represents a difficult case being unsuitable for anaerobic degradation as monosubstrate. The problem of poultry waste management has been an increasing concern in many parts of the world due to the huge amount of this waste stream. Chicken manure (CM) contains two main forms of nitrogen: uric acid and undigested proteins, which represent 70% and 30% of the total nitrogen in CM, respectively. Anaerobic decomposition of uric acid and undigested proteins in CM results in high amounts of unionized ammonia and ammonium ions, which inhibits methanogenesis. The possibility of water extraction of a portion of the inhibitory nitrogen content from CM was tested. After two days of soaking solid CM in tap water at room temperature, the liquid and solid phases could be separated by centrifugation or sedimentation. Anaerobic fermentation of the solid fraction became sustainable when the reactors were fed with treated CM (T-CM) as monosubstrate. Although treated CM had increased C/N ratio, co-fermentation could further improve the anaerobic digestion process. Corn stover is the by-product of corn derived starch production, which is usually incinerated or plough back in the soil. Co-digestion of T-CM and corn stover was investigated in batch-, and fed-batch fermentations. The results showed that corn stover efficiently stabilized anaerobic processes, which may have important ramifications for practical application. The simple and inexpensive method of removing nitrogen-rich water soluble components from CM, however, resulted in a large volume of water with high nitrogen, phosphorus and other dissolved compounds as a residual waste. The separated liquid phase (chicken manure supernatant - CMS) was therefore used as a nutrient solution for algae cultivation assuming that algae could utilize the nitrogen and phosphorus sources from their environment for growth in CMS in photoheterotrophic growth mode. A *Chlorella* sp. strain, contaminated with various bacteria in a mixed culture, were cultivated in these experiments on CMS at varying CMS concentrations and samples for metagenomic characterization of the community were removed and whole genome sequenced. In order to avoid light limitation, CMS was diluted. At the optimal dilution the *Chlorella* culture grew vigorously. In addition to removing significant amounts of nitrogen and phosphorus from CMS, additional beneficial features of the microalgal biomass include versatility and the utilization of algae for biohydrogen and biogas production.

Support: Hungarian National RDI Found projects GINOP-2.2.1-15-2017-00065, GINOP-2.2.1-15-2017-00081. R.W and Z.B received support from the Hungarian NKFIH fund Project no. PD 121085 and FK 123902.

NEUROINVASIVE PORCINE ASTROVIRUSES: NEWLY RECOGNIZED ROLES OF PREVIOUSLY KNOWN ENTERIC RNA VIRUSES

ÁKOS BOROS[1], MIHÁLY ALBERT[2], PÉTER PANKOVICS[1], HUNOR BÍRÓ[3], PATRICIA A. PESAVENTO[4], GIA PHAN TUNG[5], ERIC DELWART[5, 6], GÁBOR REUTER[7]

[1]Laboratory of Virology, Public Health Department, Government Office of Baranya County, Pécs, Hungary; [2]Ceva Phylaxia Ltd, Budapest, Hungary; [3]SHP Ltd, Kaposvár, Hungary; [4]School of Veterinary Medicine,

University of California Davis, Davis, CA, US; [5]Blood Systems Research Institute, San Francisco, CA, US; [6]University of California, San Francisco, CA, US; [7]Department of Medical Microbiology and Immunology, Medical School, University of Pécs, Pécs, Hungary

Members of family Astroviridae contain diverse, enteric, single-stranded RNA viruses infecting humans and other vertebrate. The first astrovirus was described in 1975 from a faecal sample of a gastroenteritic child. Human astroviruses (AstVs) are now recognized as the third most common viral agents of non-bacterial acute gastroenteritis after rota-, and caliciviruses. Unexpectedly, two novel "neuroinvasive" (Ni) AstVs were detected in brain biopsy from encephalomyelitis cases in humans and mink, in 2010. To date several additional, novel Ni-AstVs have been detected in central nervous system (CNS) of humans, cattle and sheep with encephalomyelitis strongly indicating the causative association between Ni-AstVs and disease. Majority of the Ni-AstVs belong to the VA/HMO phylogenetic clade suggests the common origin of these viruses. Although knowledge about the genomic diversity and host spectra of Ni-AstVs is rapidly increasing little is known about their transmission, disease course and viral neuropathology due to the lack of available in vivo animal model system. Our research group recently discovered a novel Ni-AstV which belongs to the porcine AstV-3 genotype (Ni-PoAstV-3) of the VA/HMO clade in multiple newly weaned pigs showing signs of encephalomyelitis, weakness and paralysis (posterior paraplegia) during prolonged outbreaks in Hungary took place between 2011 and 2017. The highest viral loads of Ni-PoAstV-3 were measured in brain and spinal cord samples by RT-qPCR method. According to our results Ni-PoAsV-3 can cause viremia and disseminated infection involving the brain, spinal cord and multiple organs during the acute phase of encephalomyelitis. Interestingly, Ni-PoAstV-3 is generally absent or present only at low viral loads in the faeces/intestines while it could be detectable frequently in the respiratory system of paraplegic pigs raising the possibility of the respiratory route of transmission/infection of Ni-PoAstV-3. Histologically, the observable histopathological changes (neuronal degeneration / necrosis with microgliosis) as well as the neuronal localizations of PoAsV-3 RNAs by in situ hybridization in CNS samples are comparable to astrovirus-associated encephalitis cases of minks, humans and bovine suggesting a general course of Ni-AstV neuroinfection. Therefore pigs could be promising in vivo animal models to study the pathogenesis of Ni-AstVs.
Astroviruses are known to could cause outbreaks of gastroenteritis, but until now astrovirus-associated encephalitis cases have been reported mostly sporadically. Based on our observations Ni-PoAstV-3 can cause persistent epidemics of porcine encephalomyelitis and could constitute an economically important agent threatening livestock.

EFFECTS OF MUCOLITICS IN IN VITRO AND IN VIVO *CHLAMYDOPHILA PNEUMONIAE* INFECTION

KATALIN BURIÁN, BETTINA MAGYARI, TÍMEA MOSOLYGÓ, VALÉRIA ENDRÉSZ, DEZSŐ VIRÓK, DÁVID KÓKAI

Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Hungary

Chlamydophila pneumoniae belonging to the Chlamydiaceae family is a Gram-negative intracellular bacterium with a special biphasic life-cycle. It is a common cause of acute respiratory infection, including community-acquired pneumonia, sinusitis, pharyngitis, bronchitis, and exacerbations of chronic bronchitis. *C. pneumoniae* is responsible for approximately 10% of pneumonia cases. N-acetyl-l-cysteine (NAC) is a rather commonly used pharmaceutic. It has several therapeutic uses in psychiatry, as a mucolytic agent in respiratory diseases, or in case of an acetaminophen overdose.

Mechanism of action as a mucolytic is due to the free thiol group, which breaks down the disulphide bonding in the mucus, therefore decreasing its viscosity but the anti-microbial effect against chlamydia species was not described. In this study, our aim was to investigate whether the NAC influences the replication of *C. pneumoniae* in in vitro condition. Moreover the in vivo efficacy of NAC was analyzed in *C. pneumoniae*-infected mice. To be awake to the detrimental effect of NAC, an alternative mucolytic agent, ambroxol was investigated both in vitro and in vivo conditions. Surprisingly NAC treatment resulted in 6-times higher chlamydia replication in tissue culture comparing to the untreated control cells and this effect based on the increased binding of the pathogen to the host cells as was revealed by indirect immunofluorescence staining. The *C. pneumoniae* infected mice to whom NAC was given had prolonged and more severe infections than the control mice. To provide better alternative mucolytic instead of NAC, ambroxol was found, which did not increase, rather decreased the chlamydia replication in vitro conditions thank to the increasing expression of indolamine 2,3-dioxygenase partially. In animals using the empiric usual quantity of human dose, the ambroxol did not alter the number of the recoverable *C. pneumoniae*. On the basis of our result, it is strongly recommended applying of a mucolytic agent other than NAC in respiratory diseases caused by *C. pneumoniae*, such as ambroxol.
Support: GINOP-2.3.2-15-2016-00012 and EFOP-3.6.1-16-2016-00008.

INVESTIGATION OF THE ARTIFICIALLY SPIKED HUMAN SERUM

DOMINIKA CSABÓK, VIKTÓRIA O. JUHÁSZ, FERENC SOMOGYVÁRI

Institute of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Hungary

Sepsis is defined as systemic inflammatory response syndrome to infection. The causative agents of sepsis can be bacteria which are the most common cause, and it can also be a fungal infections. Early detection and adequate treatment of infections are critical for successful outcome for patients with systemic infections. In the clinical setting, the gold standard in the diagnosis of a septic patient has been a haemoculture to identify the source of infection. Clinically, sepsis biomarkers and molecular diagnostic methods are favored as an initial diagnosis of septic patients. The DNA content of the plasma is investigated beside the C-reactive protein (CRP) and procalcitonin (PCT) which are traditionally used as biomarkers of sepsis. The destroyed pathogens and immune cells are the origin of the DNA in the plasma. Previously, this free DNA successfully used in the diagnosis of the Asperdillus infections. The aim of this study is to check a relevance of the serum DNA investigation in the case of bacterial and fungal infections with in vitro spiked samples. Recently, PCR-mastermixes containing special components have appeared in the market to enable direct DNA amplification of highly inhibitory samples. The major advantage of this method is the exclusion of a DNA preparation step, which significantly reduces the time expenditure and work load of the testing. Here we amplified the serum samples with and without DNA preparation steps, too. We have used bacterial and fungal specific primers and the real-time PCR for the investigations. The whole serum is suitable for the investigation but the sensitivity of the reaction is extremely low. This is the consequence of the inhibitors and in the case of the fungal PCR the degenerated primers. The DNA preparation step is essential in the case of serum investigation. The sensitivity of the PCR with prepared, clean DNA is better than 10 copy / reaction. The DNA preparation of the serum samples is far more quick and easier than the preparation of the whole blood. Tus, the serum investigation looks suitable for the preliminary investigation of the septic cases.

PREVALENCE STUDY OF HUMAN PLYOMAVIRUS 9

ESZTER CSOMA[1], TAMÁS GÁLL[2], MELINDA KATONA[1], LÁSZLÓ ASZTALOS[3], LÁSZLÓ BIDIGA[4], LAJOS GERGELY[5], ATTILA SZŰCS[6], LAJOS GERGELY[1]

[1]Department of Medical Microbiology; [2]Department of Pediatrics; [3]Institute of Surgery; [4]Department of Pathology; [5]Institute of Internal Medicine; [6]Department of Otolaryngology and Head and Neck Surgery, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Although human polyomavirus 9 (HPyV9) was described as a new viral genome in 2011, little is known about the pathogenesis of this virus. The number of articles about prevalence is limited (to date <15), negative results were published in most cases. The virus was found in serum or whole blood samples from kidney transplant patients, from one patient with acute myeloid leukaemia and from a patient with leukoencephalopathy; HPyV9 was detected in one urine sample from a renal transplant patient, as well. Our work team previously found HPyV9 DNA in plasma, urine and throat swab samples from pregnant and non-pregnant, healthy women. During our research project we collected different sample types from different patient groups in order to study the prevalence of HPyV9: blood (n=600), urine (n=600) and throat swab samples (n=800) from kidney transplant patients, from patients with haematologic diseases and from healthy individuals, cerebrospinal fluid (n=100) obtained for routine diagnosis of encephalitis causative agents, tonsillar (n=100) and adenoid tissue samples (n=100), and archived, formalin-fixed paraffin-embedded renal neoplasia (n=111), bladder carcinoma (n=76), lung carcinoma (n=100) and non-cancerous lung tissue samples (n=47). Different PCR methods were tested, developed and optimized, and then a nested PCR targeting the VP1 region and quantitative, real-time PCRs targeting the VP1 and LT region were used. HPyV9 DNA was detected in blood, urine and respiratory samples from kidney transplant patients, from the throat swab sample of a patient with haematologic disease, and from a tonsil tissue of a child. Most of the samples studied were negative for HPyV9 DNA which is in accordance with literature data. At the same time, seroprevalence data published revealed that similarly to other human polyomaviruses, HPyV9 seems to be also frequent in the human population (seropositivity rate is 20-70%). In order to detect the seropositivity rate in the Hungarian population, VP1 capsid protein of HPyV9 is expressed. The coding region was codon optimized, synthesized, cloned into pTriEx-4 Neo vector and expressed in Origami™ B(DE3) competent cells. Serum samples from children and adults, healthy individuals, transplant patients and patients with hematologic diseases are collected which will be examined for the presence of immunoglobulin against HPyV9 VP1 after optimization of an indirect ELISA.

Supported by the Hungarian Scientific Research Fund (OTKA-PD109108) and research activity of Eszter Csoma was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program'.

GENETIC DIVERSITY OF THE SPOILAGE YEAST *ZYGOSACCHAROMYCES LENTUS*

HAJNALKA CSOMA, HAJNALKA TÓTH, LAJOS ÁCS-SZABÓ, MATTHIAS SIPICZKI

Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen, Hungary

Species of the *Zygosaccharomyces* genus are often regarded as food spoilage yeasts. This genus includes some of the most osmotolerant yeasts that are able to resist concentrations of food preservatives vastly in excess of those normally encountered. Thus, products with low pH and high sugar concentrations, such as soft drinks, fruit juices and wine are at risk of deterioration by these

yeasts. *Zygosaccharomyces* strains are present naturally during grape-juice fermentation. However, their presence is an indicator of future spoilage problems, despite their eventual capacity of conferring useful organoleptic properties to the wine. Among the species currently assigned to the genus, *Zygosaccharomyces bailii*, *Z. rouxii*, *Z. bisporus*, and *Z. lentus* are those that pose the most serious threat of spoilage to processed food. There are hardly any data on the incidence and frequency of *Z. lentus* in wines and its genetic diversity is also little known. The aim of our study was to analyse intra-species genetic polymorphisms of this species. With this in mind, we isolated *Z. lentus* strains from sweet wines. The microsatellite oligonucleotide primers (GTG)5 and (GAC)5, and the RFLP of the mitochondrial DNA generated with HinfI digestion were used for fingerprinting assays. The band patterns of the mtDNAs and microsatellites were then used to generate dendrograms using the UPGMA method. The HinfI-based RFLP analysis yielded two patterns for the 32 *Z. lentus* strains examined. PCR fingerprinting using the microsatellite oligonucleotide primer (GTG)5 resulted in 12 different types of pattern. Using the primer (GAC)5, quite similar variability was obtained: 9 patterns. Comparing the discriminatory power of the two techniques used, RFLP analysis of mtDNA by HinfI unveiled less variation than microsatellites. We assume a relationship between the mitochondrial patterns and the chemical composition of the wines from which the strains were isolated.

SELENOCOMPOUNDS AS PROMISING ANTIBACTERIAL AGENTS

ANDREA CSONKA[1, 2], ANNAMÁRIA KINCSES[1], TÍMEA MOSOLYGÓ[1], ÁDÁM SZABÓ TÖNKI[1], CARMEN SANMARTÍN[3], JADWIGA HANDZLIK[4], ENRIQUE DOMÍNGUEZ-ÁLVAREZ[3, 4, 5], GABRIELLA SPENGLER[1]

[1]Department of Medical Microbiology and Immunology; [2]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Szeged, Szeged, Hungary; [3]Department of Organic and Pharmaceutical Chemistry, School of Pharmacy, University of Navarra, Pamplona, Spain; [4]Department of Technology and Biotechnology of Drugs, Jagellonian University Medical College, Kraków, Poland; [5]Institute of Organic Chemistry, Spanish National Research Council (IQOG-CSIC), Madrid, Spain

Multidrug resistance (MDR) is a serious problem for the treatment of bacterial infections due to the appearance of reduced or missing response of microorganisms to the applied antimicrobial agents. The emergence of MDR bacteria is considered a serious public health threat, for this reason the design of new antibacterial agents as adjuvants compounds of the antibiotic therapy is gaining more attention. Chlamydiae are Gram-negative obligate intracellular bacterial pathogens responsible for a range of diseases of clinical and public health importance. The obligate intracellular bacterium *Chlamydia trachomatis* invades host cells to replicate inside a membrane-bound vacuole called inclusion. *C. trachomatis* D is of great public health significance because of the impacts of untreated diseases on reproductive outcomes. Selenocompounds (1-11) were compared based on their antibacterial activity in AcrAB-TolC expressing *Escherichia coli* K-12 AG100 and AG100A, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212 strains. The minimum inhibitory concentrations (MICs) of selenocompounds were determined by two-fold broth microdilution method in 96-well plates. The combination effect of the compounds with antibiotics was examined using the MIC reduction assay in 96-well plates. The anti-chlamydial effect of the compounds was determined using *C. trachomatis* and HeLa cells. The number of *C. trachomatis* D inclusions was counted under an UV microscope. The tested selenocompounds showed no antibacterial effect on *E. coli* strains. The symmetric pyridine-containing diselenodiester 3 had remarkable antibacterial activity on Gram-positive bacteria, i.e. *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212, and potentiated the action of tetracycline and ciprofloxacin on *E. coli* AG100. Selenocompounds 1-11 could inhibit effectively the formation of inclusions thus these derivatives

showed anti-chlamydial effect. Based on these results it can be concluded that the selenoesters could be attractive lead structures in order to increase the efficacy of antibiotics. Moreover, the studied selenocompounds could serve as anti-chlamydial lead scaffolds for further development, however their mode of action on bacteria should be elucidated.

Supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program'.

FUNGAL BIOLOGY AND POTENTIAL FOR BIOTECHNOLOGICAL APPLICATION OF PLANT BIOMASS

RONALD P. DE VRIES

Fungal Physiology, Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands

Few aspects of fungal biology are as diverse as their strategies to obtain carbon and energy from their natural substrates. Plant biomass, and in particular lignocellulose, is a major substrate for many fungi due to its abundance in nature and its high content of monosaccharides. The availability of (post-) genomic datasets have provided an unprecedented view on the strategies of fungi to depolymerize and convert plant-based polysaccharides. Comparative genomics revealed a huge variation in genome (CAZome) content with respect to lignocellulose active enzymes and in many cases these variations could be linked to the natural biotopes of the species. However, the difference in strategy goes beyond the genomic variations. Recent studies in our lab revealed that even closely related species with similar CAZomes produce significantly different enzyme sets when exposed to the same substrate. This suggests significant differences in the regulation of the genes encoding these enzymes as well as a preference for different components of lignocellulose by the different species. In this presentation, major differences observed across the fungal kingdom will be presented and placed into context of biotope and life style and how these differences can be used to develop better or new biotechnological applications for plant biomass.

EFFECT OF FOOD RELATED BACTERIAL ISOLATES ON SOME FOOD-BORNE PATHOGENIC BACTERIA

BERNADETT DEÁK, ÁGNES BELÁK

Microbiology and Biotechnology Department, Food Faculty, Szent István University, Budapest, Hungary

The microbiota of different fresh or processed food products can contain either harmful or useful microorganisms. Therefore, the aims of our work were the isolation and characterization of potential antagonistic bacteria from food processing environments and food products, and test their effect on growth of different food-borne pathogenic bacteria. Using TSA or TSB media (depending on the tested surface) 78 bacterial isolates were collected from four different food processing environments: 20 from an abattoir, six from vegetable processing environment, 18 from surface samples of an egg processing plant, and 34 from a diary product plant. After examining the colony morphologies of the isolates on TSA and WL agar plates 64 different bacteria were selected for further analyses. Searching for the antagonistic effect of the isolates, all selected bacteria were screened for growth inhibiting activities against four food-borne bacterial pathogens (*E. coli*, *Listeria monocytogenes*, *Salmonella* Hartford and *Yersinia enterocolitica*) by co-culturing on TS agar surfaces. The incubation took place

at eight different temperatures (5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 37°C, 42°C) for one, two, three and six days to be able to determine the optimal temperature and growth phase for inhibition. Out of the tested 64 isolates, 20 could inhibit at least one of the tested pathogens, while the most effective inhibition was detected between 15°C and 30°C. The most sensitive pathogenic bacterium proved to be *Y. enterocolitica*, as out of the 20 antagonistic isolates 14 had negative effect on its growth. Biochemical (KOH, OF, catalase, oxidase) and physiological (growth on different temperatures and pH) tests were also done with the potential antagonistic isolates.
Examination of cell-free supernatants of the inhibitory isolates using Multiscan Ascent resulted in partial inhibition of growth of the pathogens, however in some cases decline in the growth could be detected. After concentrating the supernatants (10x), better inhibitions could be observed, which reflects on the extracellular nature of the inhibiting compound(s) and the necessity of its(their) higher concentration for more efficient inhibition. The antagonistic isolates were identified with miniaturized identification tests as well as sequencing, while molecular typing was done by RAPD-PCR using M13 and D8635 primers. The results showed that there were eight potential antagonistic strains from *Pseudomonas* genus, while *Bacillus* (3), *Macrococcus* (3), *Serratia* (2), *Staphylococcus* (2), *Paenibacillus* (1) and *Rothia* (1) genera were also presented.
Realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 „National Excellence Program – Elaborating and operating an inland student and researcher personal support system" The project was subsidized by the European Union and co-financed by the European Social Fund."

COMPARATIVE HIGH RISK HPV TYPES ANTIBODY DETERMINATIONS AMONG GYNAECOLOGICAL PATIENTS AND HEALTH CARE WORKERS

JUDIT DEÁK[1], GYÖRGY BÁRTFAI[2]

[1]Institute of Clinical Microbiology; [2]Department of Obstetrics and Gynaecology, University of Szeged, Szeged, Hungary

Participation rate of adult women and men in different preventive health care screening control is relatively low in our country. 300-500 women die per year from the consequences of human papillomavirus (HPV) infections. The HPV vaccine is not obligatory but complimentary among 13-14 years age groups. In spite of this fact the parents do not prefer the free vaccination. For determination of HPV type 16 and 18 antibodies ELISA kits (CUSABIO Biotech CO., LTD. Wuhan, China) were used. Both kits detected HPV 16 and 18 specific IgG antibodies without cross-reactivity or interference. The kits were developed for the detection of naturally acquired anti-HPV 16 and HPV 18 serum antibodies against L1 capsid protein. Women, who were seen at the outpatient care unit of the Department of Obstetrics and Gynaecology (N=48), University of Szeged, health care workers (HCW) of two laboratories (N=13 and N=49) and nurses of a surgery department (N=75), all working at the University of Szeged. Women who participated in this study did not get HPV vaccine. Average of age in the four groups: 30.9-43.2 years. Antibodies were detected against HPV 16 and 18 types among gynaecological patients and HCW (N=185). The most HPV 16 antibody was detected in the 1st group, (Outpatient Clinics) among the gynaecological patients (N = 38) and in the 3rd group, among laboratory assistants "2" (N = 25). HPV 16 and 18 simultaneously and HPV 18 alone were detected in the 4th group, among the registered nurses, (N = 18 and N = 14 respectively). HPV antibodies are the markers of prior exposure to the HPV. The detection of antibodies in serum can be used to study the epidemiology of HPV. The serological results are not sufficient for diagnosis but

give a direction for the necessity of clinical examinations with cytological and molecular genetic diagnosis. Educating and informing the youth, teenagers and adults are essential.

CULTIVATION-BASED IDENTIFICATION OF BACTERIAL COMMUNITIES INHABITING THE RADIOACTIVE HYDROTHERMAL SPRING CAVES OF GELLÉRT HILL, HUNGARY

NÓRA TÜNDE ENYEDI[1], DÓRA ANDA[1], ANDREA K. BORSODI[1], SÁRA E. PÁL[1], MIHÁLY ÓVÁRI[2], KÁROLY MÁRIALIGETI[1], PETRA BODOR[3], JUDIT MÁDL-SZŐNYI[3], JUDIT MAKK[1]

[1]Department of Microbiology; [2]Department of Analytical Chemistry; [3]Department of Physical and Applied Geology, ELTE Eötvös Loránd University, Budapest, Hungary

The Buda Thermal Karst System is an active hypogenic karst area where speleogenetic processes are maintained by microbially mediated sulphuric acidogenesis. In the region of Gellért Hill, the microbial communities colonize the completely thermal water-filled caves, forming red iron-rich biogeochemical layers and calcite precipitations characterized by strong adsorption capacity of trace elements including radium. Adsorbed radium might contribute to the measured radon activity up to 1005.8 Bq/l. The aim of this study was to examine the structure and microchemical composition of intact biogeochemical layers developed in the spring caves of the Diana-Hygieia and Török springs. Elemental analysis of biogeochemical samples deposited at discharge areas was performed by total reflection X-ray fluorescence. To isolate and identify radioresistant bacterial strains, the biofilm samples were irradiated by gamma radiation at various doses (5, 10, 15 kGy) using NORATOM equipment with a 60Co source. A variety of morphologically different cell types were observed adhering to mucous extracellular polymer substance based on scanning electron microscopy. Images reveal the presence of reticulated structures with hexagonal mesh and numerous filaments covered with mineral particles often identified as sheaths and stalks encrusted by the deposition of iron and manganese oxides of iron-oxidizing genera. A comparison of average background soil metal concentrations with our data indicate that most of the biofilm samples were from 1.5 to 60 times higher than typical background levels for Sn, Hg, Sr, As, Cu, Mn, Zn and Pb in uncontaminated soils. More than 450 pigment and/or endospore producing bacterial strains were isolated from the samples using oligotrophic and ferrous-sulphate containing media. Among the strains, representatives of phyla Firmicutes, Bacteriodetes, Proteobacteria, *Deinococcus-Thermus* and Actinobacteria were identified. The irradiated biofilm samples proved to be more diverse than the non-irradiated samples. From the highest dose irradiated samples members of the genera *Bacillus*, *Mycobacterium*, *Kytococcus*, *Dermacoccus*, *Micrococcus*, *Marmoricola*, *Paracoccus*, *Deinococcus*, *Brevibacterium* and *Microbacterium* were identified. The majority of the closest relatives of isolated bacterial strains were previously identified from radioactive environments and UV-exposed habitats, as nuclear waste-contaminated soils, deserts and the stratosphere. This is also the first report of several genera that might be resistant to elevated doses of gamma radiation.

This research was supported by the Hungarian Scientific Research Fund (OTKA) Grant NK101356.

ENRICHMENT OF NITRATE REDUCER TOLUENE DEGRADING BACTERIA FROM A HYPOXIC BTEX CONTAMINATED SITE.

MILÁN FARKAS[1], FRUZSINA RÉVÉSZ[1], BALÁZS KRISZT[1], SÁNDOR SZOBOSZLAY[1], ANDRÁS TÁNCSICS[2]

[1]Department of Environmental Safety and Ecotoxicology; [2]Regional University Center of Excellence in Environmental Industry, Szent István University, Gödöllő, Hungary

The Siklós benzene, toluene, ethylbenzene, and xylene (BTEX)-contaminated area is one of the best characterized petroleum hydrocarbon-contaminated sites of Hungary. This shallow aquifer have high diversity of catechol 2,3-dioxygenase (*C23O*) genes encoding subfamily I.2.C-type extradiol dioxygenases. The microbial community at this site was constantly dominated by microorganisms affiliated to the Comamonadaceae and Rhodocyclaceae. Both betaproteobacterial lineages are known to harbor aromatic hydrocarbon degraders. Last year a stable isotope probing (SIP) experiment was performed under microaerobic conditions, to identify the hypoxic degraders. We found that some of the I.2.C-type *C23O* genes were only detectable in light fractions. Nevertheless, bacteria harboring these *C23O* genotypes did not take part in the degradation of toluene in the microcosms. A possible explanation of this phenomenon is that these bacteria may prefer nitrate as electron-acceptor under oxygen-limited conditions while incorporating the available oxygen into the ring-structure of an aromatic hydrocarbon using oxygenases. Unfortunately these subfamily I.2.C *C23O* genotypes still cannot be linked to any bacterial lineage. However, the major genotype which was the predominant in the initial sediment samples and in the "light" DNA fractions, correlated with the abundance of Azoarcus-related 16S rDNA sequences in these samples. To enrich and identify these *C23O* harboring microbes and understand their role in the degradation of BTEX, toluene-degrading enrichment cultures were set up under nitrate reducing conditions. After 5 constitutive transfers still high microbial diversity was observable. I.2.C-type *C23O* and benzylsuccinate synthase (BSS) genes were constantly detected during the experiment.

Milán Farkas was supported by the NTP-NFTÖ-16 project by the Human Capacities Grant Management Office and the Hungarian Ministry of Human Capacities.

SELENOESTER DERIVATIVES AS NOVEL ANTIFUNGAL AND ANTIVIRAL AGENTS

MÁRIÓ GAJDÁCS[1], TÍMEA MOSOLYGÓ[1], EDIT URBÁN[2], CARMEN SANMARTÍN[3], JADWIGA HANDZLIK[4], ENRIQUE DOMÍNGUEZ-ÁLVAREZ[5], GABRIELLA SPENGLER[1]

[1]Department of Medical Microbiology and Immunobiology; [2]Department of Clinical Microbiology, Faculty of Medicine, University of Szeged, Szeged, Hungary; [3]Department of Pharmaceutical Chemistry, University of Navarra, Pamplona, Spain; [4]Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Kraków, Poland; [5]Institute of Organic Chemistry, Spanish National Research Council, Madrid, Spain

The emergence of multidrug-resistant microorganisms is becoming a worrying challenge to medicine, both as a significant factor of mortality and as an increasing economic burden to health systems. The modulation of oxidative stress has been described as a novel and attractive target in the field of antimicrobial research. Se-mediated reversal of multidrug resistance has received reasonable attention, both as inorganic selenium salts, organoselenium compounds or selenium-nanoparticles (SeNPs). The aim of our study was to determine the potency as antimicrobial agents of novel selenoester derivatives with different chemical functional groups. Fifteen compounds were evaluated in our study (1: cyclic selenoanydride, 2-11: selenoesters, 12-15: reference chalcogen-containing compounds). The antimicrobial activities of the tested compounds against reference strains of anaerobic bacteria and yeasts were evaluated using disk diffusion test and when warranted, broth microdilution, according to EUCAST standard methods. A double-disk synergy test was used for the

detection of synergism between the selenocompounds and reference antibiotics against multiresistant bacterial strains. The anti-HSV activity of the tested compounds was evaluated using MTT assay on Vero cells. The selenocompounds did not exhibit antibacterial activity against the tested anaerobic reference strains, and they did not show synergistic interactions with the tested antibiotics in the double-disk synergy test. The cyclic selenoanhydride (1) and the ketone-containing selenoesters (9-11) presented antifungal activities against a variety of yeast strains. Compounds 1 and 4 showed the highest anti-HSV activity, with cell recovery values of 78% and 96%, respectively. The reference chalcogens did not present any antimicrobial activity.

While the selenocompounds did not exert antibacterial activity in an anaerobic atmosphere, which could be due to their inability to form reactive metabolites in absence of oxygen, compounds with a specific structure (methyl-ketone selenoesters) showed antifungal and potent antiviral activity with promising selectivity. As their mechanism of action is still not clear, their derivatization and further biological evaluation should be considered.

Gabriella Spengler was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. The authors of this paper received funding from the Márton Áron Research Programme financed by the Hungarian Ministry of Foreign Affairs and Trade. Márió Gajdács has received input for the study/project through ESCMID's mentorship programme by Edit Urbán.

COMPARISON OF ORGANIC AND CONVENTIONAL LAND USE IN NYÍREGYHÁZA BASED ON SOIL MICROBIOLOGY AND CATABOLIC ACTIVITY

ORSOLYA GAZDAG, TÜNDE TAKÁCS, MÁRTON MUCSI, LÁSZLÓ KÖDÖBÖCZ, ILONA VILLÁNYI, TIBOR SZILI-KOVÁCS

Soil Biology, Institute for Soil Sciences and Agricultural Chemistry, Centre for Agricultural Research, Budapest, Hungary

Sustainable land use and the conservation of soil biodiversity is very important because soil microorganisms have an essential role in soil productivity, biogeochemical cycling and ecological stability. Therefore more attention has paid today to improve land managements considering soil life. The soil microbial community was investigated in a sandy soil at Nyíregyháza (Hungary). The main physical, chemical and microbiological (colony forming unit (CFU) of the aerobic soil microbes and fluorescein diacetate (FDA) enzyme activity) properties were determined from organic and conventional land use management. Physiological profile characterized by catabolic activity of the whole soil community using MicroRespTM with 22 different substrates (simple sugars, amino acids and carboxylic acids) was carried out. The above parameters were analyzed in relation to the physical and chemical properties of soils. There was a significant difference between the two different land managements considering the soil physical, chemical and microbial properties. According to the multivariate statistical analyses the samples of the two groups were clearly separated from each other and each sample was placed to their own group in 100% according to the different substrate utilization pattern and land uses as well. Soil organic carbon (SOC) content, pH and the vegetation can affect the rate of CO_2 evolution and the catabolic activity profiles of the samples.

Supported by the OTKA (K108572) and Research Institute of Organic Agriculture (2011-2013), and National Research, Development and Innovation Fund of Hungary (GINOP-2.3.2-15-2016-00056).

BIOLOGICAL DIVERSITY OF YEAST SPECIES FROM MUST SAMPLES OF THE BADACSONY WINE REGION

ANNAMÁRIA GERŐCS[1], SÁRA PÁL[1], KATALIN NEMES-BARNÁS[1], JÁNOS MÁJER[2], BARNA SZŐKE[2], FERENC OLASZ[1]

[1]Agricultural Biotechnology Institute, Gödöllő, HUNGARY; [2]Research Institute for Viticulture and Enology, Badacsonytomaj, Hungary

Wine is a popular alcoholic beverage in Hungary as in other countries as well. It has some beneficial effect for human body in small quantities. In Hungary there are lots of famous wine regions, one of them is Badacsony, located on the northern shore of Lake Balaton. The climate of the region is sub-mediterranean and the soil is a mixture of clay, loess and sand on top of basalt. The white wines from the Badacsony are specially, full bodied and rich tasted. Nowadays are there two main strategies for wine making all over the world: spontaneous or controlled fermentation, but in both cases *Saccharomyces cerevisiae* plays an important role. In spontaneous fermentation is noticeable change of yeast species, the early stages characterize appiculate yeast species (*Hanseniaspora*, *Candida*) and increase of ethanol level appears genus *Saccharomyces* (*S. cerevisiae*, *S. bayanus*). In modern winery practice, use of starter yeast to reduce of spoilage, avoid risk of slow or incomplete fermentation and make good quality wine is a standard procedure. However, the utilization of commercial yeast led to the loss of complexity in the final product. On the opposite, indigenous strains were adapted better to local conditions and local wine fermentation highlighting the specific character of the wine. Several investigations studied naturally *Saccharomyces* and non-*Saccharomyces* populations to use in fermentation process. In the present study we collected data about the biodiversity in the selected vineyard (Badacsonytomaj, RIVE-NARIC) and characterized a collection of indigenous yeast strains. The 480 isolated yeast from must samples (Kéknyelű grape juice, Kéknyelű must samples from different phases of wine fermentation) were categorized by their morphological, biochemical and physiological characteristics. These tests included the examination of colony and cell morphology, utilization of carbon and nitrogen sources. Identification of 80 selected yeast isolates was carried out sequencing the ITS4-ITS5 region. The following species were identified: *Candida californica*, *Candida zemplinina*, *Hanseniaspora uvarum*, *Pichia fermentas*, *Pichia kluyveri*, *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, *Saccharomyces pastorianus*, *Saccharomyces bayanus* x *Saccharomyces cerevisiae* (hybrid), *Saccharomyces cerevisiae/paradoxus* (hybrid). These 80 yeast strains were compared against 35 commercial strains using interdelta PCR methods and our results showed that 10 isolates have similar pattern compared to the commercially used starter strains. According to the pattern of delta PCR, the strains were divided into 70 groups, which indicating the very pronounced diversity of yeast population in must samples of this winery.
In the future the isolates will be selected according to their useful features (ethanol tolerance, killer phenotype, fermentation rate, etc.) for wine making process and we will develop “terroir” starter cultures to use in the production of “organic” wines.

SPECIES-SPECIFIC PCR FOR THE DETECTION OF *MYCOPLASMA ANATIS*, *M. ANSERIS* AND *M. CLOACALE*

DÉNES GRÓZNER[1], KINGA M. SULYOK[1], BARBARA FORRÓ[1], ZSUZSA KREIZINGER[1], SZILVIA MARTON[1], KRISZTIÁN BÁNYAI[1], ZSUZSANNA RÓNAI[2], SZILÁRD JÁNOSI[2], MIKLÓS GYURANECZ[1]

[1]Veterinary Institute, Agricultural research Institute, Hungarian Academy of Sciences; [2]Directorate for Animal Health Diagnostics, National Food-chain Safety Office, Budapest, Hungary

Mycoplasma anatis, *M. anseris* and *M. cloacale* are waterfowl pathogen bacteria. The main symptoms in the affected duck or geese flocks are inflammation of the cloaca and the reproductive organs, decreased egg production, increased embryo mortality and respiratory diseases. Co-infections can aggravate the economic losses caused by *Mycoplasma* species. Information about mycoplasmosis in waterfowl is scarce; diagnosis of the infections is based on growth inhibition test, indirect haemagglutination or the sequencing of the product of a universal *Mycoplasma* PCR (polymerase chain reaction) system targeting the 16S/23S rRNA intergenic spacer region in Mycoplasmatales. However, serological assays have low sensitivity, while sequencing is a reliable but expensive method. The aim of the present study was to improve the diagnosis of mycoplasmosis in waterfowl by the development of PCR methods specific for *M. anatis*, *M. anseris* or *M. cloacale*. *M. anatis* (NCTC 10156), *M. anseris* (ATCC 49234) and *M. cloacale* (NCTC 10199) type strains were sequenced with Illumina NextSeq500 next generation sequencer. Trimmed reads were de novo assembled with the St. Petersburg genome assembler toolkit (SPAdes). Manual curation and control mapping were processed with the Geneious software. Free online annotation programs were used to annotate the coding and non-coding regions of the genomes. The analysis of the genomes revealed that *M. anseris* and *M. cloacale* have very similar genome structure and gene repertoire, whilst *M. anatis* belongs to a distinct clade. Previously described genes that are targeted in other *Mycoplasma* species-specific PCRs were not present in the waterfowl pathogen *Mycoplasma* genomes or were not suitable for designing specific primers. Therefore, essential genes were selected and aligned in order to locate mutations differentiating species and used for primer designing. The gene analysis and aligning were performed with Geneious. Primer design and general suitability were determined using NetPrimer and BLAST (Basic Local Alignment Search Tool) programmes. Performing PCRs with the designed primers revealed that the DNA polymerase III subunits gamma and tau coding (*dnaX*) gene was found appropriate for the identification of *M. anatis* and *M. cloacale* and the ATP-dependent DNA helicase coding (*uvrD/pcrA*) gene was found acceptable for the *M. anseris*-specific PCR. The primers were tested in conventional and SYBR green real-time PCR systems as well.
The specificity of the PCR assays was tested on 15 avian *Mycoplasma* type strains, 29 clinical isolates and 26 clinical samples. The sensitivity of the real-time and conventional PCRs was 101-103 DNA template copies and no cross-reactions were detected in the assays. The assays developed in the study have potential to be used in routine diagnostics as they are suitable for the cost-effective, rapid and reliable identification of waterfowl pathogen *Mycoplasma* species.

ANTIMICROBIALS AND ECOSYSTEM SERVICES – RISING QUESTIONS

MÁRTA EMŐKE GULYÁS

National Food Microbology Reference Laboratory, Food and Feed Safety Directorate, National Food Safety Office, Budapest, Hungary

Global antimicrobial/biocide use in the agriculture, food industry and hospitals, run to a mass of $>10^5$ (concentrated) tons per year of wide-spectrum, synthetic drugs. Presence of their metabolites, affecting rhizosphere and living water ecosystems, resulted not only in antimicrobial resistance, but may also alter the integrity of ecology processes, those keep Earth living and habitable. In 1999, heavy rain washed poultry litter into Chesapeake Bay, with antibiotic residues, causing toxic algal

bloom. Such metabolites (e.g. 25 for Tetracycline), may have positive antimicrobial activity and different stability, accumulate in soils and sludge, impairing the biodiversity and functions of microbial communities. Knowledge on statistics of AMR, resistance genes, and the infection routes these follow, led EFSA to develop a new One Health Action Plan against Antimicrobial Resistance (July 2017) which outlines a complex regulation for the interconnected human, animal and environmental processes. However, maybe more activities – and more information are needed to manage this problem on its real place. 1.) Do we have information on how antibiotic residues affect microbial ecosystem services, such as optimizing soil water capacity and nutrient balance by the rhizosphere; biogeochemical cycles of Earth global household; natural remediation processes in soils and living water? 2.) In 1987 Carlson, Lovelock, Andreae and Warren wrote: "The major source of cloud condensation nuclei appears to be dimethyl-sulfide"… Now we know that reef-building corrals are important source of DMS, many niches may be home of a series of DMS producing algae, and bacteria turning it to DMSO; remote sensing shown that natural aerosols explain seasonal and spatial patterns of ocean cloud albedo. Are there data on how these niches are altered by direct exposition of biocides, impairing diversity and DMS production? 3.) How are microorganisms, participating in oil remediation, influenced by antimicrobial residues? What do we know on their alkil-halide production and the regulation of their activities? Impaired cloud formation and stability, extreme precipitation patterns, can be explained as the result of temperature changes. However, water as greenhouse gas is unique as it is able to precipitate - a process that needs condensation nuclei. "Weather is a transport of energy" - now, sudden water substance shifts inside a cloud, manage two/three logs more energy as compared to air/wind itself. These processes have a role in the maintenance of our global climate and regional weather conditions. Maybe the maintenance of antibiotic residue-free environment may help Mother Nature to keep earth living and habitable more we ever thought.

PHYLOGENETIC ANALYSIS OF HUMAN PAPILLOMAVIRUS (HPV) 33 LONG CONTROL REGION (LCR)

ESZTER GYÖNGYÖSI, ANITA SZALMÁS, BRIGITTA LÁSZLÓ, JÓZSEF KÓNYA, GYÖRGY VERESS

Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

High-risk HPVs (HPV 16, 18, 31, 33, 45, 52, 58, etc.) are the etiological agents of cervix carcinoma. The LCR of the virus genome has a very important role in the regulation of the viral replication and transcription, and contains several transcription factor binding sites. The intratypic nucleotide variants of HPVs has different oncogenic potential, as we could see in the case of HPV 16, HPV 18 and HPV 31, but this phenomenon has not been investigated in the case of other high-risk types, such as HPV 33. The goal of this study was to see the natural genetic variants of HPV 33 in Hungary, and to investigate the functional differences between the variants.

Exfoliated cell samples were collected from the cervix of HPV 33 positive women with cytological and colposcopical abnormalities. After DNA isolation, HPV 33 LCR specific PCR was performed on the specimens and the PCR products were sequenced with the PCR primers. Multiple sequence alignment and phylogenetic analysis were carried out on the sequences. The sequences of our samples were compared to HPV 33 variants published in the literature. Several variants were identified from our samples with single nucleotide changes and a 79 bp deletion. According to the constructed phylogenetic tree, our variants belongs to the A lineage, A1 and A2 sublineages, which are the most frequent lineages in Europe. The nucleotide changes in the LCR can affect transcription factor binding sites and might result in altered transcriptional activity of the regulatory region. So, after

cloning the HPV 33 variants into luciferase reporter plasmids, we are planning the functional analysis of the LCR with transient transfection experiments and luciferase tests.

PREVALENCE OF HUMAN PAPILLOMAVIRUS (HPV) AND TORQUE TENO VIRUS (TTV) IN SALIVA AND TUMOR BIOPSY SAMPLES OF HEAD AND NECK CANCER PATIENTS IN HUNGARY

ANDREA HETTMANN[1], ANETT DEMCSÁK[2], ÁDÁM BACH[3], GÁBOR DECSI[4], ÁGNES DENCS[1], DÓRA PÁLINKÓ[3], LÁSZLÓ ROVÓ[3], GABRIELLA TERHES[5], EDIT URBÁN[5], KATALIN NAGY[4], MÁRIA TAKÁCS[1], JÁNOS MINÁROVITS[2]

[1]Virology, Hungarian Public Health Institute, Budapest; [2]Department of Oral Biology and Experimental Dental Research, Faculty of Dentistry; [3]Department of Otorhinolaryngology and Head-Neck Surgery; [4]Department of Oral Surgery; [5]Institute of Clinical Microbiology, Faculty of Medicine, University of Szeged, Szeged, Hungary

Among European countries the highest incidence and mortality rates of head and neck cancer types were recorded in Hungary. Apart from classical etiological factors, namely tobacco and alcohol consumption, several virus infections, especially HPV and TTV infections may contribute to tumorigenesis. Data about the prevalence of different viral infections in head and neck cancers is scarce in Hungary. We collected biopsy and saliva samples from patients diagnosed with head and neck squamous cell carcinomas (HNSCCs) or oral potentially malignant disorders (OPMDs) and tested them for the presence of HPV and TTV. HPV was detected in 18% of all HNSCC samples. All of the HPV positive tumors carried HPV type 16. Tumors located to the oropharynx had the highest HPV positivity rate with 50%. OPMD and control samples showed significantly lower rate of HPV prevalence and other, low-risk HPV types (HPV 10, 11, 13, 81) were found mainly.
TTV prevalence was higher in head-and neck cancer patients than in the control group (saliva: 38 vs 14%; tumor biopsy: 30 vs 5%, respectively). TTV prevalence was also high in patients with premalignant lesions of oral carcinoma (saliva: 50%; biopsy, 24%). By phylogenetic analysis, TTV belonging mostly genotypes 1 and 2 was found in HNCC patients.

ROLE OF CHROMATIN-ASSOCIATED H1 HISTONE AND HMGB PROTEINS IN *ASPERGILLUS NIDULANS*

ZSUZSANNA HAMARI

Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Aspergillus nidulans is a widely studied model organism, however its chromatin organization has been only studied *ad hoc* in comparison to that of *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*. The essential core histones together with H1 histone(s) and high mobility group B (HMGB) proteins contribute to the establishment of chromatin functions. In this talk we will focus on the role of the H1 histone and chromatin associated HMGB proteins in *A. nidulans*. Function of H1 histones vary widely between lower eukaryotes and their functional impact seems to correlate with the nucleosome repeat length of the species. *A. nidulans* harbors a particularly short nucleosome repeat length and inactivation of the H1 histone coding *hhoA* gene has no detectable effect on chromatin functions, cell physiology or the sexual and vegetative development. The lack of H1 histone function is intriguing, because the protein is produced in all life stages with a similar abundance as the core

histone H4. In the talk we will show the results of the latest studies on the role of H1 histone (transcriptome analysis and recombination frequency analysis during meiosis). *A. nidulans* possesses three HMGB proteins showing a characteristic feature, on which base they can be assigned to architectural chromatin functions through their interactions with DNA and various proteins of remodeling complexes or direct acting factors.
Study of these three putative chromatin associated HMGB proteins of *A. nidulans* revealed one dually localized mitochondrial nucleoid associated protein HmbB, a nuclear localized protein HmbA which is an orthologue of the Nhp6 protein of *S. cerevisiae* and a third HMGB protein, HmbC, which is homologous to the Hmo1 protein of *S. cerevisiae*. We present here the novel structural and functional properties of the mitochondrial HmbB compared to similar proteins of other eukaryotes and show that all three HMGB proteins play role in the sexual development of *A. nidulans*.

LISTERIOSIS IN PREGNANT WOMEN IN CROATIA

BLAZENKA HUNJAK[1], DIANA BRLEK GORSKI[2], ZDENKA PERŠIĆ[1], ANAMARIJA PEJNOVIĆ[1], ANDI EREGA[1], ANAMARIJA FABRIS[1]

[1]Microbiology; [2]Ecology, Croatian Institute of Public Health, Zagreb, Croatia

Listeriosis is often unrecognized and under-diagnosed infection. It is caused by *Listeria monocytogenes* (LM), which is considered as the most pathogen isolate in the genus of *Listeria* in human population. Certain food have been described as „high risk“ such as: raw (unpasteurised) milk, foods made from milk, uncooked meats, vegetable, seafood and processed food. Psychotrophic (temperature of refrigerator +4°C) and anaerobic characteristic give LM ability to survive, grow and spread in contaminated food, especially Ready To Eat foods (RTE) and vacuum products. In Croatia, annual average is less than 10 case reports. Pregnant women represent 60% of all cases of infection in the population under the age of 40. According to literature, about 22% perinatal infections end with neonathal death or stillborn. In neonates, mortality rate approaches around 50%. Late onset listeriosis in pregnant women occurs particulary as newborn meningitis in 2 - 4 weeks after delivery.The Croatian National Institute of Public Health (CIPH) conducted serological testing, cultivation or automated identification in human and non- human samples. In the period from 2014 to 2106, 54 samples were received for serological testing. Out of 54, in 25 samples was confirmed presence of antibodies IgG types 1/2a and 4b. Although in 13/54 was confirmed presence of antibodies IgM types, acute infection was not confirmed. CIPH received 2 LM isolates to confirm, previously diagnosed in samples of newborns. In this period, listeriosis was not confirmed nor in one sample obtained from the urogenital system of pregnant women. Out of 2817 food samples, LM was confirmed in 3 samples, in accordance with ISO 11290-2: 1999/A1: 2008.The most important recommendations for the prevention of listeriosis in pregnant women are avoiding food that is potentially a source of listeria, thorough heat treatment of animal products, and avoiding unpasteurized milk and products made from raw milk.

BIOLOGICAL PRETREATMENT AND ANAEROBIC DIGESTION OF BIOMASS FROM SHORT ROTATION CROPPING OF TETRAPLOID WILLOW PLANTS

BALÁZS KAKUK[1], MÁRK SZUHAJ[1], ZOLTÁN BAGI[1], GÁBOR RÁKHELY[1,2], KORNÉL KOVÁCS[1,3]

[1]Department of Biotechnology, University of Szeged; [2]Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences; [3]Department of Oral Biology and Experimental Dental Research, University of Szeged, Szeged, Hungary

Concerns over climate change and energy security urge the increase of biomass production for heat and biofuel production. Biogas is the most versatile among renewable energy carriers; it can be stored and transported easily and after upgrading the resulting biomethane can be utilized in every way natural gas is utilized. Maize silage is the main substrate used in biogas facilities in Europe. It is however, relatively expensive to produce and requires lands that could be utilized for food and feed production. Short rotation cropping willow plants may serve as alternative substrates, as the volumetric production cost of willow biomass is around one-fifth of that of maize silage and they can be cultivated on marginal lands. They yield in green and woody biomass as raw material for energetic use, but in contrast to burning wood pellets, biogas production is carbon neutral. In order to widen the use of green biomass, the improvement of the biomass yield by genetic means and an optimal pretreatment strategy for more efficient biogas production are appreciated. Breeding willow plants with multiplied genome size has already shown that it improves organ size, photosynthesis, and hormone composition (Dudits et al. 2016). The resulting tetraploid strain, noted Poli Plus (PP), was compared to the parent diploid strain (Energo, EN) in our experiments.

Our goal was to evaluate the biomethane potential (BMP) of these substrates, mainly the green biomass, under different conditions and to find an efficient pretreatment strategy to further increase the potential of this promising substrate. We compared sludges of different origins and found significant differences in them; a sludge that was adapted to lignocellulosic feeding showed almost 30% increase in BMP compared to one that was fed with maize silage. As the PP strain has a higher leaf to stem ratio in terms of mass, we compared the different parts of the green biomass and found only slight differences between the strains, suggesting that in terms of overall BMP regarding the whole plant, PP gives a higher amount. We also utilized Phanerochaete chrysosporium, a lignin-degrading white-rot fungus for biological pretreatment in batch tests and found that with a 3-day pretreatment we could enhance the BMP with almost 20%. Our results showed that in terms BMP, untreated green willow biomass can give about 70% of maize silage, but this can be increased either with a better adapted sludge or an effective pretreatment, in our case a biological one, to reach around 90%. The tetraploid strain also performed better in almost every comparison, suggesting that strain improvement combined with appropriate fermentation techniques can yield a strategy that has the potential to replace maize silage in industrial biogas fermentations.

Supported in part by the Hungarian National Research, Development and Innovation Found projects GINOP-2.2.1-15-2017-00065, GINOP-2.2.1-15-2017-00081 and OTKA-FK-123902.

INTERSPECIES GENOME CHIMERISATION IN *SACCHAROMYCES*: EVOLUTION OF MOSAIC "*CEVARUM*" AND "*KUDVARUM*" GENOMES BY GARME (GENOME AUTOREDUCTION IN MEIOSIS)

EDINA KARANYICZ, ZSUZSA ANTUNOVICS, MATTHIAS SIPICZKI

Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen, Hungary

Over the long history of yeast taxonomy, the number of species assigned to the genus *Saccharomyces* has been changing continuously. The currently accepted 7 (8) natural *Saccharomyces* species have been delimited by the application of the biological species concept. Their hybrids are viable but sterile, unable to produce viable ascospores, the equivalents of gametes of higher eukaryotes. By

analysing a large number of synthetic "*cevarum*" (*S. cerevisiae* x *S. uvarum*) and "*kudvarum*" (*S. kudriavzevii* x *S. uvarum*) hybrids, we revealed that the sterility of the hybrids is ensured by a double sterility barrier (the sterility of allodiploids and the F1 sterility of allotetraploids). Alloploid sterility can be attributed to the differences between the parental sets of chromosomes which prevent precise prophase I chromosome pairing essential for meiosis. Occasional spontaneous genome duplication overcomes this barrier and allows viable spore formation because each chromosome will have a homologous partner to pair with. But the spores will be sterile, unable to function as gametes because they will not be able to conjugate (mate, fertilise) (F1 sterility). We found that F1 sterility is due to the autodiploidisation of the tetraploid meiosis resulting in *MATa/MATα* heterozygosity in the allodiploid spores. The simultaneous presence of both *MAT* alleles represses the conjugation pathway. This barrier can occasionally be broken down by malsegregation and loss of autosyndetically paired chromosomes carrying the *MAT* loci (loss of *MAT* heterozygosity). The alloaneuploid spore receiving only one *MAT*-carrying chromosome will regain the conjugation activity. Malsegregation and loss of additional autosyndetically paired chromosomes in subsequent meiotic divisions gradually reduce the size of the hybrid genome. Chromosomes are preferentially, but not exclusively, lost from one of the parental subgenomes. Gross analysis of the whole-genome sequences of certain *kudvarum* hybrids and their filial spore clones indicates that allosyndetic interactions also occurs, mainly between chromosomes of the subgenomes which have homologous segments. The loss of chromosomes and the allosyndetic interactions result in chimeric mosaic genomes. The uniparental transmission of the mitochondrial DNA to the hybrids indicates that nucleo-mitochondrial interactions might affect the direction of the genomic changes. The process which we call GARMe (Genome AutoReduction in Meiosis) opens a way for exchange of genetic information between species to combine their properties without the application of GMO technology.

LINK BETWEEN ANTIBIOTIC CONSUMPTION AND OCCURRENCE OF MULTIRESISTANT GRAM POSITIVE BACTERIA: A TIME-SERIES ANALYSIS

GÁBOR KARDOS, BENCE BALÁZS, ADINA FÉSŰS, ORSOLYA GORÁCZ, HAJNALKA TÓTH

Department of Medical Microbiology, University of Debrecen, Debrecen, Hungary

The effect of antibiotic consumption on occurrence of methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (VRE) was investigated between October 2004 and August 2016. Monthly drug consumption was expressed as the number of defined daily doses consumed per 100 bed-days for beta-lactamase inhibitor combinations (BLI), cephalosporins (CEF), fluoroquinolones, aminoglycosides, macrolide-clindamycin group (MLSK) and glycopeptides. Vancomycin and teicoplanin were also tested separately. Resistance data as well as *Clostridium difficile* prevalence were expressed as incidence densities per 1000 bed-days. Linear transfer models and vector autoregressive models (VARs) were built in Eviews and in R, respectively. VARs were interpreted using impulse response functions. As the last datum point influences VAR models significantly, these were built in a rolling window manner with series ending in January 2016 to August 2016. Significant correlation between the impulse and the response was accepted when present in at least half of all rolling windows. MRSA prevalence was affected by CEF (with a lag -4, coefficient 0.01, p=0.002) and MLSK use (with 0 and -1 lags, sum of coefficients 0.03, p=0.03) in linear transfer models; other drugs had no effect. In VAR, in contrast, only MLSK consumption (significant at lags 1-6 of the response horizon) acted as a provoker, use of CEF or any other drug class did not affect MRSA prevalence. A reciprocal effect was observed, i.e. increasing MRSA

prevalence led to increasing CEF use. MRSA prevalence was associated with glycopeptide usage both in linear transfer (at lags 0, -1, -3 and -5, sum of coefficients 1.87, p=0.02-<0.001) and in VAR models (significant at lags 3-12 of the response horizon). Glycopeptide use was also linked with *C. difficile* in linear transfer models (at lags 0 and -4, sum of coefficients 0.80, p=0.003). Valid VAR model could not be built with the *C. difficile* series. VRE prevalence increase was provoked by glycopeptide use in linear transfer models (lag 0 and -2, sum of coefficients 0.04, p=0.02). In VARs, VRE prevalence was strongly provoked by CEF (significant at lags 4-12 of the response horizon) and glycopeptide use (significant at lags 1-12 of the response horizon); but not by any other drug group. Both vancomycin and teicoplanin showed provoking effect, vancomycin acted faster than teicoplanin (significant at lags 2-12 vs. 5-12 of the response horizon).
When MRSA and VRE was included into a common VAR model, provoking effect of MLSK on MRSA disappeared, an effect of BLI appeared instead. MRSA weakly provoked teicoplanin but not vancomycin use. The effect of glycopeptides was similar. Glycopeptide use is probably driven by prevalence of MRSA and *C. difficile*, as expected, and is a strong provoker of VRE spread.
G. Kardos was supported by János Bolyai Resarch Scholarship of the Hungarian Academy of Sciences. H. Tóth was supported by the New National Excellence Program.

DETECTION OF DIFFERENT dsRNA PATTERNS IN VARIOUS FUNGAL ISOLATES

TÜNDE KARTALI[1], DANISH SHAHAB[1], ILDIKÓ NYILASI[1], LÓRÁNT HATVANI[1], LÁSZLÓ KREDICS[1], CSABA VÁGVÖLGYI[1], TAMÁS PAPP[2]

[1]Department of Microbiology; [2]MTA-SZTE Fungal Pathogenicity Mechanisms Research Group, Hungarian Academy of Sciences and Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Detection of double-stranded RNA (dsRNA) elements in fungal isolates suggests the presence of mycoviruses. According to the previous studies, most fungal viruses have linear dsRNA genomes, but mycoviruses with single-stranded RNA and single-stranded DNA genomes can also occur. The lack of extracellular route for infection and virion formation distinguish them from animal and plant viruses, hence they are transmitted only intracellularly with hyphal anastomosis and with sexual or asexual spores. To date, more than 90 mycoviruses have been identified and all major fungal phyla (Ascomycota, Basidiomycota, and the different groups of the former Chytridiomycota and Zygomycota) contain hosts for mycoviruses. Usually, their presence is asymptomatic on their hosts, although, there is an increasing number of data indicating that the presence of certain mycoviruses causes smaller or greater changes in the phenotype and/or induces hypovirulence or hypervirulence of their fungal hosts. The ability of mycoviruses to reduce their fungal host's virulence is one of the most important effects, due to hypovirulence has the potential to significantly decrease the losses of agricultural crops and forests which are caused by plant-pathogenic fungi. During our research, we have screened different fungal strains belonging to different fungal groups, e.g., *Umbelopsis* (Umbelopsidaceae, Mucorales), *Mortierella* (Mortierellaceae, Mortierellales), *Lecanicillium* (Cordycipitaceae, Hypocreales), *Mycogone* (Hypocreaceae, Hypocreales) and *Armillaria* (Physalacriaceae, Agaricales) genera. CF-11 cellulose chromatography was used to purify dsRNA elements from total nucleic acid extracts. The nature of dsRNA elements was confirmed by their resistance to DNase I and S1 nuclease digestions. We found 27 dsRNS-harbouring strains from the total 221 investigated isolates. We found dsRNA elements in 23% of the investigated *Umbelopsis*; in these isolates four different patterns were observed with 1 to 4 discrete and different sized (2,8-5,3

kb) dsRNA bands. In 12% (14 *Mortierella* and 1 *Dissophora* strains) of 124 tested *Mortierella* and closely related strains were detected dsRNA elements; almost all patterns were different with 1 to 6 discrete and different sized (0,5–10 kb) dsRNA fragments. Of the 19 screened *Lecanicillium* strains, 21% of isolates were characterized with almost the same dsRNA pattern (3 to 5 discrete dsRNA molecules; sizes: 0,7–8 kb). Both examined *Mycogone* strains contained dsRNA elements, but their dsRNA patterns were different. Screening of 60 *Armillaria* strains is in progress. Compared to the viruses of other organisms, we have limited information about mycoviruses. Therefore, it is important to investigate their distribution and their biological role in the host fungal cells.

Supported by the Hungarian Government and the European Union within the frames of the Széchenyi 2020 Programme (GINOP-2.3.2-15-2016-00052).

CAN THE FUNGAL BIOMASS CONTAMINATED WITH COPPER AND LEAD BE INTEGRATED IN THE FOOD CHAIN IN SOIL?

ADRIEN KASZAP[1, 2], ANIKÓ SERES[2], PÉTER I. NAGY[2], FLÓRA SEBŐK[1], KLÁRA SZENTMIHÁLYI[3], SÁNDOR SZOBOSZLAY[1], BALÁZS KRISZT[1], CSABA DOBOLYI[1]

[1]Department of Environmental Safety and Ecotoxicology; [2]Department of Zoology and Animal Ecology, Faculty of Agricultural and Environmental Sciences, Szent István University, Gödöllő; [3]Plasma Chemistry Research Group, Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

Soil biota plays an important role in transformation of the compounds of toxic metals. The biomass of soil fungi rating 35-40% of total biomass can adsorb the majority of these substances, at the same time it forms a significant source of food chains there. We followed the composition of the mycobiota of Ramman's brown forest soil and its copper-tolerating and lead-tolerating members by culturing method. No propagula could be cultured on PDA loaded by 150 mg/L Cu ions, but 4000 CFU/g propagula were found on PDA of 37.5 mg/L. On PDA loaded by 5000 mg/l Pb ions, 35000 CFU/g propagula could be detected. 21 isolates of different species were obtained via the PDA media loaded by high doses of the two toxic metals. The taxonomic identification of each isolates was based on their phenotypic features and it was confirmed by the sequence analysis of the ITS region of the rDNA. The most frequent genera were *Acremonium*, *Aspergillus*, *Chrysosporium*, *Mucor*, *Paecilomyces*, *Penicillium* and *Trichoderma*. Three strains of them belonging to the species *Acremonium sclerotigenum*, *Aspergillus niger* and *Penicillium spinulosum* were preferred by the collembollan *Folsomia candida* in feeding experiments.

Collembollan populations could be maintained on the sole diet of mycelia containing 3.9 mg/g copper or 23.5 mg/g lead. It is concluded that the toxic metals copper and lead can be accumulated in the biomass of different soil fungi that are consumed by the collembolan *F. candida*.

GENESIS AND LOSS OF SPLICEOSOMAL TWIN INTRONS ("STWINTRONS"), AND THEIR LINK WITH ALTERNATIVE SPLICING AND POST-TRANSCRIPTIONAL REGULATION

NAPSUGÁR KAVALECZ[1], MICHEL FLIPPHI[1], NORBERT ÁG[1], LEVENTE KARAFFA[1], GUSTAVO CERQUEIRA[2], CLAUDIO SCAZZOCCHIO[3], ERZSÉBET FEKETE[1]

[1]Deptartment of Biochemical Engineering, University of Debrecen, Debrecen, Hungary; [2]Broad Institute of MIT & Harvard, Cambridge MA, US; [3]Department of Microbiology, Imperial College London, London, UK

We have identified primary transcripts with unconventional intervening sequences from which full-length ORFs could only be obtained after two coupled excision events: removal of a canonical "internal" U2 intron from the primary transcript would generate the donor or acceptor of a new, canonical "external" U2 intron excised subsequently. This stwintron hypothesis predicts specific intermediates which we have demonstrated experimentally in various Pezizomycotina species. The phylogenetic distribution of older stwintrons suggests that fungal intron/exon structure displays a remarkable evolutionary plasticity, where either the internal intron or the whole stwintron can be gained or lost within related clades. For [D1,2] and [A2,3] stwintrons, the primary transcript carries two bona fide U2 introns that overlap by one G which could serve as either the last nt of the acceptor of upstream intron or the first nt of the donor of the downstream one.
In a certain constellation, such stwintrons are removed alternatively - either as [D1,2] or as [A2,3]. However, also when alternative stwintron splicing is impossible, both extant U2 introns are (mutually exclusively) excised from the primary transcript. When the "wrong" one is excised, a RNA is produced carring noncoding sequences that can no longer be removed. In vertebrates, "intron retention" is a form of alternative splicing often linked to nonsense-mediated mRNA decay constituting an important mode of post-transcriptional regulation.

SURVEY OF *ESCHERICHIA COLI* STRAINS WITH A PATHOGENIC POTENTIAL IN WILD BOAR POPULATIONS OF HUNGARY AND THE EUROPEAN UNION

TIBOR KERESZTÉNY[1], BALÁZS LIBISCH[1], BEÁTA VITÁNYI[1], ZOLTÁN KERÉNYI[2], RÓBERT KOCSIS[2], FERENC OLASZ[1], PÉTER PAPP[1]

[1]Microbiology Laboratory, Department of Genetics, NARIC-Agricultural Biotechnology Institute, Gödöllő; [2]Hungarian Dairy Research Institute Ltd., Mosonmagyaróvár, Hungary

The wild boar population in Hungary and in Europe has shown a continuous growth based on the available statistical data of the past two decades, despite that hunting has also increased. Hunted wild boar constituted 40% of the farmed and hunted game processed in the wild game processing plants in Hungary in 2013. In the same year out of the 128365 hunted wild boars 89906 animals were sold and 36564 boars were processed locally in Hungary. In spite of the increasing numbers and considerable utilization of wild boars, there are only few scientific studies concerning the occurrence of *Escherichia coli* strains with a pathogenic potential in this animal population. In our currently ongoing project, the microbiota of free living wild boars are examined, and among the shooted wild boars one animal showed signs of diarrhoeal disease. Metagenomic analysis revealed that the abundance of the family Enterobacteriaceae in its ileum content was over 70%. The dominant species was *E. coli*, as determined by species-specific PCR and partial sequencing of the 16S rRNA and *rpoB* genes of some isolates recovered from VRBG agar plates. Because of these observations, we have surveyed available data in the annual reports of the EFSA (European Food Safety Authority) and in the scientific literature regarding the identified potentially pathogenic *E. coli* strains among wild boars and wild boar meat. These data analyses included the overview of the occurrence of Shiga toxin-producing *E. coli* (VTEC/STEC) isolates in the member states of the European Union, and the occurrence of *E. coli* strains with other pathotypes as well, such as enteropathogenic and enterotoxigenic *E. coli* (EPEC and ETEC, respectively). The available data indicate that infections by

or carriage of various pathotypes of *E. coli* have been identified in wild boars in all major regions of the EU, including VTEC strains with different antibiotic resistance patterns in 2010 in Hungary.
Supported by the National Agricultural Research and Innovation Centre, Gödöllő, Hungary.

CONSTITUENTS OF *CLEISTOCHLAMYS KIRKII* AS ANTIBACTERIALS AND EFFLUX PUMP INHIBITORS

ANNAMÁRIA KINCSES[1], GABRIELLA SPENGLER[1], SZILVIA VARGA[1], BORISZ VARGA[1], ÁKOS CSONKA[2, 1], SHIRLEY SANCHA[3], SILVA MULHOVO[4], ANA MARGARIDA MADUREIRA[3], MARIA-JOSÉ U. FERREIRA[3]

[1]Department of Medical Microbiology and Immunobiology; [2]Department of Traumatology, Faculty of Medicine, University of Szeged, Szeged, Hungary; [3]Research Institute for Medicines, Faculty of Pharmacy,University of Lisbon, Lisbon, Portugal; [4]Faculty of Mathematics and Natural Sciences, Mozambican and Ethnoscience Study Center (CEMEC), Pedagogic University, Maputo, Mozambique

Multidrug resistance (MDR) to antibiotics has become a serious problem in antibacterial chemotherapy. *Cleistochlamys kirkii* (Benth) Oliv. (Annonaceae) is an African medicinal plant traditionally used in Mozambique for the treatment of wound infections, tuberculosis, and rheumatism. Eight compounds (CK 1-8) isolated from *C. kirkii* were investigated in Gram-positive bacteria concerning their antibacterial and MDR reversing effects. Minimum inhibitory concentrations (MIC) of CK compounds were determined on reference and methicillin resistant *Staphylococcus aureus* strains. The combined effects of antibiotics (tetracycline and ciprofloxacin) and compounds (CK3 and CK5) were assessed using the checkerboard microdilution method in order to evaluate the type of interaction with antibiotics in methicillin susceptible and resistant *S. aureus* strains. The efflux pump inhibiting activity of compounds on the accumulation of the general efflux pump substrate ethidium bromide was assessed by real-time fluorimetry on *S. aureus* strains. The relative gene expression level of efflux pump genes was determined by real-time reverse transcriptase quantitative polymerase chain (RT-qPCR) reaction. Compounds CK3 and CK5 were very active against the *S. aureus* strains. The combined effect of the antibiotics and CK3 or CK5 on reference *S. aureus* resulted in synergism. On the other hand, against the methicillin resistant *S. aureus* strain, when combined with antibiotics CK3 showed antagonistic effect with tetracycline, although a slight synergism has been observed with ciprofloxacin. CK5 acted synergistically with tetracycline on the MRSA strain, however in combination with ciprofloxacin the type of interaction was moderate antagonism. Concerning the ability of CK compounds as efflux pump inhibitors (EPI), the most potent compound on reference *S. aureus* was CK3. No significant activity was obtained for the methicillin resistant *S. aureus*. The derivatives CK3 and CK5 could increase the expression of efflux pump genes norA and mepA after 4 h of exposure in the MRSA strain. These results suggested that *C. kirkii* constituents could be effective adjuvants in the antibiotic treatment of infections caused by *S. aureus*. Furthermore, the compounds are promising EPIs in Gram-positive bacteria.
Supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program' and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. This work was supported by the project GINOP-2.3.2-15-2016-00012 (New ways in the natural product-based drug discovery—system metabolomic approaches to discover biologically active terpenoids of herbal and microbial origin).

ANTICANCER ACTIVITY OF DITERPENES ISOLATED FROM *EUPHORBIA TAURINENSIS*

ANNAMÁRIA KINCSES[1, 2], GRÉTA SÁTORI[3], KÚSZ KÚSZ[3], TÍMEA MOSOLYGÓ[1, 2], KATALIN BURIÁN[1, 2], JUDIT HOHMANN[2, 3], DÓRA RÉDEI[2, 3], GABRIELLA SPENGLER[1, 2]

[1]Department of Medical Microbiology and Immunobiology; [2]Interdisciplinary Centre of Natural Products; [3]Department of Pharmacognosy, University of Szeged, Szeged, Hungary

The development of multidrug resistance (MDR) to chemotherapy is a major obstacle regarding the effective anticancer treatment. It has been described that one of the most common mechanisms of cancer MDR is the over-expression of the efflux pumps (e.g. P-glycoprotein), which are membrane proteins that can recognize and extrude anticancer drugs out of the cells. Modulation of P-glycoprotein (ABCB1) can be a promising approach to overcome multidrug resistance (MDR) in cancer. Diterpenes from *Euphorbia* species have shown to have potential MDR reversing activities. Diterpenes isolated from *Euphorbia taurinensis* were investigated using L5178Y mouse T-lymphoma cell in order to describe their anticancer activities. After the determination of the cytotoxic activity, the isolated diterpenes were measured at two concentrations (2 μM and 20 μM) in rhodamine 123 accumulation assay that is based on the intracellular accumulation of the ABCB1 substrate rhodamine 123. The samples were evaluated by flow cytometry and the fluorescence activity ratios (FAR) were determined using the ABCB1-transfected cell populations over-expressing P-glycoprotein and its parental counterparts. Two derivatives had moderate cytotoxic activity (IC50 values above 50 μM), the other compounds showed no cytotoxic properties. Four of the isolated diterpenes showed remarkable ABCB1 modulating activity on the MDR mouse T-lymphoma cells with FAR values between 34 and 59 at 20 μM concentration. This study highlights the anticancer and MDR reversing activity of *Euphorbia* diterpenes, because these derivatives could be lead candidates for the development of MDR reversal agents of natural origin with low toxicity.

Supported by the project GINOP-2.3.2-15-2016-00012 (New ways in the natural product-based drug discovery-system metabolomic approaches to discover biologically active terpenoids of herbal and microbial origin).

THE EFFECT OF LIGHT ON THE SEDIMENTATION OF *CHLORELLA VULGARIS*

BERNADETT KISS, ÁRON NÉMETH

[1]Budapest University of Technology, Budapest, Hungary

Green microalgae are very small and often unicellular microorganisms and this cause problem in the harvesting process. In the industry different methods can be tailored toward each step depending on the algae size and type, the lipid content, cell density of algae suspension and the desired end product [1]. Centrifugation is one of the most common techniques to separate the algae cells, with recovery in excess of 90%. Energy consumption is high and may be too costly for large scale reactors or pounds. Gravity forced sedimentation has a lower operational cost but it takes a long time for the cells to be concentrated. Different sedimentation pound and separator are used to increase efficiency. Coagulation is another downstream operation, in which the industry use bacterial, fungal, chemical and algal coagulants aid in the flotation or settling of microalgae. [1-3] In this research we made microscale fermentations in a 24 well microplate. The volume of a well was 3 ml and we put inside 1 ml algae suspension. *Chlorella vulgaris* suspension was lighted with an RGB LED module allowing regulation of the illumination of every single column.

In our previous experiments we use just white color three different intensity. In this cultivation we used blue, red, green light and theirs combinations (turquoise, purple, yellow, white). In this study we try to measure the effect of lighting on sedimentation during cultivation.

[1] Cheruvu, S. et al. (2016). Algae harvesting 13. Membrane Technologies for Biorefining, pp: 309-328
[2] Chatsungnoen, T., and Chisti, Y. (2016). Algal Research 13, 271–283.
[3] Sim, T. S., et al. (1988). Biomass 16, 51-62.

PRODUCTION OF ITACONIC ACID FROM D-XYLOSE BY *ASPERGILLUS TERREUS*

ISTVÁN SÁNDOR KOLLÁTH[1], ÁKOS P. MOLNÁR[1], ERZSÉBET FEKETE[1], ERZSÉBET SÁNDOR[2], ÁRON SOÓS[2], BÉLA KOVÁCS[2], CHRISTIAN P. KUBICEK[3], LEVENTE KARAFFA[1]

[1]Department of Biochemical Engineering; [2]Department of Food Science, University of Debrecen, Hungary; [3]Institute of Chemical, Environmental and Biological Engineering, TU Wien, Austria

The fermentation industry is in a perennial search for cheap, renewable raw materials to be employed as carbon sources. Hence, research interest over the efficient utilization of non-food, lignocellulosic plant biomass is soaring. Lignocellulosic biomass consists of a diverse mixture of monomers, of which D-glucose and D-xylose are the two major sugars. Itaconic acid (2-methylenesuccinic acid; IA) is a five-carbon dicarboxylic acid, with one of the carboxyl groups being conjugated to a methylene group – a feature that makes IA easy to (co)polymerize. It is thus used as a building block chemical for the synthesis of a variety of compounds with a broad range of applications, such as manufacturing plastics, coatings and resins. IA is commercially produced by large-scale submerged fermentations employing the filamentous Ascomycete fungus *Aspergillus terreus* and using molasses or hydrolized corn starch as primary carbon sources. Despite of appearing a reasonable option, IA production on D-xylose as a sole source of carbon has never been critically investigated. Hence, the objective of this study was to test whether IA can be produced on D-xylose in concentrations and specific yields (Yp/s) similar to D-glucose by *A. terreus*. Production of IA is the result of the metabolic overflow of primary metabolism. High (Yp/s>0.8) molar yields on D-glucose require high (>10%, w/v) concentrations of carbon, strong aeration and carefully set cultivation parameters, of which Mn(II) ion limitation is the most prominent. When D-glucose was replaced with D-xylose under identical fermentation conditions, the plot depicting specific IA yield vs. initial carbon concentration was notably different. Maximum IA yield was significantly reduced (Yp/s=0.55), but it was achieved at a relatively low (5%, w/v) initial D-xylose concentration. Any further increase above this level did not affect yield, which was, however, subject to severe Mn(II)-related regulation. Mn(II) ion concentrations as low as 5 ppb decreased IA yield on D-xylose by 15%. In contrast to the situation on D-glucose, IA yield did not drop below 0.3 on D-xylose even in the presence of 1000 ppb of Mn(II) ions. In conclusion, while it is possible to produce IA from D-xylose by *A. terreus*, the technology has to be improved considerably to be competitive with traditional glucose-based fermentations.

ISOLATION AND IDENTIFICATION OF NOVEL PLASMIDS FROM *ACINETOBACTER* SP.

JÁNOS KÖMÜVES, JÁNOS KISS, FERENC OLASZ, ALEXANDRA VERESS

Agricultural Biotechnology Institute, National Agricultural Research and Innovation Centre, Gödöllő, Hungary

Honey has been used in traditional medicine since ancient times and regarded as valuable in keeping human's good health. The beneficial effects of honey are partially attributed to the metabolic products of bacteria living in the gastrointestinal tract of honeybees (*Apis mellifera*) and occurring in honey, as well. The primary aim of our project was to isolate lactic acid bacteria from honey and honey stomach of *Apis mellifera* for probiotic use. However, many other, yet undescribed strains have also been found and these species can be potential sources of previously unknown plasmids harbouring novel genes of special interest. Based on the results of 16S rDNA-amplicon profiling and restriction analysis of plasmid DNA preparations, several strains have been selected for whole genome sequencing. To identify plasmid sequences, the annotated scaffolds of the genome sequence of strain M.2.a (*Acinetobacter* sp.) were searched for plasmid-related genes such as *repA* (coding for key protein of plasmid replication initiation). Several putative repA genes were located on scaffolds that could be converted into circular plasmid sequences. Oligos facing outward of the ends of scaffolds containing putative plasmid sequences were designed to prove that the native sequences are circular. Altogether, five novel complete plasmid sequences have been validated by PCR. To date, pAVAci84, pAVAci117, pAVAci145 (sequence variant of pRGRH0231) and pAVAci147 have been cloned and transferred into *E. coli* for subsequent shuttle vector development. The mobilization test of 45kb pAVAci14, which appears to have a complete set of conjugation genes, is ongoing.

ANTIFUNGAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM FAECES OF EXOTIC ANIMALS

JUDIT KOSZTIK, ILDIKÓ BATA-VIDÁCS, ERZSÉBET BAKA, KATALIN INOTAI, CSABA DOBOLYI, JÓZSEF KUKOLYA

Department of Applied and Environmental Microbiology, Agro-Environmental Research Institute, National Agricultural Research and Innovation Centre, Budapest, Hungary

Exotic animals living in zoo have special gut microflora. It contains bacteria that are originated from the maternal birth canal and bacteria from their environment. In our department we have built a culture collection of lactic acid bacteria (LAB) isolated from the faeces of exotic animals and ruminants. According to the literature many LAB have antifungal activity. Moulds (fungi) are major contaminants of forage plants like maize, wheat, or rice. Among them *Aspergillus flavus* is the most undesirable mould species, as many of their strains are able to produce mycotoxins. Aflatoxin is a very dangerous and harmful mycotoxin; it can cause liver cancer and other serious health problems. The aim of our research is to study the antifungal activity of our LAB strains against *Aspergillus flavus* strains in order to select LAB for controlling moulds during silage process. During this study 4 *Aspergillus flavus* strains (Zt31, Zt41, Zt55, and Zt80), isolated in 2009 from maize, with the ability to produce aflatoxin, and near 100 LAB strains obtained from 14 exotic animals were used. In the experiments, agar modified diffusion technique was applied for the co-cultivation of LAB strain and mould strain. The mould strain was mixed into the fluid MRS agar and plated. Into the middle of the solidified agar a well was cut, and the LAB strain was added to that well. After 2 days at 37 °C, the inhibition zone around the well was measured. Against the studied four aflatoxin producing *Aspergillus flavus* strains, *Lactobacillus salivarius* and *Pediococcus pentosaceus* strains have the best inhibition ability. According to our results, strain Zt31 proved to be the least resistant mould strain to LAB. The most LAB resistant strain, Zt80, overall had a 75% higher resistance. The largest inhibition zone was 5 mm in case of *L. salivarius* strain, which was isolated from *Casuarius casuarius*. Other

LAB strains as *Pediococcus lolii*, *Lactobacillus paracasei* or *Lactobacillus amylotrophicus* have also shown inhibitory properties against *Aspergillus flavus*, though by many LAB species, for example *Lactococus lactis*, *Lactobacillus equi*, or *Lactobacillus johnsoni*, no inhibition ability could be found.
This work was supported by the NVKP-16-1-2016-0009 and OTKA K116631 projects.

ARBUSCULAR MYCORRHIZAL COMMUNITY OF MYCORRHIZOSPHERE IN RELATION TO VEGETATION TYPES OF A SOLONCHAK GRASSLAND IN APAJPUSZTA

RAMÓNA KOVÁCS[1], TIBOR SZILI-KOVÁCS[1], ISTVÁN PARÁDI[2], ANNA FÜZY[1], PÉTER CSONTOS[1], TÜNDE TAKÁCS[1]

[1]Institute for Soil Sciences and Agricultural Chemistry, Centre for Agricultural Research, Hungarian Academy of Sciences; [2]Department of Plant Physiology and Molecular Plant Biology, Institute of Biology, Faculty of Science, ELTE Eötvös Loránd University, Budapest, Hungary

Arbuscular mycorrhizal fungi (AMF) are endosymbionts, that can help their host plants in water and nutrient uptake and to cope with several kinds of stress. Therefore they influence the species composition and dominance relations in plant communities. Our knowledge of AM fungal community in saline soils of the Hungarian steppe is very limited. The goal of our work was to study the AMF communities in a typical saline solonchak grassland of Hungary. Four typical plant communities (Lepidio crassifolii - Puccinellietum limosae - *Puccinellia* sward, Artemisio santonici - Festucetum pseudovinae - *Artemisia* steppe, Lepidio crassifolii - Camphorosma annuae - saline bare spot and Achilleo - Festucetum pseudovinae - shortgrass pasture) were examined in saline-sodic soils of Apajpuszta (Kiskunsági National Park; Hungary). Chemical and physical properties of soils were analysed and the indigenous plant composition of the communities was investigated. Mycorrhizal status of host plants was measured by Trouvelot method. The density of AMF infective propagules was estimated by the most probable number (MPN) test. The extraradical presence of AMF was detected by measuring the amount of glomalin-related soil proteins (GRSP). The diversity of AMF communities from the most abundant host plants was examined with PCR-RFLP method. The DNA samples extracted from roots were tested by taxon specific primer systems for the ribosomal RNA gene sequences of the fungi. The salt concentration and the pH of the soils showed descending gradient and the humus content showed growing gradient in the order of saline bare spot - *Puccinellia* sward *Artemisia* steppe - shortgrass pasture communities. In saline bare spot mostly non-host plants were found. In *Artemisia* steppe and shortgrass pasture live more mycotroph plants, with higher colonization values. In *Puccinellia* sward the less mycorrhiza dependent and less colonized *Puccinellia limosa* plant was the most abundant. The saline bare spot community had the lowest amount of infective AMF propagules and GRSP, while shortgrass pasture had the highest values. We were able to identify more AMF species by the molecular methods from the roots of dominant plant species of every community, such as the generalist *Rhizophagus intraradices* and *Funneliformis mosseae*, which can be found in many different soils. Furthermore the specialist *Funneliformis geosporum* and the less common *Rhizophagus fasciculatus* and *Glomus indicum* were also found.
This research was supported by the Hungarian Scientific Research Fund (K108572).

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF POTENTIAL MICROBIAL BIOCONTROL AGENTS OF *ARMILLARIA* SPECIES DAMAGING TREE CROPS

LÁSZLÓ KREDICS[1], BETTINA BÓKA[1], LIQIONG CHEN[1], ORSOLYA KEDVES[1], VIKTÓRIA IMRE[1], GYÖRGY SIPOS[2], CSABA VÁGVÖLGYI[1]

[1]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged; [2]Department of Forest Protection, Institute of Sylviculture and Forest Protection, University of Sopron, Sopron, Hungary

Among the basidiomycetous fungi, the most relevant soil-borne tree pathogens from an economical point of view are the honey mushrooms from the genus *Armillaria* (Physalacriaceae, Agaricales, Basidiomycota), causing root diseases in fruit trees, nut crops and timber trees in both hemispheres of the world under temperate, boreal and tropical climates. Symptoms of the diseased plants include dwarfed foliage, wilting, premature defoliation and stunted shoots in the case of conifer hosts, while dwarfed fruits can be observed in the case of fruit and nut crops. Biocontrol agents are promising, environment-friendly alternatives to chemical pesticides for controlling *Armillaria* species in soil. Bacteria from the genera *Pseudomonas* and *Bacillus* produce various biologically active metabolites with the potential to successfully inhibit the growth of fungal plant pathogens, while *Trichoderma* species are efficient biocontrol fungi applying various antagonistic mechanisms (competition, antibiosis, mycoparasitism, plant growth promotion, induction of systemic resistance in plants) against a wide range of fungal plant pathogens. During this study, culturable bacteria and fungi were isolated from *Armillaria*-infested soil samples. *Pseudomonas* strains and other bacteria were identified based on a region within the *rpoB* gene and the 16S rRNA gene, respectively, while the identification of the *Trichoderma* isolates was carried out by ITS sequence analysis. A total of 56 *Trichoderma*, 34 *Pseudomonas*, 6 *Bacillus* and 1 *Streptomyces* strains were obtained and identified. *Trichoderma asperellum, T. atroviride, T. citrinoviride, T. gamsii, T. hamatum, T. harzianum, T. koningii* and *T. virens* were frequently occurring in the soil samples. *Pseudomonas mandelii, P. fluorescens, P. putida* and *P. jessenii* were frequent representatives of the *Pseudomonas* communities, while from the genus *Bacillus*, *B. mycoides* and *B. cereus* were identified. *In vitro* antagonism and antibiosis experiments were performed to determine the potential of the *Pseudomonas* and *Trichoderma* isolates to control *A. gallica*. The examined biocontrol candidate strains were also screened for plant growth promoting abilities: we examined the siderophore production, phosphorous mobilization and the indol-3-acetic acid production abilities.

Some of the *Pseudomonas*, *Bacillus* and *Trichoderma* strains were shown to produce siderophores, to mobilize phosphorous and to produce indole-3-acetic acid. Based on the results of this study we can conclude that *Trichoderma, Bacillus* and *Pseudomonas* are microbial genera containing potential antagonists of *Armillaria* spp., and also have plant growth promoting properties which could be exploited during the biological control of *Armillaria* root rot.

Supported by the Hungarian Government and the European Union within the frames of the Széchenyi 2020 Programme (GINOP-2.3.2-15-2016-00052).

THE DEVELOPMENT OF COMPLEX MULTICELLULAR STRUCTURES IN FUNGI

KRISZTINA KRIZSÁN[1], ÉVA ALMÁSI[1], ARUN PRASANNA[1], BRIGITTA KISS[1], GYÖRGY SIPOS[2], BALÁZS BÁLINT[3], ISTVÁN NAGY[3], LÁSZLÓ G. NAGY[1]

[1]Biological Research Center, Hungarian Academy of Sciences, Szeged; [2]Institute of Silviculture and Forest Protection, University of West-Hungary, Sopron; [3]Seqomics Ltd, Mórahalom, Hungary

The complex multicellular structures of fungi can be characterized by a 3-dimensional appearance; their best-known forms are the fruiting bodies which are involved in sexual spore production. Fruiting bodies evolved independently in major clades but their most complex representatives can be found in the Agaricomycetes. Despite the indispensable information of pioneering gene expression studies about fungal fruiting body development the general principles and conservation of gene families involved in fruiting body formation are yet to be understood.
The aim of our study is to identilfy the most important structural and regulator genes underlying the development of fungal fruiting body. In a comparative transcriptomic view, we determined the expression profiles of equivalent developmental stages (vegetative mycelia, early and late primordial, young and mature fruiting bodies) of 6 species (*Armillaria ostoyae*, *Coprinopsis cinerea*, *Lentinus tigrinus*, *Phanerochaete chrysosporium*, *Rickenella mellea*, *Schizophyllum commune*) and identified the most important developmentally regulated gene families involved in the fruiting body initialization and the fruiting body development. In a comparative phylogenomic view, we aimed to reveal the origin and biological relevance of genes already known to play role in fungal morphogenesis with the analysis of a dataset containing 242 genomes covering the whole Kingdom Fungi using phylostratigraphic approach. Our results contribute to unraveling the general principles during the fungal fruiting body initialization, which is the most interesting step during the fruiting body development in evolutionary term and get an important insight into the origin of the genetic toolkit underlying the fungal fruiting body development.

COMPARATIVE BACTERIAL METAGENOMIC ANALYSIS OF SEVEN DIFFERENT HUNGARIAN PROPOLIS SAMPLES

HEDVIG D. KURNÁSZ[1], PÉTER URBÁN[1, 2], SARRA BOUCHELAGHEM[1], RÉKA MESZÉNA[1], CSABA FEKETE[1, 2], ZOLTÁN GAZDAG[1, 2], DÁVID NAGY[3], GÁBOR PAPP[1, 2]

[1]Department of General and Environmental Microbiology, Institute of Biology, Faculty of Sciences; [2]Microbial Biotechnology Research Group, János Szentágothai Research Centre; [3]Department of Ecology, Institute of Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary

Propolis is a brownish-yellow, sticky substance produced by bees, which is mostly used to protect the hive against intruders. The main source is the resinous coating found on plant buds. Due to its antimicrobial, antiviral and antitumor activity propolis has long been used in folk and alternative medicine. The aim of this study was to determine the bacterial community of 7 Hungarian propolis samples from different geographical regions (Csikóstőttős, Héhalom, Lengyeltóti-Tatárvár, Aranyosgadány, Somogybabod, Szolnok, Szombathely) by a new generation sequencing method. The metagenomic sequencing targeted 7 hypervariable regions of the eubacterial 16S rRNA coding gene, which enables high throughput data acquisition and taxa identification. The sequencing was performed on the Ion Torrent PGM platform, and for bioinformatic and statistical analysis the Ion Reporter and PAST softwares were used, respectively. The sequencing resulted a total of 3,044,319 reads. Metagenomic analysis of the propolis samples revealed strongly diverse microbial communities among the different samples from different places. Altogether Cyanobacteria (38.9%) and Proteobacteria (29.5%) represented the most prevalent phyla followed by Firmicutes (20.5%), Actinobacteria (9.4%) and Bacteroidetes (1.5%). At genera and species level 390 operational taxonomic units (OTUs) were identified, with the number of genera in the different samples varying

between 45 and 133, and between 21 and 105 at species level. There was no identifiable OTU at species level that could be found in all 7 samples. Only two genera (*Microbacterium* sp., *Lactobacillus* sp.) were detected in each sample and 7 genera were present at least in 6 samples. Based on the Shannon and Simpson indices the most diverse samples were originated from Héhalom, Csikóstőttős and Lengyeltóti-Tatárvár followed by the samples from Szolnok, Somogybabod, Szombathely and Aranyosgadány. The origin of some bacteria could be traced back to environmental, animal- and human sources. Among the identified species numerous human pathogens (eg. *Klebsiella pneumoniae*, *Clostridium perfringens*, *Vibrio* sp., *Enterococcus faecalis*, *Haemophilus parainfluenzae*), antibiotic producers (e.g. *Saccharomonospora azurea*, *Streptomyces* sp., *Nocardioides islandensis*), bee pathogens (e.g. *Paenibacillus larvae*, *Frischella perrara*), plant- and animal symbionts (e.g. *Nostoc* sp., *Methylobacterium populi*), and extremophiles (*Rhodoferax antarcticus*, *Virgibacillus halotolerans*, *Sphingomonas glacialis*) were present. Our investigations have shown that, despite the favorable antimicrobial effect of propolis, there is a wide variety of microbial entities and the presence of potential human pathogens, which should be taken into consideration when processing and using propolis and its derivatives.

This work was connected to the project Hungarian Scientific Research Fund (OTKA) 116631.

PARALLEL SURVEY OF TWO WIDESPREAD RENAL SYNDROME-CAUSING ZOONOSES: *LEPTOSPIRA* SPP. AND HANTAVIRUSES IN URBAN ENVIRONMENT, HUNGARY

KORNÉLIA KURUCZ[1], MÓNIKA MADAI[1], DOMINIKA BALI[1], DÁVID HEDERICS[1], GYŐZŐ HORVÁTH[2], GÁBOR KEMENESI[1], FERENC JAKAB[1]

[1]Szentágothai Research Centre; [2]Faculty of Sciences, University of Pécs, Pécs, Hungary

About 75% of human pathogenic infections are caused by zoonoses. Several factors drive the emergence and/or re-emergence of these pathogens. From all of these, the most important is growing human population, that results in increased urbanization and consequential environmental alteration. It also induces changes in both food processing and in the distribution of animal/vector populations. Furthermore, it procures a closer co-existence between animals and humans, therefore potentially induces changes in zoonotic disease transmission, too. Rodents are important reservoirs for a large number of zoonotic pathogens such as *Leptospira* spp., among others. Leptospirosis is considered the most widespread zoonotic disease worldwide. As it has the clinical manifestation of acute renal failure, it mimics hantavirus infection in humans, a disease mainly caused by hantavirus. Hantavirus infections have great clinical and epidemiological significance in Europe, however no data exists for urbanized areas in Hungary, so far. Identification of reservoir hosts of zoonotic agents is a prerequisite for an effective prevention of human infections. Therefore, this study was conducted to investigate the occurrence and prevalence of *Leptospira* spp. and hantaviruses in small wild rodents living in urban habitats. Furthermore, we have performed the total molecular biological characterization of detected pathogens to broaden our knowledge about human health risk related to rodents living in close proximity to humans. Altogether, 338 small rodents representing 5 different species (*Apodemus agrarius*, *A. flavicollis*, *A. sylvaticus*, *Microtus arvalis*, *Myodes glareolus*) were captured in the city of Pécs and screened for pathogens by different types of PCR methods (qRT-PCR/PCR, RT-PCR/PCR). A total of 19.3 % of the rodents were positive for *Leptospira kirschneri*, *L. interrogans* and *L. borgpetersenii*. Nucleic acid of Tula and human pathogen Dobrava-Belgrade virus were detected in 8.8% of tested specimens. In addition, co-infections with both *Leptospira* and hantaviruses were shown in nine animals (2.6 %), suggesting that the same rodent host can be

infected with several pathogens at the same time, thus representing a serious threat to public health. Overall, this study provides important surveillance data on the prevalence of *Leptospira* spp. and hantaviruses from rodents in urbanized environment for the first time in Hungary, and emphasizes the importance of furthere co-epidemiological investigations.

ANAEROBIC BIODEGRADATION OF COMPLEX HYDROCARBON POLLUTIONS

KRISZTIÁN LACZI[1], ATTILA BODOR[1, 2], ÁGNES ERDEINÉ KIS[1, 2, 3], NAILA BOUNEDJOUM[1, 2], KATALIN PEREI[1, 2], TAMÁS KOVÁCS[4] , GÁBOR RÁKHELY[1, 2, 3]

[1]Department of Biotechnology; [2]Institute of Environmental and Technological Sciences, University of Szeged; [3]Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, Szeged; [4]Department of Biotechnology, Nanophagetherapy Center, Enviroinvest Corp., Pécs, Hungary

Crude oil pollutions are usually complex mixtures of various linear, branched, cyclic and aromatic hydrocarbons. These compounds are widespread contaminants in both aquifers and soil, their elimination is in the primary focus of environmental sciences. Microorganisms are key components in biological removal of pollutants. Strains capable of utilizing hydrocarbons usually appear at the contaminated sites, but their metabolic activities are often restricted by the lack of nutrients, electron acceptors and/or they can only metabolize one-two components of a mixture. Frequently, the oil pollutions occur at the level of anoxic ground water. In these cases, either alternative electron acceptors are used for the oxidation of the target compounds or other anaerobic pathways should be activated. A metagenomic approach was used to monitor the microbes and gene doses at potentially anaerobic contaminated sites. Apparently, the abundance of the strains and genes depended on the composition of the pollutions and the depth of sampling. Substantial amount of hydrocarbons could be detected at 7,5-8 m depths. At two sampling points similar pollutant patterns composed of branched alkanes were detected. More diverse chemical components of higher concentrations were identified in the third sampling point which contained substituted aromatic/diaromatic compounds, as well. The metagenomic analysis revealed dominant microbes in all contaminated samples which have the gene sets for anaerobic alkane degradation. In the sample containing substituted aromatic compounds, other microbial strains capable of anaerobic degradation of aromatic compounds were also abundant. In the most contaminated sites, substantial amounts of methanogens could be detected indicating a potential biochemical coupling of hydrocarbon degradation to biogas formation. The presence of the relevant genes predicted by the metagenomic approach was validated by qPCR.

Support of TÁMOP 4.2.4.A/2-11-1-2012-001 "National Excellence Program" is gratefully appreciated. The project was supported by the Norway Grant (grant agreement no. HU09-0044-A1-2013).

A VIRAL SUPPRESSOR OF RNA SILENCING INHIBITS ARGONAUTE 1 FUNCTION BY PRECLUDING TARGET RNA BINDING TO PRE-ASSEMBLED RISC

LÓRÁNT LAKATOS

University of Szeged, Szeged, Hungary

In most eukaryotes, RNA silencing is an adaptive immune system regulating key biological processes including antiviral defense. To evade this response, viruses of plants, worms and insects have evolved viral suppressors of RNA silencing proteins (VSRs). Various VSRs, such as P1 from Sweet potato mild mottle virus (SPMMV), inhibit the activity of RNA- induced silencing complexes (RISCs) including an ARGONAUTE (AGO) protein loaded with a small RNA. However, the specific mechanisms explaining this class of inhibition are unknown. Here, we show that SPMMV P1 interacts with AGO1 and AGO2 from *Arabidopsis thaliana*, but solely interferes with AGO1 function. Moreover, a mutational analysis of a newly identified zinc finger domain in P1 revealed that this domain could represent an effector domain as it is required for P1 suppressor activity but not for AGO1 binding. A comparative analysis of the target RNA binding capacity of AGO1 in the presence of wild-type or suppressor-defective P1 forms revealed that P1 blocks target RNA binding to AGO1. Our results describe the negative regulation of RISC, the small RNA containing molecular machine.

MICROBIOLOGICAL INVESTIGATIONS ON THE WATERS OF TWO THERMAL BATHS IN BUDAPEST USING NGS TECHNIQUE

ANETT LIPPAI[1, 2], ATTILA SZABÓ[1], ERIKA TÓTH[1]

[1]Department of Microbiology, Faculty of Science, ELTE Eötvös Loránd University; [2]KVI-PLUSZ Ltd., Budapest, Hungary

In Hungary there is only limited information on the natural microbial communities of thermal baths. During our studies the well and three pool waters (indoor charging-unloading 38°C, indoor charging-unloading 20°C, outdoor turning 38°C) were investigated in Dandár and Széchenyi bath (Budapest) using NGS technique. The total cell counts of water samples were also determined. The total bacterial counts were as follows: in the well water of Dandár bath $1.43*10^4$ cell/ml, in the indoor charging-unloading 38°C pool $4.94*10^6$ cell/ml, in the indoor 20°C pool $2.6*10^6$ cell/ml and in the outdoor 38°C pool $7.83*10^5$ cell/ml; In the well water of Széchenyi bath $6.14*10^3$ cell/ml, in the indoor charging-unloading 38°C pool $1.39*10^6$ cell/ml, in the indoor charging-unloading 20°C $3.65*10^5$ cell/ml and in the outdoor turning 38°C pool $8.99*10^5$ cell/ml values were detected. Results of our molecular taxonomic studies showed that the water of both thermal baths has unique but diverse bacterial communities. Members of Proteobacteria were dominant in the water samples of both thermal baths though the ratio of Alpha- and Betaproteobacteria differed strongly in the samples. *Hydrogenophaga* was characteristic mainly in Dandár bath samples while *Methylobacterium* was detected only in Széchenyi bath samples. The nitrogene fixing members of Rhizobiales were present in the water of both baths. Their presence is not surprising in these nutrient deficient environments. In the water of the charging-unloading 38°C pool of Széchenyi bath the phylum Aquficae was domininant, with a hydrogen- or sulfur/thiosulfate-oxidizing, thermophilic chemolithoautotroph bacteria, named *Sulfurihydrogenibium*. This taxon was present in all Széchenyi bath sample but not in Dandár bath samples. Chloroflexi were detected only in the well water of Dandár bath. The frequency of Actinobacteria varied but they were also detected almost in all water samples. Members of Bacteroidetes were characteristic mainly in Dandár bath samples.

DE NOVO GENOME PROJECT OF *MICROCOCCOIDES HYSTRIXII* TSL3

SZABINA LUZICS[1], ÁKOS TÓTH[1], DOROTTYA SÁRKÁNY[1], ILDIKÓ BATA-VIDÁCS[1], BALÁZS BÁLINT[2], BÁLINT MÁRK VÁSÁRHELYI[2], ISTVÁN NAGY[2, 3], JÓZSEF KUKOLYA[1]

[1]Department of Applied and Environmental Microbiology, Agro-Environmental Research Institute, National Agricultural Research and Innovation Centre, Budapest; [2]SeqOmics Biotechnology Ltd., Mórahalom; [3]Sequencing Platform, Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

A Gram-positive bacterium designated TSL3 was isolated from feces of the porcupine *Hystrix indica* in the Budapest Zoo and Botanical Garden, Hungary. According to the recent polyphasic taxonomic investigation TSL3 represents the type strain of the newly formed genus *Micrococcoides* under the name of *Micrococcoides hystrixii* gen. nov., sp. nov. On the basis of 16S rRNA gene sequence analysis, the strain is phylogenetically related to the family Micrococcaceae. The highest 16S rRNA gene sequence similarity was found with *Micrococcus terreus* V3M1T (96.50 %) followed by *Arthrobacter humicola* KV-653T (96.43%). TSL3 cells cultivated at 27 °C, 200 rpm for two days in TSA liquid medium were used for total DNA isolation. Whole genome sequencing and genome assembly Mate Pair Sequencing library was generated, with an average insert size of 7.5 kbp, using Illumina Nextera Mate Pair Kit (Cat. No.: FC-132-1001) as per manufacturer's instructions. DNA sequencing was performed on an Illumina MiSeq machine using V2 sequencing chemistry. Raw reads were processed following the manufacturer's recommendations (Data Processing of Nextera® Mate Paired Reads on Illumina Sequencing Platforms). De novo assembly was performed with CLC Genomics Workbench Tool (v8.5.1, CLC Bio). Contigs were arranged into scaffolds using SSPACE 3.0 (Boetzer et al., 2011). Gaps in scaffolds were closed with Spades v3.1.1 driven by an in-house R script. The draft genome of TSL3 consists of 2.527.433 nt with 2.276 putative coding sequences. The GC content is 59.63%. The contig number is 11, within the range of 1.430 nt-738.220 nt. According to the whole genome comparisons the most similar genome is from the representative of the *Micrococcus* genus: *Micrococcus luteus* SUBG006.

The analysis of the annotated genome of TSL3 revealed the existence of 5 putative glycoside hydrolases belonging to different GH-families, 9 different glycosyltransferases, 10 alpha-beta hydrolases, 28 proteases belonging to 8 different classes and 23 oxygenases representing mono- and di-oxygenases, a P450 enzymes. *Micrococcoides hystrixii* TSL3 is a moderate sterigmatocystin degrading microbe, and the identified oxygenases may take role in the detoxification process.

This study was supported by OTKA-K116631.

FIRST SEROLOGICAL EVIDENCE OF CRIMEAN–CONGO HAEMORRHAGIC FEVER VIRUS IN HUNGARIAN POPULATION

NÓRA MAGYAR, BERNADETT PÁLYI, ANNA NAGY, IVELINA DAMJANOVA, MÁRIA TAKÁCS, ZOLTÁN KIS

Directorate of Clinical and Public Health Microbiology, National Public Health Institute, Budapest, Hungary

The Crimean-Congo haemorrhagic fever virus (CCHFV) is considered to be a major emerging disease threat causing serious symptoms with an alarming fatality rate of up to 40%. The virus is spreading to and within the European region following the expanding distribution of the main vector, the Hyalomma marginatum tick. Due to climatic, environmental and economic changes the tick has appeared in Hungary and there is also serological evidence of CCHFV infection in the European hare.

However, no data on seroprevalence are available denoting any previous exposure of humans to CCHFV infection. Therefore, the aim of our study was to assess the seroprevalence of CCHFV infection in the Hungarian population using a retrospective panel of blood donor sera collected in 2008 and in 2015. We received samples from 14 counties, mainly from the southern, southern-eastern and central regions of Hungary. The gold standard whole-virus containing immunofluorescent assay (IFA) slides were used to determine seropositivity. To exclude cross-reactivity, the CCHFV IFA slides were tested for Hanta virus, Tick-borne encephalitis virus, Cytomegalovirus, Epstein-Barr virus, Leptospira sp., Mycoplasma sp. and Coxiella burnetii. We found seven seropositive donors in four county regions of Hungary. This is the very first serological evidence of CCHFV infection in the Hungarian population using the gold standard whole-virus IFA. Considering our results, it would be strongly recommended to set up and launch an extended serosurveillance program focusing on both the human and animal population, and also to evaluate the distribution of the ticks in Hungary. Our results also attach great importance to increase the awareness of clinicians and the at-risk population (foresters, hunters) about the potential emerging threat of CCHFV.

POSTANTIFUNGAL EFFECT OF MICAFUNGIN AGAINST *CANDIDA ALBICANS* COMPLEX

LÁSZLÓ MAJOROS[1], ZOLTÁN TÓTH[1], TAMÁS KARDOS[2], FRUZSINA NAGY[1], RENÁTÓ KOVÁCS[1], GÁBOR KARDOS[1], ALÍZ BOZÓ[1]

[1]Medical Microbiology; [2]Department of Pulmonology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

We compared micafungin killing rate and postantifungal effect (PAFE) at 4, 16 and 32 mg/L in RPMI-1640 and 50% serum against *C. albicans*, *C. dubliniensis* and *C. africana* reference strains and clinical isolates. In RPMI-1640 MIC value ranges were 0.015-0.03, 0.015 and 0.015-0.06 mg/L for *C. albicans*, *C. africana* and *C. dubliniensis*, respectively. In 50% serum MICs increased by 16-64 folds. In RPMI-1640 one hour exposure of micafungin yielded fungistatic effect against the *C. albicans* complex; 50% growth reduction was never reached for any isolate. In RPMI-1640 PAFE were 1.5 - >19.4, 9.7 - >20.1 and 15.9 - >18.5 hours for *C. albicans*, *C. africana* and *C. dubliniensis*, respectively. In 50% serum the three species behaved similarly; the duration of PAFEs decrease sharply to 0 - 1.7 hours; one hour exposure of micafungin produced only weak and transient CFU decreases with always negative killing rate values. The prolonged PAFE measured in RPMI-1640 may not be a good predictor of PAFE in vivo as indicated by PAFE in 50% serum. Short growth inhibition without killing in 50% serum suggests that micafungin PAFE has a limited role in the eradication of the *C. albicans* complex from the bloodstream.

INTERNALISATION OF ENDOPHYTIC, ENTERIC AND HUMAN PATHOGENIC BACTERIA IN SWEET PEPPER (*CAPSICUM ANNUUM* L. VAR. *GROSSUM*)

ANNA MARÁZ[1], ZOLTÁN FÜSTÖS[2], ÁGNES BELÁK[1]

[1]Department of Microbiology and Biotechnology, Faculty of Food Science, Szent István University; [2]Department of Nutritional Physiology, Food Science Research Institute, NARIC, Budapest, Hungary

High number of putative endophytic bacteria were isolated from vegetative and reproductive organs of sweet pepper (*Capsicum annuum* L. var. *grossum*), which comprised 200 genotypically different strains. Identification and phylogenetic analysis of the endophytes indicated an extended diversity; majority of the strains belonged to *Pseudomonas*, *Bacillus*, *Rhizobium*, *Pantoea*, *Microbacterium* and *Enterobacter*, while several genera were represented only by single strains [1]. As the continuation of this work we aimed to determine the real endophytic lifestyle of selected putative endophytic bacteria by looking for microscopic evidence of their residence inside the plant tissues, which is considered as one of the most important prerequisites of real endophytes [2]. Beside studying the internalization of putative endophytic bacteria we intended to check whether a commensal *Escherichia coli* and a food-borne human pathogen *Listeria monocytogenes* cells are able to enter and colonize sweet pepper under the same conditions as the endophytic bacteria. We used seed bacterization and combination of cultivation dependent and independent (PCR and FISH-CLSM) techniques for studying the infiltration, persistence and colonization of endophytic as well as *E. coli* and *L. monocytogenes* in sweet pepper. We proved the real endophytic lifestyle of the seed germination promoting *Chryseobacterium hispalense* and *Pseudomonas* sp. putative endophytic strains, because their cells were visualized by FISH–CLSM in the internal tissues of 6-7 weeks old pepper seedlings and their genomes were detected by specific PCR reactions.

On the contrary, we could not detect stably or transiently colonized *E. coli* and *L. monocytogenes* in the endosphere, which suggested that there is a low risk associated with internalized enteric or human pathogenic bacteria via germinating seeds in sweet pepper, not even a transient persistence could be suspected by PCR amplification of DNA being present in dead or VBNC cells.

This work was funded by the Hungarian project OTKA K-101716.

[1] Füstös Z. et al. (2014). Annual Meeting of the Hungarian Microbiological Society. Book of Abstracts p. 18.
[2] Reinhold-Hurek, B., and Hurek, T. (1998). Critical Rev. Plant Sci. 17, 29-54.

ISOLATION OF *FRANCISELLA NOVICIDA*-CONTAINING PHAGOSOME FROM INFECTED HUMAN MONOCYTE DERIVED MACROPHAGES

VALENTINA MARECIC[1], OLGA SHEVCHUK[2], MATEJA OZANIC[1], MIRNA MIHELCIC[1], MIHAEL STEINERT[2], BEGONJA ANTONIJA JURAK[3], YOUSEF ABU KWAIK[4], MARINA SANTIC[1]

[1]Department of Microbiology, Faculty of Medicine, Rijeka, Croatia; [2]Institute for Microbiology, TU Braunschweig and Helmholtz Center for Infection Research, Braunschweig, Germany; [3]Department of Biotechnology, University of Rijeka, Rijeka, Croatia; [4]Department of Microbiology and Immunology and Center for Predictive Medicine, Louisville, US

Francisella is a gram-negative bacterial pathogen, which causes tularemia in humans and animals. A crucial step of *Francisella* infection is its invasion of macrophage cells. Biogenesis of the *Francisella*-containing phagosome (FCP) is arrested for ~15 minutes at the endosomal stage, followed by gradual bacterial escape into the cytosol, where the microbe proliferates. The crucial step in pathogenesis of tularemia is short and transient presence of the bacterium within phagosome. Isolation of FCPs for further studies has been challenging due to the short period of time of bacterial residence in it and the characteristics of the FCP. Here, we will for the first time present the method for isolation of the FCPs from infected human monocytes-derived macrophages (hMDMs). For

elimination of lysosomal compartment these organelles were pre-loaded with dextran coated colloidal iron particles prior infection and eliminated by magnetic separation of the post-nuclear supernatant (PNS). We encountered the challenge that mitochondria has similar density to the FCP. To separate the FCP in the PNS from mitochondria, we utilized iodophenylnitrophenyltetrazolium, which is converted by the mitochondrial succinate dehydrogenase into formazan, leading to increased density of the mitochondria and allowing separation by the discontinuous sucrose density gradient ultracentrifugation. The purity of the FCP preparation and its acquisition of early endosomal markers was confirmed by Western blots, confocal and transmission electron microscopy. Our strategy to isolate highly pure FCPs from macrophages should facilitate studies on the FCP and its biogenesis.

A SURVEY ON THE SPECIES DIVERSITY OF CELLULOSE AND XYLAN HYDROLYSING SOIL BACTERIA IN SOIL SAMPLES TAKEN FROM AGRICULTURAL AREAS, BY CULTURING METHODS

RÓZSA MÁTÉ[1], ÁKOS TÓTH[2], ÉVA KÁRPÁTI[3], JÓZSEF KUKOLYA[4], JÓZSEF KUTASI[5]

[1]BioFil Microbiological, Biotechnological and Biochemical Ltd; [2]Department of Applied and Environmental Microbiology, Agro-Environmental Research Institute, National Agricultural Research and Innovation Centre; [3]Saniplant Biotechnological Research and Development Ltd.; [4]Department of Applied and Environmental Microbiology, Agro-Environmental Research Institute, National Agricultural Research and Innovation Centre; [5]BioFil Microbiological, Biotechnological and Biochemical Ltd., Budapest, Hungary

The presence of crop residues is a major issue in soil management, due to the effect it has on soil structure and nutrient availability. Decomposing stalk residues can provide nutrient supply for both plants and soil microbes and improve physical properties of the soil. Without treatment, however, large volumes of crop residues cause problems in tilling and can temporarily disrupt nutrient supply. Undegraded organic matter has adverse effects on soil structure, water storage capacity, and hinders agrotechnical operations. The presence of cellulose and xylan (NSP, non-starch polysaccharide) hydrolyzing microbes can enhance crop residue degradation. The aim of our study was to identify the cellulase and xylanase producing microbial species in soil samples from agricultural lands with crop residues. Samples were taken from the Zala hills and the Great Plain regions in Hungary, from corn fields following harvest. From the Great Plain region, both organically fertilized (stable manure) and unfertilized areas were sampled. Eight different culture media were used for isolation and the purification of cultures. A total of 250 isolates were tested for the presence of polysaccharidase enzyme activity by Congo-red staining. Incubation times and colony morphology were also recorded. 34 strains were positive in the Congo-red test. DNA was extracted for 16S rDNS PCR profiling for molecular taxonomy. 14 polysaccharidase positive strains were isolated from the Zala hills region and 20 strains from the Great Plains region. From areas without fertilization, 7 different genuses were identified, and the number of strains were double than those from fertilized lands, representing 3 bacterial genera. Strains isolated from the Zala hills region belonged to 8 different bacterial genera.
Supported by KFI_16-1-2016-0214 grant of the National RDI Office, Hungary.

ONCOVIRUS-INDUCED EPIGENETIC ALTERATIONS IN HOST CELLS

JÁNOS MINÁROVITS

Department of Oral Biology and Experimental Dental Research, Faculty of Dentistry, Universuty of Szeged, Szeged, Hungary

The genomes of DNA tumor viruses and proviral genones of RNA tumor viruses regularly undergo epigenetic modifications in host cells and the epigenetic marks deposited by cellular enzymes to the viral chromatin affect the activity of viral promoters. In parallel, viral oncoproteins interacting with the cellular epigenetic machinery induce changes in the cellular epigenome and transcriptome. Thus, it was suggested that oncoviruses may cause malignant transformation by „epigenetic reprogramming" of host cells. Distinct viral oncoproteins alter DNA methylation patterns, redistribute euchromatic or heterochromatic histone marks and relocate epigenetic regulators along the cellular chromatin. All of these mechanisms may change the gene expression pattern, phenotype and behaviour of target cells and facilitate tumorigenesis. Human oncoviruses frequently cause epigenetic dysregulation. Epstein-Barr virus (EBV) encodes nuclear proteins and transmembrane proteins expressed in a host cell phenotype-dependent manner. EBNA1 (Epstein-Barr nuclear antigen 1) induced site-specific DNA demethylation whereas other EBNAs interacted with enzymes involved in histone modification. Latent membrane protein 1 and 2 (LMP1 and 2) upregulated cellular DNA methyltransferases (DNMTs) causing promoter hypermethylation and silencing. Genome-wide alterations of the cellular methylome were also observed in immortalized B cell lines and in various neoplastic cells carrying latent EBV genomes. LANA, the latency-associated nuclear antigen of Kaposi's sarcoma-associated herpesvirus (KSHV) interacted with MeCP2 (methylcytosine-binding protein 2) and other epigenetic regulators and affected the position of chromosomal domains in interphase nuclei. LANA was involved in both gene silencing and activation events. Tax, the transactivator protein of human T-lymphotropic virus type I (HTLV-1) upregulated a set of cellular genes but induced silencing and hypermethylation of SHP1, a putative tumor suppressor gene encoding a tyrosine phosphatase. The hepatitis B virus (HBV)-encoded HBx oncoprotein also activated a diverse set of cellular genes and silenced, in parallel, selected tumor suppression genes. HBx-mediated gene-silencing was attributed to the upregulation of cellular DNA methyltransferases DNMT1 and DNMT3A1. In HBx-transfected liver cell lines, local hypermethylation events were accompanied with global demethylation of cellular DNA, due to the downregulation of DNMT3B involved in de novo methylation of repetitive sequences.

Hepatitis C virus (HCV) does not code for a typical viral oncoprotein. The HCV core protein mediated, however, tumor suppressor gene silencing in hepatocarcinoma cells by activating cellular DNMTs. E6 and E7, key oncoproteins of high-risk human papillomaviruses (HPVs) induced complex epigenetic changes via the modulation of DNA methylation and histone modifications.. Epigenetic dysregulation was also documented in Merkel cell polyomavirus (MCPyV)-associated tumors.

ANTIBACTERIAL EFFECT OF ARYLMETHYL SELENOCYANATES WITH VARIOUS AROMATIC MOIETIES

TÍMEA MOSOLYGÓ[1], YUNSU JANG[1], MUHAMMAD JAWAD NASIM[2, 3], ANNAMÁRIA KINCSES[1], GABRIELLA SPENGLER[1], KATALIN BURIÁN[1], JADWIGA HANDZLIK[3], CLAUS JACOB[2]

[1]Department of Medical Microbiology and Immunology, University of Szeged, Szeged, Hungary; [2]Department of Pharmacy, Saarland University, Saarbruecken, Germany; [3]Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Kraków, Poland

Members of the Chlamydiaceae family are obligate intracellular bacteria that can cause both human and animal diseases. *Chlamydia trachomatis* causes trachoma and genitourinary infections. Although antibiotic therapy eliminates chlamydial infections, there is emerging evidence that *C. trachomatis* is developing antibiotic resistance, with some clinical isolates having single and multidrug resistance when cultured in vitro. Development of new antibiotics is required to overcome this problem. Selenium-containing compounds are an interesting chemical group promising for therapy of various dangerous diseases. Especially, an increasing interest of antibacterial potency of selenium compounds has been observed since an excellent bactericidal activity of ebselen against Gram-positive ESKAPE pathogens was found recently. Our study was designed to evaluate the antibacterial activity of a series of arylmethyl selenocyanates that, in contrast to an endocyclic ebselen, represent a group of exocyclic selenocompounds. The minimum inhibitory concentrations (MICs) of compounds were determined by two-fold broth microdilution method in 96-well plate on Gram-positive and Gram-negative strains. Elementary bodies (EBs) of *C. trachomatis* D were incubated with different selenocompounds at various concentrations (10, 1 μM). To quantify the anti-chlamydial effects of the compounds, HeLa cells were seeded in tissue culture plates with coverslips. After 24h, the confluent cells were infected with EBs or compound-treated EBs. After 48h the cells were fixed and the titre of the infectious EBs was determined by indirect immunofluorescence assay.

The number of *Chlamydia* inclusions was counted under a UV microscope. Five compounds exerted antibacterial activity against *C. trachomatis* D, in particular the nitrobenzyl derivative (MJ-16) caused total growth inhibition of at the concentration of 1 μM. The p-methylbenzyl- and both unsubstitued compounds, benzyl– and α-naphthylselenocyanates, displayed significant activities, as well. Four compounds had any antibacterial effect on the Gram-positive and Gram-negative strains. Based on our results it can be concluded that selenocompounds could be used as effective antimicrobial agents against *C. trachomatis* D and other bacteria.

Supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program' and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *NIGELLA SATIVA* FIXED AND ESSENTIAL OILS

AHMAD MOUWAKEH

Department of Microbiology & Biotechnology, Szent István Egyetem, Budapest, Hungary

Plant extracts of spices contain various phytochemicals having anti-bacterial, anti-fungal and medicinal properties. *Nigella sativa* (Black cumin) is a spicy plant which belongs to the family of "Ranunculaceae" and it is well known for its benefits in the field of traditional medicine in several areas such as Arabic countries and Eastern Asia. The microdilution method was used to determine the minimal inhibitory concentration (MIC) of *Nigella sativa* fixed oil (FO) and essential oil (EO) against 4 Gram-positive (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Listeria monocytogenes*) and 3 Gram-negative (*Salmonella* Hartford, *Pseudomonas aeruginosa* and *Escherichia coli*) food-borne pathogenic and spoilage bacteria occurring in food products. The FO (total fatty acid composition) were analysed by GC while the EO obtained by steam distillation out of

the cold pressed oil were analysed by GC/MS to detect its active compounds. Ten fatty acids and ten compounds were determined in the FO and EO respectively. The major fatty acids were palmitic (C16:0) and stearic (C18:0) as saturated fatty acids, however oleic (C18: 1) and linoleic (C18: 2n-6) were the main unsaturated fatty acids. While the major compounds of the EO were p-cymene and thymoquinone. EO of *Nigella sativa* showed 10 times higher inhibition on all tested bacteria than the FO and the lowest concentration value was observed in case of *Bacillus subtilis* at 0.003%. Hence, results reinforce the ambitious to apply the EO of *Nigella sativa* in food as a natural preservative.

SURFACE DISPLAY OF HETEROLOGOUS PROTEINS IN YEAST – FROM UNDERSTANDING BASIC CONCEPTS OF CELL WALL BIOSYNTHESIS TO BIOTECHNOLOGY APPLICATIONS OF SURFACE ENGINEERING

VLADIMIR MRŠA

Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia

Yeast cell wall is a complex extracellular organelle that requires sophisticated molecular mechanisms for its biosynthesis and remodeling. Most of the biochemical reactions involved in these cellular events have been revealed in the last several decades but their precise regulation is still largely unknown. It includes formation of regulatory protein complexes at the cell surface and proteolytic activation of most probably sets of proteins whose role is to a large extent still unexplained. Studies of microbial cell envelopes and particularly cell surface proteins and mechanisms of their localization brought about new biotechnological applications of gained knowledge in surface display of homologous and heterologous proteins. By fusing surface proteins, or their anchoring domains with different proteins of interest their so called *genetic immobilization* is achieved. Hybrid proteins are engineered in a way that they are expressed in the host cells, secreted to the cell surface and incorporated into the wall/envelope moiety. In this way laborious and often detrimental procedure of chemical immobilization of the protein is avoided by letting the cells do the whole procedure. Both bacterial and yeast cells have been used for this purpose and a number of potential biotechnological applications of surface displayed proteins have been reported. Examples range from microbial whole cell biocatalysts, biosorbents, biosensors and biostimulants development to design and screening of protein and peptide libraries. When surface immobilized enzymes are used, substrates do not need to cross membrane barriers, i.e. enzymes are free to access any externally added substrate. Thus, often complex and expensive purification of the enzymes used on an industrial scale is bypassed. In addition, the multi-step transformation can be performed using microbial cells displaying different enzymes that catalyze cascade reactions.

In recent years particular attention has been paid to yeast systems for surface display of proteins since most yeasts are generally regarded as safe (GRAS) microorganisms, yeast cell walls are capable of binding more proteins, and the cells are bigger. Besides, yeasts are generally more suitable for expression of proteins originating from higher eukaryotes. In this talk our current knowledge on molecular mechanisms for yeast cell wall biosynthesis will be summarized. Besides, the application of knowledge gained through rather basic molecular research for surface display of proteins on yeast cell surfaces and their use in biotechnology will be discussed.

STUDY OF STRUCTURAL COMPOSITION AND CATABOLIC ACTIVITIES OF MICROBIAL COMMUNITIES OF SALINE-ALKALINE SOILS AT APAJPUSZTA, HUNGARY

MÁRTON MUCSI[1, 2], ORSOLYA GAZDAG[1], GERGELY KRETT[1, 2], PÉTER CSONTOS[1], ANDREA K. BORSODI[2], TIBOR SZILI-KOVÁCS[1]

[1]Institute for Soil Science and Agricultural Chemistry, Centre for Agricultural Research, Hungarian Academy of Sciences; [2]Department of Microbiology, Faculty of Science, ELTE Eötvös Loránd University, Budapest, Hungary

While soil salinization is a serious problem in many parts of the world, in Hungary, it is also relevant from an environmental protection point of view. Naturally saline and alkaline areas provide a double-extreme habitat, in which a quite unique extremophile biota could develop. While the plant associations of these areas are well-studied and relatively easy to examine, the microbiota of these soils remained largely unknown. Our project aims to study the structure and functioning of these microbial communities with classical and molecular microbiological methods. Sampling was done in the dry and wet seasons in a salinized area at Apajpuszta, Kiskunság NP. Based on the vegetation types, four sites in different stages of salinization were selected. Three parallel samples were taken from each site from three depths (0-10 cm, 10-30 cm, 30-60 cm). Physical and chemical parameters (e.g. soil pH, water content, texture, salt content etc.) and major nutrient contents of the samples were determined. The structure of the soil microbiota was examined by cultivation on agar media and denaturing gradient gel electrophoresis (DGGE) based on the 16S rRNA gene from environmental DNA. Metabolic activity of soil samples were examined by biomass estimation using the substrate induced respiration (SIR) method, general catabolic activity using fluorescein diacetate hydrolysis (FDA), and catabolic fingerprinting using the MicroResp method. Our results showed that while the structure of the bacterial communities were mostly influenced by the sampling site (vegetation type), the activity of the microbial communities showed a better correspondence with the depth of the sampling. This can be explained by the degree of salinization, as samples from deeper layers have more extreme conditions. Therefore, communities with similar genetic structure were showed to have different catabolic properties at different levels of salinization. Some seasonal differences could also be shown, however, it had a lesser influence on the examined microbial properties.

This research was funded by the Hungarian Scientific Research Fund (OTKA, K108572) and the National Research, Development and Innovation Fund of Hungary (GINOP-2.3.2-15-2016-00056).

FIRST DETECTION OF TICK-BORNE ENCEPHALITIS VIRUS IN CLINICAL SPECIMENS OF ACUTELY ILL PATIENTS IN HUNGARY

ANNA NAGY, ORSOLYA NAGY, KATALIN TARCSAI, ÁGNES FARKAS, MÁRIA TAKÁCS

Division of Virology, National Public Health Institute, Budapest, Hungary

Tick-borne encephalitis virus (TBEV) is one of the endemic flaviviruses in Hungary, that is responsible for human infections every year. Neurological involvement of the disease is characterized by meningitis, encephalitis or meningoencephalitis which can result long-term neurological and neuropschichiatric sequelae too. Microbiological diagnosis of acute cases is predominantly based on serological tests due to the limited duration of viremia and long incubation period, however the application of molecular methods can supplement the serological diagnosis and provide epidemiological data. The aim of this study was to determine how successfully could viral RNA be detected from different body fluids of serologically confirmed acute cases. From the beginning of the

2016 seasonal period until July 2017 serum, whole blood, cerebrospinal fluid (CSF) and urine samples of a total 30 patients were investigated by using RT-PCR method. Two sera, one urine and one CSF sample of four patients tested positive and the European subtype of TBEV could be identified. As far as we know this was the first time that TBEV could be detected from clinical samples in Hungary. Our finding highlights that the application of molecular methods besides serological tests can be a valuable tool in differential diagnosis especially in areas like Hungary, where two or more flaviviruses are co-circulating.

IN VITRO SUSCEPTIBILITY PATTERN OF *CANDIDA KEFYR* PLANKTONIC CELLS AND BIOFILMS AGAINST TRADITIONAL ANTIFUNGAL AGENTS

FRUZSINA NAGY[1], ALIZ BOZÓ[1], ZOLTÁN TÓTH[1], LAJOS DARÓCZI[2], GÁBOR KARDOS[1], LÁSZLÓ MAJOROS[1], RENÁTÓ KOVÁCS[1]

[1]Department of Medical Microbiology, Faculty of Medicine; [2]Department of Solid State Physics, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

Candida kefyr is a rare but emerging nosocomial pathogen with higher prevalence among patients with hematologic malignancies. Furthermore, it may have a potential role in catheter-related infections. Nevertheless, data regarding biofilm production and antifungal susceptibility of *C. kefyr* biofilms are very limited. Therefore, the antifungal susceptibility of *C. kefyr* clinical isolates was evaluated against fluconazole (FLU), amphotericin B (AMB), caspofungin (CAS) and micafungin (MICA) using XTT-based fungal damage assay. Based on XTT-assay, median MICs of planktonic cells of isolates were 0.25 mg/L, 0.25 mg/L, 0.5 mg/L and 0.06 mg/L for FLU, AMB, CAS and MICA, respectively. FLU showed lower activity against biofilms compared to AMB, CAS or to the tested MICA concentrations. Fifty percent fungal damage caused by FLU was detected from 4 mg/L (55%±6.97%) and from 128 mg/L (57.88%±9.2%) against one-day-old and three-day-old biofilms, respectively. AMB produced higher anti-biofilm effect compared to FLU at ≥0.5 mg/L (64.63%±6.79%) and at ≥0.12 mg/L (77.63%±8.43%) for early and mature biofilms, respectively. In case of MICA, 50% fungal damage was observed from 0.06 mg/L (66.88%±10.16%) and from 0.25 mg/L (81.38%±6%) against one-day-old and three-day-old biofilms, respectively. CAS-exposed cells showed a strange unexpected susceptibility pattern. Planktonic cells had significantly reduced susceptibility at caspofungin concentrations ranging from 0.015 mg/L to 1 mg/L compared to sessile population. However, the damage in planktonic cells and biofilms was comparable at higher concentrations. In case of planktonic and sessile cells, 50% fungal damage was observed first at 0.5 mg/L (59.75%±3.16%) and at 0.06 mg/L (70.25%±10.95%), respectively. This unusual susceptibility pattern was confirmed using scanning electron microscope. The unexpected susceptibility pattern observed at lower CAS concentrations may give an explanation to the poorer outcome of CAS-treated *C. kefyr* infections in patients with haematological malignancies. Our data suggest that MICA may be a more effective option than CAS, AMB or FLU against *C. kefyr* infections.

FUNCTIONAL ANALYSIS OF GENOME POLYMORPHISMS IN HUMAN PAPILLOMAVIRUS 11 ASSOCIATED WITH RECURRENT RESPIRATORY PAPILLOMATOSIS WITH DIFFERENT CLINICAL SEVERITY

ZSÓFIA NAGY[1], GÁBOR KARDOS[1], MIHÁLY SZINAI[1], ATTILA SZŰCS[2], ZOLTÁN PETHŐ[3], KRISZTINA SZARKA[1]

[1]Department of Medical Microbiology, [2]Clinic of Otorhinolaryngology and Head & Neck Surgery, Faculty of Medicine; [3]Department of Biophysics and Cell Biology, Faculty of Science and Informatics, University of Debrecen, Debrecen, Hungary

Polymorphisms of the long control region (LCR) of human papillomavirus (HPV) 11 may modify severity of respiratory papillomatosis (RRP). We also found unique amino acid polymorphisms in virus proteins. We analysed a HPV11 genome from a novel patient, and earlier reported and newly found polymorphisms in silico to assess their role in the clinical course of RRP. The new patient has an adult-onset RRP with dysplasia. All LCRs were examined for transcription factor binding sites using the tool. In silico protein modelling (Molecular Operating Environment 2014.9) was performed on the following. i) The two polymorphisms (A476V and S486F) in L1 present in the virus from the papilloma but not from the healthy mucosa of Patient 5 with aggressive RRP. ii) E1 polymorphisms A72E and N100T in Patients 4 and 9, respectively, with moderately severe RRP. iii) E2 polymorphisms Q86K in Patient 4; S245F and N247T in Patient 9; K308R in multiple patients. Complete genome of the newly enrolled Patient 10 contained 21 polymorphisms (16 silent, A45S in E7, S78L in E4, K308R in E2, I28F and V41L in E5a), a deletion and an insertion in the LCR; all known from other genomes. It also contains a large unique deletion in ORF E2-E4 (NT3381-3438) causing a frameshift with an early stop codon. The identified seven LCR polymorphisms resulted in 28 new binding sites of 24 transcription factors, modified 66 binding sites of 52 transcription factors; no binding site was lost. L1 polymorphisms are located in C-terminus playing a role in capsomer formation and possibly in heparan-sulphate binding of virions. In protein models both were involved in binding between monomers; S486F led to a new interaction with E382 and with A385 of the neighbouring monomer of the capsomer. Both E1 polymorphisms are in the N-terminal regulatory region, but do not involve nuclear transport signal sequences. As this part of the protein is unstructured, structure modelling was not possible. E2 polymorphism Q82K is located in the E1 binding domain, but due to lack of E1 model, this interaction could not be modelled. Polymorphisms S245F and N247T are in the hinge region of E2 containing phosphorylation sites involved in E2 stability and chromatin binding. As they lead to loss of one phosphorylation site and to gaining another, and alter the surface charge of E2, they may affect phosphorylation patterns. Polymorphism K308R is in the DNA binding domain, but is positioned distantly from the DNA during interaction. The truncated E2 of the HPV11 from Patient 10 lost a large part of its DNA binding domain, and though the alpha-helix serving as DNA binding motif is preserved, its surface charge alters from basic to acidic, most probably impairing DNA binding. In summary, protein modelling supports the role of some of these unique polymorphisms in pathogenesis of RRP.

K. Szarka was supported by János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

APPLICATION OF IMPEDIMETRIC MEASUREMENTS IN LACTIC ACID, PROPIONIC ACID AND SUCCINIC ACID FERMENTATION

ÁRON NÉMETH

Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Budapest, Hungary

An impedimetric equipment (BacTrac, Sy-Lab, Austria) was adapted for primer metabolites (namely lactic acid, propionic acid and succinic acid) fermentation researches. Since these products are

grown-associated and their ionic behaviour changes the media and/or the electrode characteristics, through impedance measurements 40 parallel experiments can be followed online, which may replace the shaking flask experiments generally used for screening and optimization tasks in fermentation researches. The successful optimization results were successfully verified in common shaking flask experiments, and now scale-up is ongoing in fermenters. The application of this methods saved 90% of media utilization, meanwhile it provided more data as well.

MICROBIOLOGICAL CONDITION OF LIQUID WHOLE EGG PRODUCTS WHICH ARE PRESERVED BY VARIOUS METHOD

CSABA NÉMETH[1], ADRIENN TÓTH[2], JÓZSEF SURÁNYI[2], FERENC HORVÁTH[2], DÁVID LÁNG[1], TAMÁS NÉMETH[1], TAMÁS BÉTÉRI[1]

[1]Capriovus Ltd., Szigetcsép; [2]Departmen of Refrigeration and Livestock Products' Technology, Szent István University, Budapest, Hungary

The shelf life of liquid eggs is relatively short since the proteins responsible for microbial resistance of shell egg is denatured during pasteurization, and in case of the mixture of white and yoke of egg provides a medium of excellent composition for microbial growth. Therefore, liquid egg production plants use various preservatives to increase the shelf life of their products. Such substances include citric acid and other additives according to the Hungarian Codex Alimentarius that approves these products such as sodium benzoate and potassium sorbate. The total concentration of the two substances together can be up to 5000 mg/l. In the selection of the amount of citric acid the pH sensitive proteins of egg represent the main limitation; these proteins are denatured at a relatively high rate at the pH lower than 5. However, the adjusted acidity highly effects on the efficiency of the preservatives that can be used in liquid egg products. But potassium sorbate and sodium benzoate do not have appropriate effect at nearly neutral pH values. Sodium benzoate and potassium sorbate can be added to liquid egg products at any portions up to a concentration of 5000 mg/l (one of them can even be omitted); while the experiments with foods prepared from egg showed that they can reduce microbial growth significantly only in combinations. However, it should be noted that in addition to correct selection of preservatives, adequate storage temperature and microbial contamination and composition of the fresh product from the production line also significantly effect on the shelf life of products. Our purpose with this work was to determine how the total viable count changes in liquid whole eggs during storage in refrigerator depending on the storage temperature, pH value of samples and their preservative content. In general pH and storage temperature of liquid eggs significantly affects the change in viable cell count during storage. However, our measurements did not clearly demonstrate that the mixture of Na benzoate and K sorbate added to liquid egg at the approved concentration range would significantly inhibit microbial growth. We introduced storage time into our experiments as the fourth variable. This we could obtain a model closely correlated with our results (r2=0.97) by which the storage time to a specific increase in viable cell count in various liquid whole egg products can be calculated with good approximation.

TESTING OF THE MICROBIOLOGICAL EFFICIENCY OF DISINFECTANTS USED IN FOOD INDUSTRY IN HUNGARY

ZSUZSANNA NÉMETH, MÁRTA GULYÁS, JUDIT FARAGÓ, ANNA ZOLTAI, NÁNDORNÉ KOLBL

Food and Feed Safety Directorate, National Food Chain Safety Office, Budapest, Hungary

Disinfectants used in food industry play an important role in the prevention of foodborne diseases. They perform their fullest potential when they are applied in the proper concentration, at the proper temperature, for the proper contact time, as determined by preliminary studies. Since 2009 National Food Chain Safety Office has Monitoring programme to control commercially available disinfectants. Also provides insight into the efficiency of disinfectants sampled in connection with food infection cases, in the everyday practice (since 2011). The poster presents the summary test results (monitoring and foodborne disease) during the last 8 years. Checking food disinfectants we have experienced a futher microbiological problem. A few disinfectants had bacterial contamination. This problem has the highest impact on consumers when the contaminant occurs in hand disinfectants. We controlled the bacteriae survival in these products, data shown on the poster too. Our experiences of disinfectant control in last 8 years led to some observations when the quality of some disinfectants used in food industry were not properly suitable to EU Standards (EN 1276; EN 1650).

THE ROLE OF EFFLUX PUMPS AND ENVIRONMENTAL pH IN BACTERIAL MULTIDRUG RESISTANCE

MÁRTA NOVÉ[1], ANNAMÁRIA KINCSES[1], ORSOLYA VÁSÁRHELYI[1], VIVIEN UNGER[1], VIKTOR TÓTH[1], JÓZSEF MOLNÁR[1], LEONARD AMARAL[1, 2], GABRIELLA SPENGLER[1]

[1]Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, Szeged, Hungary; [2]Travel Medicine, Institute of Hygiene and Tropical Medicine, Universidade Nova de Lisboa, Lisbon, Portugal

The phenomenon of multidrug resistance (MDR) has a great importance concerning the therapy of infectious diseases. One of the most studied bacterial resistance mechanisms is the one that is related to efflux pumps, which promotes the elimination of toxic compounds out of the cells, including antibiotics as well. RND transporters of Gram-negative bacteria are common MDR efflux pumps operating with the proton motive force (PMF). It has been described previously that the pH of the external environment can influence the activity of efflux pumps. In our study we examined the activity of RND pump at neutral and acidic pHs in the presence of the phenothiazine type efflux pump inhibitor (EPI) promethazine (PMZ), in addition the influence of PMZ treatment on the relative expression of the efflux pump genes and their regulators at neutral and acidic pHs were examined as well. In our experimental model Escherichia coli K-12 AG100 strain expressing the RND type AcrB pump as part of the AcrAB-TolC efflux system was used. The activity of the pump system on the accumulation of the general efflux pump substrate ethidium bromide was assessed by real-time fluorimetry. The relative gene expression levels were determined at pH 5 and pH 7 in the presence and absence of PMZ. The *E. coli* AG100 strain was cultured at pH 5 and pH 7 and total RNA was isolated at various time points (0, 1, 2, 4, 8, and 18 hours). The relative expression levels of the efflux pump genes *acrA* and *acrB*, in addition the regulators *marA*, *marB*, *marR*, furthermore the *soxS* stress gene were determined by real-time quantitative reverse transcriptase polymerase chain reaction (RT-qPCR). It can be concluded that the EPI activity of PMZ was more effective at neutral pH. However, the acidic pH and PMZ treatment induced a significant stress response in the bacterium, for this reason the *marB*, *marR*, *acrA*, *acrB* and *soxS* genes were upregulated compared to the neutral pH. The experiments justified that the activity of efflux pump is pH dependent and the explanation is the different H+ concentration, namely at acidic pH the PMF is higher compared to the neutral pH, for this reason the EB accumulated in the presence of PMZ was lower at pH 5.

Supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program' and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

INFLUENCE OF THE SUGAR CONCENTRATION OF GRAPE MUST ON THE GROWTH KINETICS OF WINE RELATED STRAINS OF *STARMERELLA BACILLARIS* (SYN. *CANDIDA ZEMPLININA*)

BORBÁLA OLÁHNÉ HORVÁTH[1], VIVIEN KORMOS[2], EDINA NAGY[2], ILDIKÓ MAGYAR[1]

[1]Department of Oenology; [2]Department of Brewing and Distilling, Szent István University, Budapest, Hungary

The increasing initial sugar concentration of grape juice due to the global climatic change outlines the importance of sugar tolerance in wine yeasts. *Starmerella bacillaris* (formerly known as *Candida zemplinina*) is a wine related yeast species, that is particularly connected with sweet, bortytized wine fermentations, but it is commonly isolated from different oenological sources. From several oenological aspects, like high glycerol production, fructophylic character, *S. bacillaris* is different from *Saccharomyces* wine yeasts. Although this species has recently been in the focus of wine research and is considered a sugar tolerant yeast, little information is available about its response to the osmotic stress under fermentative conditions in wine. Our aim was to compare the change of growth parameters of three wine related species caused by the increasing initial sugar concentration. Three selected strains of *S.bacillaris* (Y1667T, Y1756 and MLO) were investigated in comparison with one strain of *Saccharomyces cerevisiae* (UvafermPM, industrial starter) and one of *Saccharomyces bayanus* var. *uvarum* (CBS395T) as reference strains. The applied synthetic medium modelled a grape must with three different sugar concentrations from the normal to the extreeme ones. As expected, *S. cerevisiae* showed faster growth than *S. bacillaris* at all sugar concentrations. Between 220 and 270 g/l sugar content the S.cerevisiae strain showed only a slight decrease (5%) in its specific growth rate (μmax), nevertheless the highest sugar amount reduced this parameter by 37%. The change of μ_{max} at *S. bacillaris* strains was somewhat bigger, with 20-30% reduction at 270 g/L and 48-53 % at 320 g/L sugar content. The performance of *S. bayanus* strain was close to that of *S. bacillaris*. In the maximum number of cells (Nmax) there was an opposite trend among the species compared to the μ_{max}. Between 220 and 270 g/l sugar there was only a slight decrease in the maximal population of two *S. bacillaris* strains (2% and 5%), whilst the *Saccharomyces* strains showed 17 and 14 % reduction. The 320g/l sugar content was a big challenge for each strain; the Nmax of the *S. bacillaris* and the *S. bayanus* strains decreased by about 35%, while that of *S. cerevisiae* by about 50%. We concluded that even though there is a bigger decrease in the μmax of the investigated *S. bacillaris* strains due to the osmotic stress, the loss in Nmax was considerably smaller in comparison with *S. cerevisiae*. At the end of the monitored period they were less influenced by a more stressful condition which means a significantly better sugar tolerance.
The fermentation kinetics was also monitored, and the strong fructophilic nature of all *S. bacillaris* strains was confirmed in this study. Altogether the fructophilic character and the good sugar tolerance of *S. bacillaris* strains confirm that it could be a promising promoter of sluggish or stuck fermentations, furthermore an advantageous contributor in multistarter wine fermentations.

METABOLISM OF PROTEINS AND PEPTIDES AS SOLE GROWTH SUBSTRATES BY *LACTOBACILLUS* AND *LACTOCOCCUS* STRAINS

MELINDA PÁZMÁNDI[1, 2], MYRIAM BENHALIMA[1, 3], ZOLTÁN KOVÁCS[2], ANNA MARÁZ[4]

[1]Department of Microbiology and Biotechnology; [2]Department of Food Process Engineering, Faculty of Food Science, Szent István University, Hungary; [3]Département I.S.S.B.A, École d'ingénieurs de l'Université d'Anger, Anger, France; [4]Department of Microbiology and Biotechnology, Faculty of Food Science, Szent István University, Budapest, Hungary

One of the most commonly used valorization technique of cheese whey is the separation of proteins by the combination of ultrafiltration (UF) and diafiltration (DF). This treatment will result in deproteinized whey permeate, which is then further processed by nanofiltration, when the lactose concentration is increased to over 200 g/dm^3. While whey proteins represent high market value, finding ways for the utilization of the lactose syrup is still a challenging research task. The long term goal of our study is to develop a new way for the valorization of UF/DF whey concentrate (lactose syrup) via the production of lactic acid fermented drinks. In our previous study, fermentation characteristics of lactic acid bacteria (LAB) were investigated in order to develop an optimal whey-based fermentation media. We found that the applied LAB strains were able to utilize a negligible amount of lactose present in the diluted UF/DF whey in the presence of whey proteins [1]. We supposed that the reason for the low lactose fermentation rate was the strains' preference for the utilization of whey proteins as a carbon source instead of lactose. Currently our focus is to understand the fermentation characteristics of the applied LAB strains during fermentation of lactose medium supplemented with various organic nitrogen sources. In our experimental work, we monitored utilization of whey protein extract and two different food-grade peptones (casein and soy) alone and in combination with lactose or diluted UF/DF whey permeate. We concluded that whey proteins and peptones do not provide enough nutrition and energy sources for the growth of the investigated LAB strains, as the cell concentrations and pH remained almost stable during the 72 h fermentation period. The number of cells remained close to the initial value of 10^7 cell/cm^3 (average $2*10^7$ cell/cm^3) and pH dropped only to approximately 6.0-6.7 from the starting pH 7 in all cases. Addition of lactose resulted in a considerable growth enhancement.

Cell concentrations increased from the initial 10^7 cell/cm^3 to approximately 10^8 cell/cm^3, while pH decreased to 6.2-3.5 during the 72 hours of fermentation. Although all the LAB strains responded positively to the addition of lactose, mostly stimulated strains were *Lb. casei* PB8, *Lb. helveticus* PB9, *Lb. rhamnosus* PB10, *Lb. sakei* DSM 20017 and *Lb. acidophilus* N2. We supposed that low protease activity of the investigated *Lactobacillus* and *Lactococcus* strains was responsible for the poor growth in proteins and peptones, therefore we investigated protease activity of strains in culture media containing different carbon and nitrogen sources.

[1] Pázmándi M. et al. (2016). Ann. Meeting of the Hung. Society for Microbiology. Book of Abstracts pp. 50-51.

Acknowledgement: This work and Z. K. were supported by the 'Marie Sklodowska-Curie' integration grant of the 7th EU FP (PCIG11-GA-2012-322219) and the Bolyai Scholarship of the HAS.

ANTAGONISTIC EFFECTS OF *BACILLUS SUBTILIS* AND *PSEUDOMONAS CHLORORAPHIS* BACTERIA ON GROWTH OF SOIL BORNE PLANT PATHOGENIC FUNGI IN LABORATORY TESTS

NIKOLETTA PÉK[1], RITA KOVÁCS[2], ILDIKÓ PUSPÁN[2], CSILLA IMRE[2], RÓZSA MÁTÉ[2], JÓZSEF KUTASI[2], ÉVA KÁRPÁTI[1]

[1]Saniplant Biotechnological Research and Development Ltd.; [2]BioFil Microbiological, Biotechnological and Biochemical Ltd., Budapest, Hungary

The sustainability of agricultural production and the ever so intensifying demand for healthy and clean foodstuff badly urge the reduction of fungicide usage in field. Environment friendly and at the same time cost effective biological solutions are highly required in field crop production to replace chemical fungicides. Microbial soil inoculants - composed of bacteria that are able to inhibit soil borne plant pathogenic fungi, like *Fusarium*, *Sclerotinia* – represent useful tool to diminish the fungal damage in field. Our aim was to study the inhibitory effect of *Bacillus subtilis* and *Pseudomonas chlororaphis* soil bacteria – isolated from Hungarian soils – on growth of important field pathogens, like *Fusarium graminearum*, *Sclerotinia sclerotiorum*, and *Alternaria alternata*, in laboratory tests. Dual inhibition assays on agar medium in Petri dishes were performed to test the inhibitory effect of liquid bacterial cultures and cell free culture filtrates on the fungal growth. Sizes and shapes of inhibition zones were evaluated. It was revealed that *B. subtilis* exhibited strong and long term inhibion on the fungal growth. The effectiveness of the liquid culture could be enhanced by changing culture medium composition and mode of culturing (with or without shaking). Inhibition zones by the cell free culture filtrates were also obtained, but in decreased extent and length of time. In contrast, *P. chlororaphis* showed weak or zero inhibitory effect against the fungi, independently on culture medium composition and mode of culturing. Although the liquid culture in King B medium showed yellow greenish pigmentation and fluorescence on 365 nm, resembling siderophore accumulation produced by the bacterium, fungal growth inhibition virtually could not be detected. Regarding our results, the *B. subtilis* isolate in liquid culture form seems to be a good candidate for the control of *F. graminearum*, *S. sclerotiorum*, and *A. alternata* in field conditions.

Supported by Vállalati KFI_16-1-2016-0214 grant of National RDI Office, Hungary.

IDENTIFICATION OF POSSIBLE DOWNSTREAM EFFECTOR PROTEINS OF THE CA^{2+}-BINDING PROTEIN CABB IN *STREPTOMYCES COELICOLOR*

ANDRÁS PENYIGE, MELINDA SZILÁGYI-BÓNIZS

Department of Human Genetics, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Streptomycetes are Gram-positive, obligate aerobe soil bacteria well known for their complex morphological differentiation process which is coupled to the production of a wide variety of secondary metabolites with medical importance. On solid medium spores of *Streptomyces coelicolor* A3(2) germinate to produce a complex network of apically growing filamentous hyphae. Subsequently this substrate mycelium gives rise to aerial hyphae, which undergo septation to form chains of monogenomic, thick walled spores. Complete understanding of the regulation of differentiation and antibiotic production would require the characterization of regulatory mechanisms and signaling pathways present in *Streptomyces*. Ca^{2+}, a key messenger in pro- and eukaryotes, is involved in mediating the effect of a large variety of signals. In *Streptomyces* Ca^{2+} was reported to be involved in the regulation of spore germination, aerial hypha formation and antibiotic production, however, the underlying mechanism of this signaling is not known. The effect [Ca^{2+}] is mediated by Ca^{2+}-binding proteins (CaBP) such as the ubiquitous calmodulin, a multifunctional Ca^{2+} sensor containing four EF-hand Ca^{2+}-binding motives. Interestingly four genes coding for EF-hand containing CaBPs with unknown biological functions are present in the *S. coelicolor* genome (*cabB-D*). By analyzing the physiological role the *cabB* gene, we have found that in the *ΔcabB* mutant

sporulation, spore morphology and actinorhodin production was affected, stress conditions enhanced the mutant phenotype. Conventionally EF-hand proteins could be divided into two groups: (1) calcium sensors, which function to translate the signal to various responses; and (2) calcium buffers, which control the level of free Ca^{2+} ions in the cytoplasm. Sequence analysis of two unique structural motifs that provide a supporting scaffold for the calcium-binding EF-hand DxDxDG sequence motif allowed us to classify CabB into the sensor/regulator group of CaBPs. Ca^{2+}-regulators are expected to interact with effector proteins to modulate their activity/function. In order to identify specific downstream effectors for CabB we have created GST-CabB fusion protein by cloning the *cabB* gene into a pGEX-4T1 plasmid by ligating it between the EcoRI and NotI restriction sites. The GST-CabB fusion protein was coupled to glutathione-Sepharose matrix and the complex was then used to pull-down CabB-binding proteins in the presence of Ca^{2+}. The captured proteins were eluted by EGTA, separated by SDS-PAGE and analyzed by an Easy nLCII nanoHPLC-4000QTRAP MS/MS system. The following proteins were identified:
Aminopeptidase N (*pepN*); Guanosine-pentaphosphate synthase (*gpsI*); Ribosomal protein S1 (*rpsA*); Adenosyl-L-homocysteine hydrolase (*achY*); Fructose 1,6-bisphosphate aldolase (*fba*); DNA-dependent RNA polymerase a subunit (*rpoA*); and the cAMP-receptor protein (CRP). These results provide indirect evidence that prokaryotic CaBPs might have more specific biological role.

POLYGENIC TRAITS IN YEAST BIOTECHNOLOGY

UROS PETROVIC

Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Saccharomyces cerevisiae is an outstanding model and host organism for biotechnology. This holds true also for production of lipid-based products, despite the fact that lipid accumulation is much less pronounced in *S. cerevisiae* than in oleaginous yeast species. Storage of neutral lipids is a polygenic trait and current knowledge about the genes that are involved in this process does not provide for a satisfactory model which could be used to accurately engineer lipid content and its regulation in yeast cells. Recently developed methods for polygenic trait analysis in *S. cerevisiae*, however, enable identification of comprehensive sets of causal genes for polygenic traits. We thus performed polygenic trait analysis to identify causal genes for high lipid content, with the aim to employ these genes in engineering high lipid production in *S. cerevisiae*.
As a prerequisite for the identification of causal alleles for high lipid content, we developed a high-throughput phenotyping method for this trait. We used allele swapping to confirm the identity of the causal genes, as well as inter- and backcrossing to expand the knowledge on the genetic variants that govern the processes leading to lipid accumulation. Additionally, follow-up experiments including protein interaction screen using barcode fusion genetics allowed elucidation of the molecular mechanism behind these effects of the causal genes. Industrial yeast strains require many different traits, from altered metabolic fluxes to increased robustness, which are most often polygenic and therefore difficult to engineer on the genetic level using traditional approaches. But identification of causal genes / alleles for biotechnologically relevant traits using polygenic trait analysis, in combination with recently developed genome editing methods, enable efficient and accurate transfer of such traits to industrial strains. Combining polygenic trait analysis approaches further with other technologies, such as cell surface display of heterologous enzymes for utilization of complex carbon sources, could lead to new bioprocesses that will be competitive with existing state-of-the-art technologies for the production of also several lipid-based products.

Supported by the grants N1-0034 and P1-0207 of the Slovenian Research Agency and by the EU funding of the YeSVitE consortium (FP7-IRSES-2013-GA no. 612441).

IMPACT OF OFF-THE-SHELF VAGINAL GELS ON THE REPLICATION OF *CHLAMYDIA TRACHOMATIS*

TÍMEA RAFFAI[1], ÁRPÁD PÁRDUCZ[2], VALÉRIA ENDRÉSZ[1], KATALIN BURIÁN[1], DEZSŐ VIRÓK[1]

[1]Institute for Medical Microbiology and Immunology, Faculty of Medicine, University of Szeged, Szeged; [2]Obstetrics and Gynecology, Pándy Kálmán County Hospital, Gyula, Hungary

Vaginal gels are used as lubricants or therapeutic gels such as prebiotics for *Lactobacillus* growth. *Chlamydia trachomatis* is an obligate intracellular bacterium propagating in the epithelial cells of the urogenital tract. *C. trachomatis* infections are the most prevalent sexually transmitted diseases. The principal target cells in women are the cervical epithelial cells, therefore the vaginal gels could have a direct impact on the propagation of the bacterium. We tested the growth-modifying effect of six off-the-shelf vaginal gels. First, the cytotoxicity of the gels was measured by a standard MTT test on HeLa human cervical epithelial cells. The maximum non-toxic concentrations were used in the subsequent experiments. *C. trachomatis* elementary bodies were preincubated for 1 hour, 37°C with the gels before infection of HeLa cells. 48 hours post infection the infected cells were lysed and the chlamydial DNA accumulation - as an indication of chlamydial growth - was measured by a recently developed direct qPCR method. Initially we screened the growth-modifying effect of six gels in one concentration and concluded that four of them had an impact of chlamydial growth. These gels were tested further in a concentration range.

From the tested four gels, two showed a prominent concentration-dependent growth-enhancing effect and one gel showed a concentration-dependent inhibitory effect. Interestingly, one gel had an inhibitory effect at high concentrations, and a significant growth-enhancing effect at a lower concentration. The growth enhancing effects of the gels were between approximately 4-32 fold. Off-the-shelf vaginal gels-lubricants may have a significant growth enhancing effect on chlamydial growth in vitro. Further animal model experiments and epidemiology data are needed to clarify the potential role of vaginal gels in the transmission of chlamydial infections.

COMPARATIVE GENOMICS OF XOP2-LIKE *XANTHOMONAS ORYZAE* PV. *ORYZAE* BACTERIOPHAGES

GÁBOR RÁKHELY[1, 2], ILDIKÓ VARGA[3], JÁNOS MOLNÁR[3], ANNAMÁRIA GAZDAG[3], DOMINIKA SZŰCS[3], ZSOLT DOFFKAY[1], SRASHAD KODERI VALAPPIL[1], SZILVIA PAPP[3], RÉKA PINTÉR[3], CASIANA M. VERA CRUZ[4], TÍMEA VIZI[5], GYÖRGY SCHNEIDER[6] TAMÁS KOVÁCS[3]

[1]Department of Biotechnology, University of Szeged; [2]Institute of Biophysics, Biological Research Center, Szeged; [3]Department of Biotechnology, Nanophagetherapy Center, Enviroinvest Corporation, Pécs, Hungary; [4]International Rice Research Institute, Los Banos, Philippines; [5]Roche Hungary Ltd., Budaörs; [6]Institute of Medical Microbiology and Immunology, University of Pécs, Pécs, Hungary

Rice (*Oryza sativa* L.) is one of the most important foodstuff globally. Diseases are one of the most important limiting factors of rice production. The bacterial leaf blight (BLB) of the rice, caused by the Gram negative bacterium *Xanthomonas oryzae* pv. *oryzae* (XOO), is a devastating disease especially in Asia and Western-Africa. The actual plant protection agents and measures (various pesticides, to

create and growth XOO resistant rice plants) often provide not satisfactory results, therefore, development of alternative ways is necessary. Bacteriophages could provide a possible solution because these agents have been used in several cases as biocides against plant pathogenic bacteria successfully. XOO phages were isolated and characterized earlier and several complete genome sequences of them are available in GenBank as well. Based on genome, morphology and serological features, the Caudovirales XOO phages could be divided in two groups: OP1-like phages (OP1, Xp10 and Xop411) and OP2-like phages (from this group only OP2's complete genome has been determined until now). We isolated and characterized 12 novel XOO phages from the Mekong-Delta (Vietnam) and from Philippines. All bacteriophages belong to the Myoviridae family. Based on laboratory trials, all 12 phages can effectively hinder XOO growth. Their complete genome sequences revealed that these are OP2-like phages. We made their genomes available in Genbank and performed comparative genomics analysis (created phylogenetic tree based on terminase large subunit gene sequences, investigated the mutation frequencies along their genomes, performed Mauve analysis, among others). Our study enables an insight of genomics of the Bcep78 virus genus.

EFFECTS OF THE BIOLOGICAL LIFE AND MANKIND ON THE EARTH (MAINLY BETWEEN 1778 AND 2015)

BÉLA RALOVICH

Balatonberény, Hungary

Components and events of timeless and endless Universe are consequences of permanent flow of energy and substance in accordance with the laws of eternal Nature which will never be totally known by man kind. All components of the Universe have lived their own physical life. The life of our Earth is exceptional because of the presence of biological life. The biological life is a biological phenomenon of a living unit which lasts till its own death. The living unit is a substantial matrix which is bordered by a permeable membrane/wall. Inside the unit and through its membrane/wall an organized and directed engergy and substance transport flows. The living unit has been affected by the outside effects and it can accomodate to those only between the borders of its own life requirements. In the same time it influences on its environment, too. Now it seems that the living unit is only present in the Bio-sphere of our Earth which is a closed system for it. In the case of any kind of closed systems it is obligatory permanently to ensure the specific life conditions which are necessary for a continuous life and reproduction of a living unit. It seems that the appearance of biological life and mainly that of men have fundamentally effected on the Earth. In consequence of the effects different periodes can be determined in the life of our Earth.

It is necessary to know about these periods because each of them had/have special energetic and substantial processes which had/have biological consequences. Now we shall only deal with a short piece of the period of the life of our Earth which has started in 1778.

ENDOPHYTIC FUNGI PRODUCING VALUABLE HOST METABOLITES

VIGNESHWARI RAMASAMY ARUNA

Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Endophytes are the plant-associated microorganisms that could be frequently found in the living tissues of different plants without causing apparent harm to their hosts. These organisms are known to produce an enormous variety of bioactive secondary metabolites, that can be exploited for human health and welfare. Furthermore, in some cases, the endophytic microorganisms could produce certain secondary metabolite, which was originally detected from the host plant. Thus the endophytes could be promising sources of known plant bioactive metabolites serving potential alternatives for the biotechnological productions of these drugs. In our present study, fungal endophytes were isolated on PDA plates from the medicinal plant Hypericum perforatum after a surface sterilization procedure The plant samples were collected from the Botanical Garden of University of Szeged (Szeged). After the purification of the strains, they were characterised via molecular techniques using PCR amplification and sequencing of ITS region of their genomic DNA and their taxonomical location were determined using BLAST search. To investigate the secondary metabolite production of the isolates, there were inoculated into liquid media and after the cultivation, both the ferment broth and mycelia were extracted with organic solvents. The prepared crude extracts were examined regarding their hypericin and emodin content by HPLC-UV and HPLC-MS techniques using standard compounds. Based on our results one fungal strain produced the hypericin in the applied medium under shake flask fermentation conditions. Emodin was also found both in the extracts of this fungus and the extracts of two other isolates. The antimicrobial activities of the extracts were also examined against different bacterial strains in bioassays. The mycelial extracts of the isolates showed significant bactericide activity, while the broth extracts showed lower inhibitory activity against tested bacteria. The effects of the most active extracts were reached to the level of the applied control antibiotic.

Supported by the project GINOP-2.3.2- 15-2016- 00012. AS was supported by the New National Excellence Program of the Ministry of Human Capacities (ÚNKP-16- 4).

COMPARATIVE ANALYSIS OF BACTERIAL ENRICHMENT CULTURES DEGRADING AROMATIC HYDROCARBONS UNDER AEROBIC OR MICROAEROBIC CONDITIONS

FRUZSINA RÉVÉSZ[1], MILÁN FARKAS[1], ISTVÁN SZABÓ[2], BALÁZS KRISZT[2], ANDRÁS TÁNCSICS[1]

[1]Regional University Center of Excellence; [2]Department of Environmental Safety and Ecotoxicology, Szent István University, Gödöllő, Hungary

In subsurface BTEX-contaminated environments the availability of oxygen is the main limiting factor of quick and complete degradation of all BTEX-compounds. Since availability of oxygen is always restricted in subsurface environments, bacteria adapted to hypoxic BTEX-degradation may have a key role in this process. Previous studies of oxygen-limited, BTEX-contaminated sites of Hungary have shown the large diversity of subfamily I.2.C-type extradiol dioxygenase (*C23O*) genes in these environments. Older literature suggests that such catabolic potentials could be associated to hypoxic degradation. However, recent results of a DNA-SIP-based investigation of an oxygen-limited, toluene-degrading enrichment culture have shown that not all subfamily I.2.C-type *C23O* genes can be linked to hypoxic degradation of aromatic hydrocarbons. To reveal those *C23O* genes which have

a key role in the hypoxic degradation of benzene and toluene, aerobic and microaerobic enrichment cultures, degrading solely benzene or toluene, were established and thoroughly characterized. Results of the study have shown that highly different microbial communities were observable under aerobic and microaerobic conditions. It was also observed that microaerobic enrichment cultures degrading benzene or toluene showed notably different community structure, while communities of the aerobic enrichment cultures were more similar. This result shows that aromatic hydrocarbon degraders adapted to aerobic conditions may have wider substrate specificity and are able to degrade both benzene and toluene, while microaerobic degraders are specialists and can degrade only either of them. All enrichment cultures were dominated by Beta- and Gammaproteobacteria, although different genera were and species were observable under aerobic and microaerobic conditions. It was observed that under aerobic conditions fluorescent pseudomonads were always detectable, but were completely missing from microaerobic enrichments. Regarding subfamily I.2.C-type *C23O* genes it was observed that completely different genotypes were detectable under aerobic and microaerobic conditions, suggesting that ecophysiological fine-tuning, rather than catabolic repertoire contributes to niche definition amongst hypoxic degraders of BTEX compounds in groundwater systems.

BIOLOGICAL ACTIVITY OF SILVER AND GOLD NANOPARTICLES PREPARED BY CELL-FREE EXTRACT OF *PHAFFIA RHODOZYMA*

ANDREA RÓNAVÁRI[1], NÓRA IGAZ[2], GOPISETTY MOHANA KRISHNA[2], BETTINA SZERENCSÉS[3], DÁVID KOVÁCS[2], CSABA VÁGVÖLGYI[3], IMRE MIKLÓS BOROS[2], ZOLTÁN KÓNYA[1], MÓNIKA KIRICSI[2], ILONA PFEIFFER[3]

[1]Department of Applied and Environmental Chemistry; [2]Department of Biochemistry and Molecular Biology; [3]Department of Microbiology, University of Szeged, Szeged, Hungary

In the recent decades, increased development of green synthesis of nanoparticles is inevitable because of its incredible applications in all fields of science. Due to the obvious advantages, the microbial assisted biosynthesis of nanoparticles is a rapidly progressing area of the nanobiotechnology. In this study, we report a simple and eco-friendly method for the synthesis of silver and gold nanoparticles using the red yeast *Phaffia rhodozyma*. The as-prepared silver and gold nanoparticles were characterized by transmission electron microscopy (TEM), powder X-ray diffraction (XRD), dynamic light scattering (DLS) and ultraviolet–visible spectroscopy (UV-Vis). Moreover, a complex biological screening was carried out to determine the toxicity of silver and gold nanoparticles to human keratinocyte cells as well as against various fungal species with a special emphasis on antifungal efficiency against dermatophytes. The results indicate that the yeast mediated green synthesis was successful in both cases, the as-synthesized silver and gold nanoparticles were phase pure, well crystalline with a face-centered cubic structure and have the characteristic peak which can be attributed to surface plasmon resonance. Furthermore, screening of their biological activities confirmed that the silver nanoparticles exhibited good antimicrobial activity against all examined microbial species whereas the gold nanoparticles were toxic only to *Cryptococcus neoformans*. None of the silver or the gold nanoparticles were toxic to human cells in the applied concentration range.

Financial support by the National Research, Development and Innovation Office-NKFIH through project GINOP-2.3.2-15-2016-00035 is gratefully acknowledged.

PROTECTION AFTER VACCINATION WITH ALUMINUM-ADJUVANTED INACTIVATED WHOLE VIRION INFLUENZA VACCINE IS INDEPENDENT OF VIRUS NEUTRALIZATION ACTIVITY AGAINST AN INFLUENZA VIRUS CHALLENGE

JÚLIA SARKADI[1], DÁVID KUTI[1], MÁTE JANKOVICS[1], ÉVA PALLINGER[2], KINGA FODOR[1], ZOLTÁN KIS[1], ÉVA GÖNCZÖL[1], ILDIKÓ VISONTAI[1], ISTVÁN JANKOVICS[1]

[1]National Center for Epidemiology; [2]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary

To investigate the protective efficacy of a seasonal trivalent inactivated whole virion influenza vaccine (TIV) adjuvanted with aluminum phosphate (Fluval AB, referred to TIV+Al here), we immunized mice with the TIV+Al, and as controls, with TIV, TIV+Al combined with Freund adjuvant (TIV+Al+F), the inactivated A/PR/8/34(H1N1) (PR8) strain or PBS, and challenged them with a lethal dose of a mouse-adapted PR8 virus. Serum pools obtained from immunized mice were passively transferred to recipient mice that then were challenged similarly. All actively immunized mice survived the challenge infection. The 80% or 100% of mice receiving serum from mice actively immunized with TIV+Al or TIV+Al+F survived, and 20% survived who received serum from TIV-immunized mice. The rate of mortality and morbidity of recipient mice was inversely proportional to the hemagglutination inhibition (HI) antibody level to the vaccine virus in the absence of detectable PR8-specific HI, neuraminidase inhibition (NI) and virus neutralization (VN) antibodies. No cross-reactivity was observed between the vaccine and the PR8 strains in HI, NI or VN assays. In splenocytes from TIV+Al-immunized mice the production of IFN-γ or granzyme-B protein and mRNA expression increased ($p<0.05$). Thus, cellular immune responses may contribute to the full protection in actively immunized mice. Antibodies independent from HI, NI and VN activity have a major role in the protection against a mismatched challenge infection.

MOLECULAR CHARACTERIZATION OF MULTIDRUG RESISTANT *BACTEROIDES FRAGILIS* GROUP ISOLATES FROM HUMAN CLINICAL SAMPLES IN HUNGARY

KÁROLY PÉTER SÁRVÁRI[1], JÓZSEF SÓKI[1], KATALIN KRISTÓF[2], MIKLÓS IVÁN[2], CECILIA MISZTI[3], KRISZTINA LATKÓCZY[4], SZILVIA ZSÓKA MELEGH[5], EDIT URBÁN[1]

[1]Institute of Clinical Microbiology, University of Szeged, Szeged; [2]Institute of Laboratory Medicine, Semmelweis University, Budapest; [3]Institute of Medical Microbiology, University of Debrecen, Debrecen; [4]SYNLAB Ltd., Budapest; [5]Institute of Medical Microbiology and Immunology, University of Pécs, Pécs, Hungary

Bacteroides species are important members of the normal human gut microbiota and can cause severe sepsis, intra-abdominal, skin and soft tissue infections. Although, MDR *Bacteroides* isolates have been rarely published so far, in the recent Hungarian antibiotic resistance survey we observed six MDR *Bacteroides* isolates. While MDR phenotypes can be quite frequent among the aerobic pathogens, this seems less occurring for the anaerobic bacteria. In our study 400 *Bacteroides fragilis* group clinical isolates collected from 2014 to 2016 were investigated. MIC values of ten antibiotics (ampicillin, amoxicillin/clavulanic acid, cefoxitin, meropenem, clindamycin, moxifloxacin, metronidazole, tetracycline, tigecycline, chloramphenicol) were determined with agar dilution method according to the CLSI. We found six MDR isolates and performed further molecular investigations.

RT-PCR was carried out to detect the most common antibiotic resistance genes (*cepA*, *cfxA*, *cfiA*, *nim*, *ermB*, *ermF*,*ermG*, *tetQ*, *tetX*, *tetX1*, *bexA*), IS4351, upstream region of *cfiA* and *cfxA* genes. In the case of *gyrA*, *gyrB*, *parC*, *parE* genes we looked for amino acid substitutions. The examined MDR isolates displayed resistance to four to six different antibiotic classes. The *B. fragilis* SZ38 isolate harboured *cfiA* gene with high level resistance to ampicillin, amixicillin/calvulanic acid, cefoxitin, meropenem. All of the strains were negative for *cepA* gene, and three *cfxA* positive isolates were detected. Four strains (*B. ovatus* D92, SZ9, *B. vulgatus* SZ34, and *B. thetaiotamicron* SZ35) expressed high level clindamycin resistance, *B. ovatus* D92 harboured *ermG* gene, *B. vulgatus* SZ4, *B. thetaiotaomicron* SZ35 *ermF* gene, and *B. ovatus* SZ9 both of them. IS4351 were detected in *B. vulgatus* SZ4 and *B. thetaiotaomicron* SZ35 strains.
All isolates harboured *tetQ* gene and three of them (*B. ovatus* D92, SZ9 and *B. fragilis* SZ38) expressed high level tetracycline resistance, *B. ovatus* SZ9, *B. vulgatus* SZ34 and *B. thetaiotaomicron* SZ35 strains contained *tetX* gene. None of the isolates harboured *nim* genes, *B. ovatus* D92 strain was metronidazole resistant. In the case of four strains moxifloxacin MIC≥32 μg/ml were detected, and among them the *B. thetaiotamicron* SZ35 harboured *bexA* gene. Ser82→Phe substitution in QRDR region of GyrA subunit of *B. fragilis* SZ38 strain was detected. Tigecycline and chloramphenicol were active against these isolates. In this study we experienced the pile-up of MDR strains isolated in Szeged with five isolates and only another one from Debrecen. The increasing number of MDR strains in the Szeged region may be caused by the local habit of antibiotic usage.

FILAMENTOUS FUNGI INHABITING REGWEED POLLEN

FLÓRA SEBŐK[1], CSABA DOBOLYI[1, 2], KATALIN INOTAI[2], MÁRTA DOBRÓNÉ TÓTH[3], SÁNDOR SZOBOSZLAY[1], BALÁZS KRISZT[1], DONÁT MAGYAR[4]

[1]Department of Environmental Safety and Ecotoxicology, Faculty of Agricultural and Environmental Sciences, Szent István University, Gödöllő; [2]Department of Applied and Environmental Microbiology, Research Institute of Agro-Environmental Sciences, National Agricultural Research and Innovation Center, Budapest; [3]Institute of Environmental Science, University of Nyíregyháza, Nyíregyháza; [4]Department of Air Hygiene and Aerobiology, National Public Health Center, Budapest, Hungary

A high proportion of allergic patients suffers from ragweed allergy in Europe. The major allergen of ragweed pollen, namely Amb a1 is responsible for serious allergic symptoms. According to the literature, the length of pollen season of this common weed governed by the photoperiod of flowering; anthesis is usually terminated by the occurrence of frost and airborne pollen levels are reduced by rain. The authors hypothesized that fungal populations may also play an important role in the termination of pollen season. Fungal colonization of pollen grains in open ragweed anthers was studied at the margin of a sunflower culture four times during the ragweed pollen season. Quantitative cultivation of fungi was performed on PDA at 25 ºC. The number of colony forming units (CFU) of filamentous fungi and yeasts was determined and filamentous fungal isolates were identified by the ITS region (ITS1, ITS2 and 5.8S rDNA) of the nuclear ribosomal DNA. In August, the CFU number of filamentous fungi was 100-1000 per gram pollen. Their quantity continuously increased during the pollen season and it reached the level of 100000 CFU/g by October. At the beginning of the pollen season the pollen samples were markedly dominated by yeasts, however an increase in the proportion of filamentous fungi was experienced; at the end of the season it was 45-50% with well-visible mycelia on pollen grains. The most frequent genera were *Alternaria* and *Cladosporium*, but other fungi, like *Botrytis cinerea*, *Coprinellus domesticus*, *Epicoccum nigrum*, *Filobasidium floriforme*, *Mortierella alpina*, *Peroneutypa scoparia*, *Phaeosphaeria herpotrichoides*, *Phialemonium*

dimorphosporum and *Pseudopithomyces chartarum* were also detected. The mycelia of fungi colonizing ragweed pollen grains usually grow onto the anthers and the inflorescence; in consequence, they can block the release of allergenic pollen in mid-autumn.

RECOMBINANT PROTEIN PRODUCTION IN *TRICHODERMA REESEI* BY TUNABLE AND CAZYME INDEPENDENT PROMOTERS

BERNHARD SEIBOTH[1, 2]

[1]Institute of Chemical, Environmental & Biological Engineering, TU Wien; [2]Austrian Centre of Industrial Biotechnology (ACIB) GmbH, Vienna, Austria

The ascomycetes *Trichoderma reesei* is a main producer of Carbohydrate Active Enzymes (CAZymes) required e.g. for plant biomass hydrolysis in the biorefinery industry. Most of the promotors used today are derived from (hemi-)cellulase-encoding genes. There is therefore a need of novel promoters that can be regulated independently from CAZyme production. Addition of L-methionine or pantothenic acid to growing cultures of *T. reesei* did not alter growth or the expression profile of hemi-(cellulases). Using whole transcriptome analysis different *T. reesei* genes were found that were either strongly induced by the addition of pantothenic acid or repressed by the addition of L-methionine. Promoter regions of selected genes were then fused to different reporter genes and proved the inducibility and repressibilty of the identified promoters under protein production conditions. The two presented promotor system are inexpensive and are therefore valuable tools for recombinant engineering of industrial *T. reesei* strains.

ASSESSING THE ANTIBIOTIC RESISTANCE LEVELS OF HUMAN INTESTINAL *BACTEROIDES* AND *PARABACTEROIDES* STRAINS ISOLATED IN EUROPEAN COUNTRIES

JÓZSEF SÓKI[1], KATALIN ÖRDÖG[1], INGRID WYBO[2], DANIEL TIERNEY[3], SAMO JEVERICA[4], NURVER ULGER[5], CATALINA-SUZANA STINGU[6], EDIT HAJDÚ[7], JOHN PERRY[3], EDIT URBÁN[1], ELISABETH NAGY[1]

[1]Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary; [2]Microbiological Laboratory, University Hospital Bruxelles, Bruxelles, Belgium; [3]Microbiology Department, Freeman Hospital, Newcastle-upon-Tyne, UK; [4]Institute for Microbiology and Immunology, University of Ljubljana, Ljubljana, Slovenia; [5]Department of Microbiology, Marmara University, Istanbul, Turkey; [6]Institute for Medical Microbiology and Infection Epidemiology, University Clinics AÖR, Leipzig, Germany; [7]Department of Medicine, University of Szeged, Szeged, Hungary

The majority of human clinical anaerobic infections are caused by the *Bacteroides* and the closely-related *Parabacteroides* species where these are not only opportunistic pathogens but important members of the intestinal microbiota. In recent years we conducted some antimicrobial susceptibility studies on domestic, and *Bacteroides* isolates from clinical specimens all over Europe. Additionally, we also detected the antibiotic resistance genes in those strains. As a follow-up we sought to investigate the situation with intestinal strains. 242 *Bacteroides* and *Parabacteroides* strains were isolated from faecal samples of healthy individuals and patients treated with carbapenems in 5 European countries (Belgium, Germany, Hungary , Slovenia and Turkey) using the novel selective Chromogenic Bacteroides Agar (CBA). Antibiotic susceptibilities for all of these strains were

recorded by agar dilution and the *cepA*, *cfxA*, *cfiA* and *ermF* genes were detected in Hungarian isolates by previously established PCR methods. The orders of the resistance rates for the examined antibiotics (ampicillin, amoxicillin/clavulanate, cefoxitin, imipenem, clindamycin, moxifloxacin, metronidazole, tetracycline, tigecycline and chloramphenicol) were similar to that obtained for clinical strains earlier but some slight differences were also encountered, decreased resistance rates for amoxicillin/clavulanate (10.4 vs. 3.7% for clinical and normal flora isolates, respectively) and higher resistance rates for cefoxitin (17.2 vs. 21,2%), imipenem (1.2 vs. 3.3%) and clindamycin (32.4 vs. 42.3%). The presence of antibiotic resistance genes were also higher in the normal flora isolates from Hungary for the *cfxA*, cefoxitin, and the *ermF*, clindamycin, resistance genes.
This latter finding explains the higher antibiotic resistance rates and invokes that the microbial and genetic composition of the normal *Bacteroides* and *Parabacteroides* microbiota in the intestine and in the strains from anaerobic infections are different, which should also be investigated in some more aspects, e.g. genetic background and virulence properties.
This study was supported by the ESCMID Study Group on Anaerobic Infections.

GENETIC RECOMBINATION IN YEAST - FROM FUNDAMENTAL RESEARCH TO STRAIN CONSTRUCTION

ANAMARIJA ŠTAFA[1], MARINA SVETEC MIKLENIĆ[1], BOJAN ŽUNAR[1], BOŽIDAR ŠANTEK[2], IVAN KRESIMIR SVETEC[1]

[1]Laboratory for Biology and Microbial Genetics; [2]Laboratory for Biochemical Engineering, Industrial Microbiology and Malting and Brewing Technology, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

Yeast *Saccharomyces cerevisiae* is one of the most important industrial microorganisms and a model organism for research of almost all biological processes of eukaryotic cell. It is also known as an organism which genome can be precisely modified by routine transformation with DNA prepared in vitro because in contrast to many other organisms, DNA introduced in the cell of *S. cerevisiae* undergo homologous recombination. The most commonly used approach for genetic modification of *S. cerevisiae* is known as gene replacement during which the transforming DNA replaces the particular region in the genome. It has been taken for granted that gene replacement is practically 100% successful in *S. cerevisiae* but we have shown that this approach can result in several aberrant, unwanted genetic events such as (i) non-targeted integration, integration of transforming DNA at random position in the genome known as illegitimate integration; (ii) integration of the transforming DNA next to the region that should have been replaced and (iii) duplication of entire targeted chromosome resulting in disomic cell having two copies of targeted chromosome, one carrying transformed and the other carrying untransformed targeted region. We have also shown that in some cases the success of gene replacement in *S. cerevisiae* can be as low as 60% expressed as proportion of transformants carrying intended/desired modification. Apart from efficiency of gene replacement, the strategies for construction of *S. cerevisiae* strains suitable for second generation bioethanol production will be presented. We have also developed the first successful procedure for genetic transformation of *Brettanomyces bruxellensis*, a non-conventional yeast recognized as promising producer of bioethanol of second generation. As expected, targeted genetic modification in *B. bruxellensis* is far less successful than in *S. cerevisiae*, presenting a great obstacle in construction of strains having desired traits. Therefore, the strategy for construction of *B. bruxellensis* strains which will be more suitable for precise genetic modification will be presented.

ANTIBIOTIC SUSCEPTIBILITY PROFILES OF *MYCOPLASMA HYOPNEUMONIAE* ISOLATES FROM HUNGARY

ORSOLYA STAMMNÉ FELDE[1], KATINKA BEKŐ[1], VERONIKA HRIVNÁK[1], KRISZTIÁN KISS[2], IMRE BIKSI[3], ZSUZSA KREIZINGER[1], KINGA MÁRIA SULYOK[1], MIKLÓS GYURANECZ[1]

[1]Veterinary Research Institute, Agricultural Research Center, Hungarian Academy of Sciences, Budapest; [2]SCG Diagnostics Ltd., Délegyháza; [3]University for Veterinary Science, Budapest, Hungary

Mycoplasma hyopneumoniae is the causative agent of porcine enzootic pneumonia, a chronic respiratory disease prevalent worldwide. The disease induces serious economic losses to the swine industry mostly through growth retardation. Vaccines are able to reduce the symptoms, but are not suitable to prevent the infection. The use of antibiotics to control or treat enzootic pneumonia is of common practice, but the inadequate usage of the antimicrobial agents can induce the development of antibiotic resistance. The aim of the present study was to describe the in vitro antibiotic susceptibility profiles of seventeen isolates, collected in 2015-2016 from Hungarian slaughterhouses. Minimum inhibitory concentration (MIC) values were determined to fluoroquinolones, an aminoglycoside and an aminocyclitol, macrolides, pleuromutilins, florfenicol and lincosamide by microbroth dilution method. MIC values were defined as the lowest concentration of the antibiotic that completely inhibits the growth in the broth (no pH and no colour change) detected when red to yellow shift was observed in the antibiotic-free bacteria cultures used as growth controls. MIC50 values are drug concentrations, which inhibit fifty percent of the examined isolates. All isolates showed similar MIC values independent from the place of origin, except one outlier isolate, which had the highest values for tylosin, tulathromycin, gamithromycin and lincomycin (32 mg/l, ≥64 mg/l, 64 mg/l and ≥64 mg/l, respectively). The MIC50 values for fluoroquinolones (enrofloxacin and marbofloxacin) were ≤0.039 mg/l and 0.078 mg/l; for the aminoglycoside, gentamicin ≤0.25 mg/l and aminocyclitol, spectinomycin 2.0 mg/l; for tetracyclines (oxytetracycline and doxycycline) 0.5 mg/l and 0.078 mg/l; for macrolides (tylosin, tilmicosin, tylvalosin, tulathromycin and gamithromycin) ≤0.25 mg/l, 2 mg/l, ≤0.25 mg/l, 0.5 mg/l and 0.5 mg/l; for pleuromutilins (tiamulin and valnemulin) ≤0.039 mg/l; for florfenicol 1.0 mg/l and for lincomycin ≤0.25 mg/l. There is no standardized procedure for MIC testing of *M. hyopneumoniae*, which makes the comparison of the results complicated. Both fluoroquinolones and pleuromutilins had low MIC50 values, as described earlier. MIC50 values for tetracyclines were higher than in a recent Spanish publication. The results for gentamicin were low as published before in Europe, but we observed an increase in the susceptibility to spectinomycin. The MIC50 values for florfenicol and lincomycin were four times higher, than in other examinations. Only a few publications were available about the susceptibility testing to macrolides, but we observed an increasing tendency compared to earlier data. The most effective antimicrobial agents based on our results are gentamicin, tylosin, tylvalosin and valnemulin against *M. hyopneumoniae*. Also, the majority of the isolates were inhibited by low concentrations of lincomycin, tiamulin and florfenicol.

MODULATION OF CELL CYCLE BY HESPERETIN ON HIV-1 INFECTED CELLS

BALÁZS STERCZ[1], KAZUNORI YOSHIMURA[2], KÁROLY NAGY[1]

[1]Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary; [2]Nihon Institute of Medical Science, Faculty of Health Science, Saitama, Japan

Upon HIV infection, cells become activated and cellular components – including cell membrane lipid rafts – express increased number of –SH groups. Earlier we demonstrated significant in vitro anti-HIV effect of our thyolated pyrimidine nucleotides which modify –SH groups. Besides this thyolated pyrimidine nucleotides inhibited the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), suggesting that these compounds may interfere with the function of the essential –SH groups of CD4 molecule (the primary receptor of HIV) as well as –SH groups in HIV viral envelope, resulting a certain antiviral effect. Hesperetin (3',5,7-trihydroxy-4'methoxyflavonon) found in citrus fruits, has an effect on cell proliferation in vivo and in vitro, but its action and molecular mechanism is not revealed. It is involved in apoptosis of cells, and also modulates GAPDH enzyme expression. The aim of this ongoing studies is to investigate the effect of hesperetin on HIV-1 activated cell cycle. The antiproliferative and acute and chronic cytotoxic effect and modification of cell cycle of hesperetin have been investigated in vitro on HeLaCD4+β-gal monolayer cells. Hesperetin was added to the cell on 96 well plates in concentration ranged 10 -300 μM in triplicate, for 24hrs and 48hrs respectively. Cytotoxic effect of hesperetin has been determined quantitatively in vitro by XTT based Toxicology Assay Kit (Sigma-Aldrich), which is a spectrophotometric measurement of cell viability based on mitochodrial dehydrogenase activity in living cell. Hesperetin in concentration of 10, 25, 50, 100, 150 μM concentration respectively had no effect on cell proliferation. However at 200 μM concentration cell proliferation was seriously affected, and morphological as well as biochemical signs of cellular cytotoxicity have been observed. Hesperetin in >200 μM dose used at 24h and 48 h time periods induced cell death and modified cell cycle.
This effect may be related to the modification of GAPD expression. Based upon this pilot studies HIV-1 infectivity assays in vitro on HeLaCD4+β-gal cells (MAGI assay), and H9 human lymphoid cell line will be performed in the presence and absence of hesperetin in <200 μM.

BIOAUGMENTATION OF THE ANAEROBIC DEGRADATION OF CORN STOVER AND SUNFLOWER STALK WITH CELLULOLYTIC MICROORGANISMS

ORSOLYA STRANG[1], ZOLTÁN BAGI[1], GÁBOR RÁKHELY[1, 2], KORNÉL L. KOVÁCS[1, 2, 3]

[1]Department of Biotechnology, University of Szeged; [2]Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences ; [3]Department of Oral Biology and Experimental Dental Research, University of Szeged, Szeged, Hungary

Substrates for second generation biofuels do not compete with food and feed production. Biogas from agricultural wastes and residues are considered as carbon neutral. Sunflower stalk and corn stover, two high carbon containing and therefore energy rich lignocellulosic substrates are produced in vast amounts annually. Their recalcitrant nature makes them difficult to use as substrate for biofuel production. In the anaerobic degradation process the rate limiting step is hydrolysis of polymeric components. The biochemical methane potentials of sunflower stalk and corn stover at mesophilic (37°C) and thermophilic (55°C) temperature were determined. There was no pronounced difference in case of the same substrate between the two temperature regimes, the methane yields were 235 and 242 mL/g oDM for corn stover and 181 and 170 mL/g oDM for sunflower stalk at 37°C and 55°C, respectively. Irrespective of the temperature, corn stover had higher methane yield. This could be the result of the different chemical composition; sunflower stalk has higher lignin content (29.2%) in

comparison to corn stover (17.6%) and lignin is considered as recalcitrant inhibitor of lignocellulosic deconstruction. Cellulolytic microorganisms can be used to pretreat the biomass or bioaugment the process and thereby promote polymer hydrolysis.
At mesophilic temperature *Clostridium cellulolyticum* and at thermophilic condition *Clostridium thermocellum* could be a suitable candidate to advance the anaerobic degradation.
Supported by the National Research, Development and Innovation Fund projects GINOP-2.2.1-15-2017-00065, GINOP-2.2.1-15-2017-00081. Z.B. is the Principal Investigator of the NKFIH-OTKA project FK 123902.

CHARACTERISATION OF NEW FOOD-BORNE T5-LIKE LYTIC BACTERIOPHAGES INFECTING PATHOGENIC *ESCHERICHIA COLI*, *SHIGELLA* AND *SALMONELLA* STRAINS

DOMONKOS SVÁB[1], LINDA FALGENHAUER[2], MANFRED ROHDE[3], TRINAD CHAKRABORTY[2], ISTVÁN TÓTH[1]

[1]Enteric bacteriology, Institute for Veterinary Medical Research, Centre for Agricultural Resarch, Hungarian Academy of Sciences, Budapest, Hungary; [2]Institute of Medical Microbiology, Justus Liebig University Giessen, Giessen; [3]Central Facility for Microscopy, Helmholtz Centre for Infection Research, Braunschweig, Germany

Recently there is a growing interest in the use of bacteriophages as biocontrol agents against foodborne enterobacterial pathogens. Here by using *Escherichia coli* K-12 derivative strain C600 as the host bacterium, we isolated and characterised 12 novel T5-like bacteriophages from confiscated food samples. All the phages showed Siphoviridae morphology. They effectively lysed *E. coli* K-12 derivative strains with an average burst size of 40 PFU/cell with a 45 minute long latent period. The host spectrum of the phages divided them into two subgroups. All phages proved to be lytic of enterotoxigenic *E. coli* (ETEC) and enteroinvasive *E. coli* (EIEC) strains, the latter from the O152 serogroup. The phages also lysed a *Shigella sonnei* strain as well as a multi-drug resistant *E. coli* of the O73 serogroup. Strains lysed exclusively by the subgroup designated 'Φ24-like' phages included enterohaemorrhagic (EHEC) of the O103:H2 serotype, multiple *S. sonnei* strains, a *Shigella dysenteriae* 1A strain, as well as strains representing *Salmonella enterica* serovars Typhimurium, Infantis and Panama. On the other hand, 'Φ130/1-like' phages lysed enteropathogenic *E. coli* (EPEC) O127:H6 in addition to strains lysed by all phages.
Whole genome sequences of the T5-like novel phages showed that in harmony with their host specificity they represent two new distinct groups among T5-like phages. The genome of the 'Φ24-like' phages is 110,652-110,662 bp long corresponding to 152 or 153 protein-encoding CDS, as well as 18 tRNA genes. The genome length of the 'Φ130/1-like' phages is 113,269 bp, with 155 or 156 protein-encoding CDS and 20 tRNA genes. The GC contents of the genomes of the two phage groups were 39.2% and 39.8%, respectively. The fact that these novel T5-like phages do not harbour any virulence related genes, as well as their broad host spectrum makes them valuable candidates to be used as bio-control agents against food-borne pathogenic enterobacteria.

ANALYSING TARGETED CHROMOSOME DUPLICATION IN YEAST *SACCHAROMYCES CEREVISIAE* BY QPCR

MARINA SVETEC MIKLENIĆ[1], ANAMARIJA ŠTAFA[1], BOJAN ŽUNAR[1], ANTONIO ZANDONA[1], NEŽA ČADEŽ[2], HRVOJE PETKOVIĆ[2], IVAN KREŠIMIR SVETEC[1]

[1]Department for Biochemical Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia; [2]Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Gene targeting is one of the basic tools in genetic engineering allowing deletion, precise modification or insertion of genes thus facilitating new strain construction for research and industry. Gene targeting is based on transformation of cells with a linear DNA fragment carrying ends homologous to the targeted region. The desired result of such transformation is replacement of the targeted region of the genome with the transforming DNA fragment.
Yeast *Saccharomyces cerevisiae* is known for extraordinarily efficient gene targeting, i.e. the occurrence of unwanted, aberrant genetic events during gene targeting in this organism is generally considered to be fairly low. The most frequent aberrant genetic event in yeast is the targeted chromosome duplication, where one chromosome copy carries the transforming DNA in the targeted region while the other chromosome copy retains the original allele. In recent study we used qPCR to analyse the targeted chromosome duplication events in more detail. We used telomere-proximal primer pairs and probes to confirm that indeed the entire length of the targeted chromosome is duplicated. Moreover, we determined not only the targeted chromosome copy number, but also the copy number of all other (non-targeted) chromosomes. Interestingly, only two out of eight analysed transformants were aneuploids carrying targeted chromosome duplication and a single copy of non-targeted chromosomes, while other six out of eight transformants were diploids. Based on this results, we proposed a two-step mechanism for genome duplication instigated by gene targeting. After the duplication of the targeted chromosome (by break-induced replication mechanism), a protein content imbalance occurs. Since the transformants were kept under selective pressure to maintain both targeted chromosome copies, the imbalance could not be relieved by loss of an extra chromosome copy. Therefore, the duplication of non-targeted chromosomes leading to diploidisation and restoration of protein content balance would be favoured in yeast cell culture.

REPROGRAMMING OF TYROSINE PHOSPHORYLATION REGULATED SIGNALLING PATHWAYS BY HPV ONCOPROTEINS

ANITA SZALMÁS[1], VJEKOSLAV TOMAIĆ[2], PAOLA MASSIMI[3], ESZTER GYÖNGYÖSI[1], JÓZSEF KÓNYA[1], LAWRENCE BANKS[3]

[1]Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; [2]Division of Molecular Medicine, Rudjer Boskovic Institute, Zagreb, Croatia; [3]Tumour Virology Research Group, ICGEB, Trieste, Italy

Activation of signaling pathways ensuring cell growth are essential for the proliferative competence of human papillomavirus (HPV) infected cells. Kinases and phosphatases are the key regulators of cellular growth control pathways. Recently identified cellular targets of HPV E7 are certain cytoplasmic protein tyrosine kinases and phosphatases, such as Src, PTPN14, and PTPN21, which have been linked to the control of multiple cellular growth pathways. We showed that the E7 proteins of both high-risk and low-risk mucosal HPV types can interact with certain non-receptor kinases and phosphatases. These interactions can happen independently of pRb regulation. We also showed that E7 can influence the stability of these regulatory proteins, for example by inducing proteasome mediated degradation of certain cytoplasmic phosphatases.

These results suggest that diverse HPV E7 oncoproteins can perturb tyrosine phosphorylation regulated pathways, and also suggest that these interactions are important for the viral life cycle and potentially also in the development of high-risk HPV infection-associated malignancy.

GENOME POLYMORPHISMS IN HPV6S FROM BENIGN RESPIRATORY AND GENITAL LESIONS

KRISZTINA SZARKA[1], ZSÓFIA NAGY[1], MIHÁLY SZINAI[1], GÁBOR KARDOS[1], ATTILA SZŰCS[2]

[1]Department of Medical Microbiology; [2]Clinic of Otorhinolaryngology and Head & Neck Surgery, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Complete genomes of HPV6s from respiratory papillomatoses and from a genital condyloma (single episode) were determined and compared to published genomes. Three adult onset respiratory papillomatoses (one solitary and two recurrent papillomas with two and six episodes) were HPV6b positive and five HPV6vcs (in the condyloma, in two juvenile papillomatoses with four and five recurrences and in two adult onset papillomatoses with seven and twelve episodes) were found, HPV6a was not encountered. In HPV6b, 25 polymorphisms were identified, 17 to 21 polymorphisms in a genome. Ten were virus-specific and fifteen were characteristic to the intratypic variant group. All three HPV6b genomes clustered separately from the prototype into three different groups. Five, two and two polymorphisms were found in E1, E5a and E6 ORFs, respectively, of which those of E5a were unique. These two resulted in amino acid alteration (E39D and P78S), others were silent. Other early ORFs were conservative. Late ORFs L1 and L2 contained four and five conservative polymorphisms, respectively. In the noncoding region one, in the long control region (LCR) six polymorphisms were detected. In HPV6vc, 22 polymorphisms were found, three to seven polymorphisms in a genome. Only one was present in all five genomes, one in three; 20 were unique. All five genomes clustered to the same large group as the reference genome, but all to different subclusters. ORFs E1, E2, E4, E5a and E6 carried three, five, one, one and two polymorphisms, respectively, of which, in contrast to HPV6b, all except two (in E1 and in E5a) were unique. Four of them led to amino acid replacement, all in the E2-E4 ORF (T116N, S144T, S246A and E380D in the E2; S246A corresponded to a S68R change in E4 ORF, the others were in the region belonging solely to E2). ORFs E5b and E7 were the same as the reference.

Late ORF L1 contained one polymorphism common to all five genomes and five unique alterations. The common polymorphism led to a Y219D change. Three of the five unique polymorphisms were silent, one led to F441L and one to K449E amino acid replacement. In the noncoding region one unique, in the LCR two unique polymorphisms were detected. HPV6vc showed considerably higher variability with multiple non-silent polymorphisms in E2-E4, while coding regions of the three HPV6bs, though different from the prototype, were more similar. LCR, in contrast, was more variable in HPV6b. These suggest that HPV6bs differ mainly in LCR activity, while in HPV6vc polymorphisms of replication proteins may be more important.

K. Sz. & G. K. were supported by a János Bolyai research scholarship of the Hungarian Academy of Sciences.

DISRUPTION OF GENES *cotH1* AND *cotH2* OF *MUCOR CIRCINELLOIDES* VIA THE CRISPR/CAS9 SYSTEM

CSILLA SZEBENYI[1], GÁBOR NAGY[1], GRACE VAZ AMANDA[1], ESZTER TÓTH[1], CSABA VÁGVÖLGYI[1], TAMÁS PAPP[2]

[1]Department of Microbiology, Faculty of Science and Informatics, University of Szeged; [2]MTA-SZTE "Lendület" Fungal Pathogenicity Mechanisms Research Group, Szeged, Hungary

In this study, we tested a transient, plasmid free CRISPR/Cas9 system for efficient gene deletion in *Mucor circinelloides*. A CRISPR/Cas9 system has been developed to disrupt the genes *cot*H1 and *cot*H2, encoding spore coat protein H in *M. circinelloides*. The system is efficient and reliable, as RNA guided mutagenesis can be achieved by transforming a target fungus with a deletion cassette, the guide RNA (gRNA), i.e. the CRISPR-RNA (crRNA) and trans-activating crRNA (tracrRNA) complex, and the Cas9 enzyme. The crRNA and trans-activating crRNA (tracrRNA) were simultaneously used (gRNA) to guide CRISPR-associated (Cas) nuclease to create double-strand breaks in the targeted DNA sequence. The components of the CRISPR/Cas9 system were introduced together into the fungal cells by polyethylene glycol (PEG)-mediated protoplast transformation. In this transformation strategy, a linearized deletion cassette constructed by PCR was used as a template DNA for the HR repair. *M. circinelloides* is a widely used model organisms in different genetic and molecular biological studies. Even if genetic transformation of *M. circinelloides* has been reported before, stable integration of the introduced DNA into the host genome can hardly be carried out and remained a great challenge in this fungal group. CotH1 and CotH2 proteins are present in Mucorales and act as fungal ligands for GRP78 during invasion of endothelial cells. Previously, directed mutagenesis was achieved by various transformation strategies using the components of the CRISPR/Cas9 system in different filamentous fungi. In this study, we have successfully developed a functioning CRISPR system to disrupt the *cot*H1 and *cot*H2 genes in *M. circinelloides* without the using of plasmids.Double-strand breaks of DNA were achieved by co-transformation of the Cas9 and the gRNA with the deletion cassette. The presence of the selection marker *pyr*G gene was detected by molecular analysis in the coding sequence of *cot*H1 and *cot*H2 genes.

The described method resulted in stable gene disruption mutants. To prove the mutation, a region containing the targeted *cot*H1 and *cot*H2 genes was amplified by PCR from the DNA of the transformants. Some features of the cotH-disrupted mutants were characterized.

The study was supported by the grants LP2016-8/2016 and GINOP-2.3.2-15-2016-00035.

CHARACTERIZATION CELLULOSE AND XYLAN DEGRADING MICROORGANISMS FROM RABBIT GASTROINTESTINAL TRACT

ÁRPÁD SZILÁGYI[1], KATALIN PEREI[1], BARBARA HÓDI[1], GÁBOR RÁKHELY[1, 2, 3]

[1]Department of Biotechnology, University of Szeged; [2]Institute of Biophysics, Biological Research Center, HAS; [3]Institute of Environmental and Technological Science, University of Szeged, Szeged, Hungary

The increasing energy demand and the serious environmental impact of the currently used non-renewable fossil fuels inspired the researchers to produce alternative energy carriers from renewable sources. Basically, one of the largest – practically unexhaustable – energy source is the Sun. The light energy can be converted directly or indirectly into various energy carriers by various physical, chemical and biological approaches. Biomass is an abundant organic substance which stores the sunlight in the form of chemical energy. Therefore, biomass is one of the most promising renewable raw materials for fuel production. Lignocellulose composed of cellulose, hemicellulose and lignin form the body of plant biomasses. Many microorganisms are able to degrade these biopolymers under aerobic or anaerobic conditions with complex enzyme systems. Hydrolysis of cellulose/hemicellulose by cellulases and xylanases results monosaccharides and/or secondary metabolites that can be further fermented by microorganisms into various alternative biofuels, such as bioalcohols, biogas, H_2 etc.

The digestive systems of herbivores might be rich resources of cellulose/hemicellulose degrading microves. Various herbivores have alternative strategies for utilization of plant biopolymers. Among these, rabbit has a special processing system in which the colon sorts the plant fibres into digestible and indigestible parts. The digestible biopolymers are broken down in the caecum containing special microbes for this process. We could successfully isolate cellulose and xylan degrading microorganism(s) from rabbit gastrointestinal tract under aerobic conditions. We isolated two strains which showed good cellulase and xylanase activity on minimal salt agar plate supplemented with microcrystalline cellulose or xylan as carbon source. The activities of enzymes were determined by Congo-red probe then strains were identified by 16S rDNA-based method. The physiological and biochemical characteristics of the isolates were determined, as well. Moreover, metagenomic approach was used to characterize the microbial community present in the caecum.

Supported by the European Union and Hungarian State (grant agreement no. TÁMOP-4.1.1.C-12/1/KONV-2012-0012, EFOP-3.6.2-16-2017-00010,).

A-FACTOR MEDIATED TRANSCRIPTOMIC CHANGES DURING ANTIBIOTIC PRODUCTION IN *STREPTOMYCES GRISEUS*

MELINDA SZILÁGYI, NIKOLETT NAGY, ÉVA MÁRTON, ZSUZSANNA BIRKÓ, SÁNDOR BIRÓ

Department of Human Genetics, University of Debrecen, Debrecen, Hungary

Streptomyces comprises Gram-positive, soil dwelling bacteria that show complex morphological differentiation terminated in sporulation. Another characteristic of streptomycetes is the production of a wide variety of secondary metabolites that occurs in parallel with morphological differentiation. This ability makes streptomycetes one of the most important industrial microbial genus since 60% of the known antibiotics derive from their cultures. The spread of antibiotic resistant bacteria encourages the search for new antibiotics. Streptomycetes are still promising candidates for these screening efforts. γ-butyrolactones, such as A-factor in *S. griseus*, play a crucial role in the regulation of morphological development and secondary metabolism in streptomycetes. However, little is known about the regulation of γ-butyrolactone biosynthesis. To shed light on the factors that influence A-factor production in *S. griseus* we studied the transcriptomic changes by RNA sequencing during A-factor mediated differentiation in the B2682 wild type strain and in its AFN mutant, which is not able to produce significant amount of A-factor. In this work the mRNA profile of a non-differentiating (26 h old) and a differentiating (50 h old) culture were compared in both of the strains. In the B2682 strain the transcription of 1140 genes changed during differentiation – 623 were upregulated and 517 were downregulated. However, in the case of the AFN strain only 598 genes showed different expression – 342 were upregulated and 256 were downregulated. The fact that only the transcription of 184 genes changed in both of the strains - among them the expression of 113 genes changed to the same direction- shows low correlation between the two strains that result in their markedly different phenotype. In the AFN strain genes linked to the primer metabolism were upregulated in contrast to the B2682 strain, in which these genes showed downregulation. The expression of genes involved in secondary metabolism were upregulated in B2682, including several genes of the streptomycin gene cluster, that was not experienced in the case of the AFN strain as expected. Several genes function in fatty acid biosynthesis showed high expression in AFN that might explain its low A-factor producing capacity since both of these processes are fueled by acetyl-coA molecules. The high activity of energy metabolism and genes related to translation in AFN are in good accordance with its intensive growth. The expression of genes coding hydrolases, membrane transporters, transcription factors and redox proteins was also affected by differentiation.

Our work helps to understand the regulation of A-factor and antibiotic production in details. This might contributes to the development of more effective antibiotic screening methods.

MOLECULAR EPIDEMIOLOGY OF *SALMONELLA INFANTIS* IN CENTRAL EUROPE

AMA SZMOLKA[1], JUDIT PÁSZTI[2], ROBERT HORTON[3], RENATA KARPÍŠKOVÁ[4], ESTELLA PRUKNER-RADOVCIC[5], JASNA MIĆUNOVIĆ[6], KRASEN PENCHEV[7], BÉLA NAGY[1]

[1]Enteric bacteriology and food-borne zoonoses, Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest; [2]Department of Phage-typing and Molecular Epidemiology, National Center for Epidemiology, Budapest, Hungary; [3]Animal and Plant Health Agency (APHA), Field Epidemiology & Surveillance, Department of Bacteriology, Addlestone, UK; [4]Department of Bacteriology,Veterinary Research Institute, Brno, Czech Republic; [5]Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia; [6]Institute for Microbiology and Parasitology, UL Veterinary Faculty, Ljubljana, Slovenia; [7]Ameta Holding, Razgrad, Bulgaria

In the last two decades, *Salmonella* Infantis became endemic in Hungarian poultry flocks leading to increased occurrence in humans. The dissemination of this serovar was associated with the emergence of the PFGE cluster B, characterized by the Nal-Sul-Tet phenotype, and the large multiresistance (MDR) prototype plasmid pSI54/04. Previously we found that plasmidic strains of the Hungarian MDR cluster B are also widespread in Europe and remained prevalent until recently in Hungary. Here, we aim to give a comparative overview of the distribution and molecular attributes of recent poultry strains of *S.* Infantis in Central European countries relative to our earlier reports from some of these and of other European countries including Hungary. For this purpose a total of116 strains of *S.* Infantis were tested, originating mostly from broilers (n:87), but strains from human clinical samples (n:25) and layers (n:4) were included for comparison. Strains were isolated between 2010-2016in Bulgaria, Czech Republic, Croatia, Germany, Poland, Romania and Slovenia. The antimicrobial resistance phenotype was determined for all strains of S. Infantis, based on which 40 strains - representing different AMR patterns were subjected for PFGE analysis and for resistance genotyping by our PCR microarray system. The co-existence of certain genes for antimicrobial resistance (*tetA-merA-intI1-aadA1-sul1-tehA*) and for virulence (*irp1-fyuA-htrE-faeI-pefC*) were used for the PCR-based detection of the prototype plasmid pSI54/04 and its variants. A 69.8 % of all the strains proved to be resistant to at least three different antimicrobials. The MDR phenotypes of Nal-Sul-Tet and Nal-Sul-Tet-Tmp were predominant (63.2% of the broiler and 48.6% of the human strains). Most of the strains with the above resistance patterns belonged to the PFGE cluster B. and 21.5% of the strains showed resistance to Nal only, or were pansensitive. Interestingly, 80.1% of the broiler strains from Croatia, and some human strains from Czech Republic represented these patterns. Carriage of the large MDR prototype plasmid pSI54/04 was typically detected in broiler strains showing the resistance patterns Nal-Su-Tet/-Tmp and belonging to the PFGE cluster B.

Results on resistance genotyping showed the association between the pSI54/04 and blaTEM-1 plasmids in strains with co-resistance to ampicillin. In conclusion, resistance phenotyping and molecular analysis has shown the stabilization and constant circulation of the former major MDR cluster B of *S.* Infantis and its characteristic plasmid pSI54/04 not only in Hungarian poultry flocks but also in Central Europe with indications of further evolution of pSI45/04 and its associations with other plasmids mediating resistance to drugs with clinical significance.

Support of OTKA K101546 and the János Bolyai stipend of the HAS to A. Sz. is acknowledged.

A NOVEL BIOTECHNOLOGICAL FUNCTION OF FERMENTATION RESIDUE: BIOCH$_4$ PRODUCTION BY MICROBIAL CONVERSION OF H$_2$ AND CO$_2$

MÁRK SZUHAJ[1], GÁBOR RÁKHELY[1, 2], ZOLTÁN BAGI[1], KORNÉL L. KOVÁCS[1, 2, 3]

[1]Department of Biotechnology, University of Szeged; [2]Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences; [3]Department of Oral Biology and Experimental Dental Research, University of Szeged, Szeged, Hungary

The accelerated technological, ecological development results in growing energy demand. The increase of the utilization of fossil energy sources leads to deterioration of climate. The only solution is the utilization of renewables, which supply an increasing share of our energy needs. The rapidly developing renewable capacities are wind and photovoltaics based electricity production, but these technologies are coupled with alarming fluctuations in the electricity grid. Finding a solution to the problem of the storage of the surplus electricity generated by these renewables is indispensable. The excess power can be employed for splitting water in electrolyzers to generate H_2 and O_2. The technologies to store and transport the H_2 are not cost-effective today, therefore conversion H_2 to CH_4 is a preferable solution. CH_4 can be transported and stored easily via the existing natural gas grid. The chemical methods to reduce CO_2 with H_2 are well developed, but the same results can be reached in an environmentally friendly and economically feasible way with the help of biological systems. Hydrogenotrophic methanogens catalyze the conversion of H_2 and CO_2 to CH_4. These microbes are present in the biogas evolving natural and man-made systems. In our study the upgrading effect of injected H_2 was tested at laboratory scale and mesophilic temperature in fed-batch reactors. The fermentation effluent from a mesophilic biogas plant was used directly as catalyst. The limiting factor in this system was the efficiency of gas/liquid mass transfer. Daily dosage of H_2 in the reactors was carried out to reduce this hurdle. Several operational conditions were tested and efficient CH_4 evolution was achieved. The addition of stoichiometric mixture of CO_2 and H_2 resulted in stable and sustained CH_4 production, as a by-product acetate was detected. Based on the results a strategic alliance between the various methods of producing renewable electricity and the biogas technology is proposed. The proposed novel strategy suggests the utilization of the biogas effluent reservoir, which is part of most industrial-scale biogas facilities and stores the digested material until its utilization as organic fertilizer. The biogas microbial community serves as efficient biocatalyst, transforming green electricity-derived H_2 into bioCH_4, and thus acquiring an entirely new function for the biogas plant.
Supported by the National Research, Development and Innovation Fund projects GINOP-2.2.1-15-2017-00065, GINOP-2.2.1-15-2017-00081. Z.B. is the Principal Investigator of the NKFIH-OTKA project FK 123902.

STUDY OF BACTERIAL STRAINS ISOLATED FROM FERTŐ: CARBON SOURCE UTILIZATION TESTS AND DETECTION OF NOVEL TAXA

SÁRA S. SZURÓCZKI, ORSOLYA SÁRKÁNY, ESZTER SÁRI, KÁROLY MÁRIALIGETI, ERIKA TÓTH

Department of Microbiology, Faculty of Science, ELTE Eötvös Loránd University, Budapest, Hungary

Fertő, the largest soda lake in Europe, is a shallow lake with slightly alkaline pH. In this study, bacterial strains isolated in November of 2015 and in July of 2016 from the open water of the Hungarian-Austrian border, from the Kis Herlakni inner lake and from the water of a reed covered area were investigated for their carbon source utilization, among them many novel bacterial taxa.

These new bacterial taxa were isolated mainly from our novel, own developed oligotrophic medium (73%). 561 bacterial strains were identified by sequencing their 16S rRNA gene after grouping them by ARDRA. The phylum Proteobacteria with classes of Alpha-, Beta- and Gammaproteobacteria were identified. Representatives of Actinobacteria, Firmicutes, Bacteroidetes and Verrucomicrobia were also detected from all samples. The utilization of Tween 80, glycogen, cellobiose, cellulose and degradation of reed-extract (originated from leaf and stalk of *Phragmites australis*) were studied on each representing strain of an ARDRA group. More than half of the strains were able to grow on the medium contains reed extract as sole source of carbon. The isolated strains from all of the three sampling sites from November grew better on this medium than the strains isolated in July. 59% of the strains hydrolysed Tween 80, and it was more characteristic in case of strains isolated in November than in July. 77% of the strains were able to grow on the medium contained glycogen as sole source of carbon, but only a few strains were able to utilize glycogen by fermentation. Bacterial strains isolated from the inner lake and the reed covered area in November utilizes cellobiose better than strains isolated in July. Members of Gammaproteobacteria hydrolysed Tween 80 in higher extent. Actinobacteria grew mostly on the ”reed extract medium” and on the cellobiose medium. The studied novel bacterial taxa were relatives of *Verrucomicrobium spinosum* (94.45%), *Luteolibacter algae* (96.53%), *Perlucidibaca psicinae* (97.24%), *Pseudorhodobacter psychrotolerans* (96.16-96.97%), *Tabrizicola aquatica* (97.12-96.47%), *Rhabdobacter roseus* (95.59-96.66%), *Citreicella marina* (96.60%) and *Belliella baltica* (97.94%) based on their full 16S rRNA gene sequence. Some of these strains have photoheterotrophic relatives. Relatives of *Rhabdobacter*, *Perlucudibaca*, *Tabrizicola* and *Belliella* could hydrolyse Tween 80. Relatives of *Pseudorhodobacter*, *Tabrizicola* and *Citreicella* were able to grow on glycogen as sole source of carbon. Only two strains (relatives of *Tabrizicola* and *Belliella*) could grow on the cellobiose containing media.

SIGNIFICANCE OF VIRUS GENOME INVESTIGATIONS

MÁRIA TAKÁCS

National Public Health Institute, Budapest, Hungary

Attention to virus genome research has increased during the last decades. Remarkable progress has been achieved in this field thanks to the theoretical and technical developments. Results achieved by molecular methods complete and extend information obtained by the classic methods (serology, virus isolation, virus culturing). The lecture focuses on two fields of virus genome research. At the beginning of the molecular research era, when the PCR and the sequencing were already available, the source of a large nosocomial hepatitis B infection had to be identified. For this purpose we applied a molecular method based on the virus genome variability, which we have used frequently since then. We amplify the nucleic acid of the viruses derived from the blood of the patients, sequence the variable regions and using the representative genotype sequences and the previously determined dominant virus sequences from Hungary a phylogenetic tree is constructed. National Reference Laboratory for Hepatitis Viruses, as part of the National Epidemiological Center has been often asked to identify the source of hepatitis B and C virus infections occurred in different hospital wards. Besides of nosocomial infections, we clarified the origin of the first proved food-borne hepatitis A virus infection in Hungary with similar methods two years ago. The diagnosis of zoonotic viruses, including West Nile virus and tick-borne encephalitis virus (Flaviviridae) used to be based only on serological methods .We had no information which genetic variants caused the human flavivirus infections in Hungary, only indirect conclusion could be drawn based on the results achieved by veterinarians. Based on the recent recognition that after the infection flaviviruses may be

present in the urine for a long time we could detect and isolate West Nile virus RNA from human urine samples for the first time in Hungary. In addition, whole genome sequence determination has become also possible. Furthermore we have applied the methods for other flaviviruses, and now the National Reference Laboratory for Viral Zoonosis can detect tick-borne encephalitis virus circulating in Hungary, as well as Dengue virus and Zikavirus imported from tropical areas.

THE EFFECT OF TEXTURAL ATTRIBUTES ON THE APPLICABILITY OF GEL-BASED MICROCAPSULES AS MICRO DELIVERY SYSTEM FOR PROBIOTIC BACTERIA

LINH TA PHUONG, ERIKA BUJNA, SZILÁRD KUN, RÉKA JUHÁSZ

Szent István University, Budapest, Hungary

Probiotic bacteria are common ingredients used in several food products, such as yogurt or other dairy products, due to their health-promoting effects in humans. For this property to be efficacious, sufficiently high number of viable probiotic cells should reach the large intestine. Cell microencapsulation (micro delivery system) provides a chance for probiotics to keep their viability and beneficial effects by being protected against harmful and often lethal external environment. One of the key issues that affects the efficiency of microencapsulation and the viability of cells is the selection of the capsule material. In this study, a probiotic bacterium (*Lactobacillus casei* 01) was incorporated into Ca-alginate, κ-carrageenan-locust bean gum and gellan gum–xanthan gum microbeads. Moreover, the Ca-alginate based microgels were modified by combining with a variety of prebiotic components like lactulose, lactosucrose (LS40L and LS55L) and resistant starch, and by coating with polymers like chitosan and DEAE Sephadex A-50. The study focuses on the texture profile of these gel capsules and its relationship with encapsulation efficiency and viability of the bacteria exposed to simulated complex digestive (acidic and bile) conditions. The texture properties of the microgels were quantified by Brookfield LFRA Texture Analyzer. For obtaining springiness and mechanical strength related information both non-destructive and destructive texture measurements were carried out, respectively. Parameters such as hardness, area cycle, fracture force (brittleness) and adhesive force values were observed in both methods. Based on the statistical analysis of all these parameters, it was found that the resistant starch-alginate and the lactosucrose LS55L-alginate beads are the hardest whereas the gellan gum-xanthan gum and the chitosan coated alginate beads have the softest texture in overall. Pearson correlation analysis highlighted that there is no significant linearity between any of the texture related parameters and the encapsulation efficiency. According to the viability study, the hardest beads showed the most effectiveness at protecting *L. casei* 01 under simulated complex gastrointestinal conditions; however not the softest ones provided the least protection. Therefore, the texture profile of beads did not appear to have an obvious effect on the viability of enclosed probiotic bacteria under simulated complex gastrointestinal conditions.

DUPLICATION AND REDUNDANCY OF BRANCHED CHAIN AMINO ACID BIOSYNTHESIS GENES AND REGULATORS IN *ASPERGILLUS NIDULANS*

RICHARD B. TODD, CAMERON C. HUNTER, JOEL T. STEYER, PIERRE A. MIGEON, DAMIEN J. DOWNES

Department of Plant Pathology, Kansas State University, Manhattan, KS, US

The branched chain amino acids (BCAA) leucine, isoleucine, and valine are essential dietary amino acids in mammals. Fungi, however, can synthesize these three amino acids. For this reason, BCAA biosynthesis enzymes have been suggested as possible drug targets for treatment of infections by opportunistic fungal pathogens. Synthesis of the three BCAAs has been well characterized in the yeast *Saccharomyces cerevisiae*. However, recent work on the BCAA pathway enzymes dihydroxyacid dehydratase in *Aspergillus fumigatus* and α-isopropylmalate synthase *Aspergillus nidulans* has shown divergence with *S. cerevisiae* in the number of genes encoding functional enzymes for these steps. The final two steps of leucine biosynthesis are carried out by β-isopropylmalate dehydrogenase (β-IDH) and BCAA aminotransferase (BAT), but the genes encoding these enzymes have not yet been characterized in the Aspergilli. In *S. cerevisiae*, there is one β-IDH gene and two BAT genes. The BATs also catalyze the final step of isoleucine and valine production. Using protein sequence similarity we identified two β-IDH encoding genes in *A. nidulans*: *leuD* and *leuE*. We deleted these genes by gene replacement and showed that *leuD*Δ, but not *leuE*Δ, causes a leaky leucine auxotrophy. A *leuD*Δ *leuE*Δ double mutant is a strict leucine auxotroph and therefore both genes encode functional enzymes. Quantitative RT-PCR showed that leuE up-regulation compensates for loss of *leuD*. We have also identified six putative BAT encoding genes, *batA-F*, by protein sequence similarity to Bat1p and Bat2p from yeast. Deletion of these six genes separately does not confer BCAA auxotrophy. However, simultaneous deletion of the two most highly expressed BAT genes, *batA* and *batB*, is sufficient to confer BCAA auxotrophy, suggesting that the other BAT genes may have evolved new roles since gene duplication. Two of the other BAT genes lie in the aspercryptins secondary metabolism gene cluster and likely catalyse biosynthesis of unusual BCAAs that are components of aspercryptins. We have characterized the regulation of leucine biosynthesis pathway genes by the transcription factor LeuB.

The *leuB*Δ mutant is a leaky leucine auxotroph. We have identified a LeuB paralog, which we named LeuR. Deletion of *leuR* does not confer leucine auxotrophy, but the *leuB*Δ *leuR*Δ double mutant is a strict leucine auxotroph indicating that LeuR also regulates leucine biosynthesis.

HPV-16 E7 PHOSHORYLATION AS A SIGNATURE OF MALIGNANCY

VJEKOSLAV TOMAIĆ

Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia

Among 200 Human papillomavirus (HPV) types which infect human epithelial cells only a small number of these are associated with human cancers at various anatomical sites, and they are referred as the high-risk types. Of these, cervical cancer is the most important disease which affects women predominantly in developing countries, causing more than 600,000 cancers annually. The malignant development results from a joint action of the two main viral oncoproteins E6 and E7. They target various host proteins which are involved in the regulation of apoptosis, cell cycle control and cell polarity. E7 was shown to interact with many cellular proteins amongst which targeting of the retinoblastoma tumor suppressor (pRb) being the most relevant. Interestingly, it has been also shown that some of the other interactions were specifically modified by posttranslational modifications of E7. A good example of that is the phosphorylation of Ser31 and Ser32 by casein kinase II (CKII) which was demonstrated to play the pivotal role in modulating E7 interactions with cellular substrates. Older data have demonstrated that CKII phosphorylation plays a crucial role in E7s transformational capacity and that it is required for its interactions with TBP, while our more recent

studies indicate that the upregulation in the phosphorylation of a variant of E7 leads to an increased capacity of E7 to target pRb and p130 for proteasome mediated degradation. In addition, our recent NMR analyses of CKII phosphorylated E7 reveal critical changes in the local conformation of the protein, thereby beginning to explain how phosphorylation might modulate E7 function.

RESPONSE OF THP-1 MONOCYTES TO *CURVULARIA* STRAINS ISOLATED FROM HUMAN INFECTIONS

ESZTER JUDIT TÓTH[1, 2], ÉVA BOROS[3], ALEXANDRA HOFFMANN[1], CSABA VÁGVÖLGYI[1], ISTVÁN NAGY[3], TAMÁS PAPP[1, 2]

[1] Department of Microbiology, University of Szeged; [2]MTA-SZTE "Lendület" Fungal Pathogenicity Mechanisms Research Group; [3]Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Members of the genus *Curvularia* are melanin producing ascomycetes fungi. Although these fungi are primarily saprotrophic or plant pathogenic organisms, certain *Curvularia* species have been recovered from human infections known as phaeohyphomycoses. In healthy individuals, these mycoses can manifest as local infections like fungal keratitis, sinusitis, cutaneous lesions while they can form invasive infections with frequent involvement of the central nervous system in immunocompromised patients. Opportunistic fungal infections represent a continuously increasing problem because of the growing population with underlying conditions. In this study, strains of *C. lunata* and *C. spicifera* isolated from human eye infections and *C. hawaiiensis* from a systemic infection were used. Capability of the strains to induce cytokine and chemokine production and activation of THP-1 monocytes were compared. Relative transcription level of genes of cell signaling, adhesion and chemotactic molecules and their receptors was measured by real-time quantitative reverse transcription PCR in response to conidial and mycelial forms of the fungi. Production of pro- and anti-inflammatory cytokines was determined by ELISA after 3 and 24 hours of infection. We blocked the DHN melanin biosynthesis during cultivation to analyze the role of melanin in hampering monocyte response to conidia. Interaction of monocytes with conidial form of the fungus were analyzed with a FlowSight Imaging Flow Cytometer.

Supported by the "Lendület" Grant of the HAS (LP2016-8/2016) and the project GINOP-2.3.2-15-2016-00035.

FIRST STEPS IN MONITORING THE MICROBIAL COMMUNITY OF MUSHROOM COMPOST AND SEARCHING FOR POTENTIALLY MYCOTOXIN DEGRADING BACTERIA

FANNI TÓTH, BALÁZS VAJNA, KÁROLY MÁRIALIGETI

Department of Microbiology, Faculty of Science, ELTE Eötvös Loránd University, Budapest, Hungary

White button mushroom (*Agaricus bisporus*) is produced on a composted substrate in which process microorganisms have a substantial role. An important risk factor is that raw materials (wheat, rape and barley straw, sunflower husk, etc.) of the substrate are potentially contaminated with mycotoxins. It is not known at present what kind of effects mycotoxins or their degradation products have on button mushroom and weather mycotoxins accumulate in the fruiting bodies. Further question is whether the mushroom compost microorganisms are able to degrade or neutralize mycotoxins. Thus in the present work we started to study the microbial community of the mushroom compost. The aims

of this work were to optimize sample processing, to examine the homogeneity of the microbial communities at the different stages of mushroom production and the isolation of bacterial strains from the raw materials of the compost. Samples were collected from different stages of mushroom compost production. They were homogenized with grinding in liquid nitrogen, than community DNA was isolated by a commercial kit using different time interval bead mill homogenization. The quantity, quality of the obtained DNA was analyzed with spectrophotometer. Bacterial community composition was revealed by Terminal Restriction Fragment Length Polymorphism (T-RFLP) based on 16S rRNA gene, whereas fungal community was studied by Automated Ribosomal Intergenic Spacer Analysis (ARISA) based on nrDNA ITS-1 region. Bacterial strains were isolated from wheat straw using two different types of media. The first one was an extended spectrum, peptone containing medium (*Corynebacterium* Agar), the other one was more specific, starch casein medium. The strains were identified with 16S rRNA gene sequence analysis. Mainly members of Firmicutes phylum (*Bacillus* sp. and *Paenibacillus* sp.) were detected on the Corynebacterium Agar, while phyla Proteobacteria, Actinobacteria (*Massilia* sp. and *Rhodococcus* sp.) and *Paenibacillus* sp. were isolated from starch casein medium. In the future we want to isolate strains from other raw materials (sunflower husk, rape and barley straw) and from mushroom compost. Furthermore, we wish to compare the microbial community changes with the mycotoxin content during mushroom production, and detect weather the compost bacterial communities are able to degrade the different types of mycotoxins.

Supported by the National Competitiveness and Excellence Programmes (NVKP_16-1-2016-0035).

THE RELATIONSHIP BETWEEN ANTIBIOTIC CONSUMPTION AND RESISTANCE IN CASE OF GRAM-NEGATIVE BACTERIA: THE DOWNWARD SPIRAL FROM CEPHALOSPORIN CONSUMPTION TO COLISTIN RESISTANCE

HAJNALKA TÓTH, BENCE BALÁZS, JULIANNA MÓZES, GÁBOR KARDOS

Department of Medical Microbiology, University of Debrecen, Debrecen, Hungary

In our former study, we demonstrated an association between the carbapenem consumption and carbapenem resistance in case of Gram-negative bacteria at the University of Debrecen. The aim of this study was to investigate the driving forces behind the increased carbapenem consumption and the effect of the increasing carbapenem resistance on the colistin usage. The 3rd generation cephalosporin-, carbapenem- and colistin consumption were described by defined daily doses per 100 bed-days (DDD). Antibiotic resistance was represented by incidence density of infections caused by resistant bacteria per 1000 bed-days (ID). Target bacteria were *Escherichia coli*, *Klebsiella* spp., *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Time series were analysed using dynamic regression and vector autoregression models (VAR). The consumption of the 3rd generation cephalosporins (2,84→5,33 DDD) induced the increasing of the cephalosporin resistance after 3-6 months (0,44→1,25 IS). The effect of cephalosporin resistance on the carbapenem consumption occurred without delay (0,95→4,03 DDD twelve-month averages between 2005 and 2016), carbapenem resistance responded after 1-3 months (0,02→0,16 IS). Colistin consumption (0,00→1,56 DDD) increased in parallel with carbapenem resistance, while the colistin resistance appeared with 1-4 months delay (0,00→0,03 IS). Dynamic regression and VAR models generally indicated similar relationships between time series, but the estimated lags tended to be longer with VAR models. nvestigating the target bacteria separately, in case of *E. coli* we found significant connection between cephalosporin consumption and cephalosporin resistance as well as between cephalosporin resistance and carbapenem consumption, but none was found with *Klebsiella* spp. In

case of *P. aeruginosa* cephalosporin resistance provoked carbapenem consumption and carbapenem consumption provoked carbapenem resistance. In case of *A. baumannii*, the effect of cephalosporins was not analysed, other relationships were significant. Colistin resistance occurred primarily in *A. baumannii* and colistin consumption increased mainly due to carbapenem resistance of *A. baumannii*. In summary, increased antibiotic resistance provoked the overuse of carbapenems and later of colistin, forming a resistance spiral, which was followed up from cephalosporin consumption to colistin resistance. As colistin resistant bacteria severely limit the antibiotic choice and threatens with panresistance, protocols relying heavily on carbapenems should be replaced by more diverse antibiotic usage to interrupt the resistance spiral and to preserve the efficacy of the last-resort drugs.

H. Tóth was supported by the New National Excellence Program. G. Kardos was supported by János Bolyai Resarch Scholarship of the Hungarian Academy of Sciences.

CORE GENOME MLST ANALYSIS OF *LISTERIA MONOCYTOGENES*: POSSIBLE SEVERAL YEAR-LONG OUTBREAK IN HUNGARY?

KINGA TÓTH[1], JUDIT PÁSZTI[2], IVELINA DAMJANOVA[2], ÁGNES FEHÉR[3], KATALIN KRISZTALOVICS[3], ÁKOS TÓTH[4]

[1]Department of Microbiology, Faculty of Science, ELTE Eötvös Loránd University; [2]Department of Phage and Molecular Typing, National Public Health Institute; [3]Department of Hospital Hygiene and Communicable Disease Control, Ministry of Human Capacities; [4]Department of Bacteriology I, National Public Health Institute, Budapest, Hungary

Listeria monocytogenes (LM), one of the important foodborne pathogens, causes mainly gastroenteritis, but severe diseases also (invasive listeriosis) particularly in the immunocompromised and elderly populations as well as in pregnant women, who may give birth to stillborn or severely infected infants. The pathogen is able to inflict extended international outbreaks existing for years. Between 2010 and 2015 the number of invasive listeriosis cases reported in Hungary increased and 38% of them (33/86) belonged to serotype IVb and have identical (Pulsed-Field Gel Electrophoresis) PFGE pattern. In order to figure out an possible spatio-temporal outbreak situation a set of 17 strains isolated from clinical samples between 2010-2015 and showing LMAPA-H020/LMAPA-H011 PFGE pattern was considered for core genome MLST (cgMLST) analysis. Whole genome sequencing (WGS) of LM isolates was performed using SureSelect QXT Reagent Kit (Agilent Genomics) and paired end sequencing (2 x2 50 basepairs) on a MiSeq platform (Illumina Inc.). Samples were sequenced to aim for minimum coverage of 60 fold. Raw reads were quality trimmed and de-novo assembled using Velvet assembler version 1.1.04 integrated in Ridom SeqSphere+. A core genome (cg)MLST scheme comprising 1701 target genes was used for WGS data interpretation using SeqSphere+. Cluster type (CT) for LM was defined as isolates attributed to the same clone with a $\leq$10 differing alleles (CT threshold) within a group. The epidemiological data for all investigated isolates were collected and possible epidemiological links were surveyed. The 17 isolates originated form eight counties of Hungary could be distinguished by cgMLST into 5 CTs. CT1285 comprises 11 isolates with distinct spatio-temporal origin displaying a maximum of 10 allelic differences. Three strains could be assigned to CT3427, where two of them originated from two different counties and isolated 5-years apart. Furthermore, one isolate each belonged to CT3333, CT4213 and CT4212. The isolates within the four minor cluster types differed at least by 12 alleles from the main cluster (CT1285). The data survey did not reveal any epidemiological link among patients. The only exception was for two isolates (CT3427) from a mother and her baby where lack of allelic differences between isolates supported the vertical transmission event.

Whole genome based cgMLST typing showed higher discrimination power than the PFGE. We identified 5 different CTs which shared identical PFGE pattern. Despite the lack of epidemiological link between isolates in CT1285 an unrecognized epidemiological event and common source of isolates could be suspected. In the light of that this suspected association could be further supported by investigation of both clinical and foodborne samples using cgMLST.

REGULATION OF THE BIOSYNTHESIS OF SECONDARY METABOLISM IN *ASPERGILLUS NIGER*

ADRIAN TSANG

Centre for Structural and Functional Genomics, Concordia University, 7141 Sherbrooke Street West, Montreal, Quebec H4A2P1, Canada

Secondary metabolites (SM), or natural products, are products of reaction pathways which are generally not included in the standard metabolic networks. They comprise mostly of bioactive, low molecular weight compounds. Filamentous fungi are known to produce a diverse range of SMs including the antibiotic penicillin, the cholesterol-lowering drug statin, the immunosuppressant cyclosporine, and the toxin/carcinogen aflatoxin. Despite the chemical diversity of SMs identified in fungi, they are made by enzyme pathways whose products can be chemically classified as: polyketides, nonribosomal peptides, terpenes, and indole alkaloids. The backbones of polyketides and nonribosomal peptides are made by the multi-domain enzymes polyketide synthases and nonribosomal peptide synthetases, respectively. The backbones of terpenes and indole alkaloids are products of terpene synthases and dimethylallyl tryptophan synthetases, respectively. The products of these backbone enzymes are typically modified further by P450 monooxygenases, oxidases, reductases, and methyltransferases to expand the diversity of SM. Detailed manual curation showed that the Aspergillus niger genome harbours 81 genes that are predicted to encode backbone SM biosynthetic enzymes. As in other microorganisms, accessory enzymes involved in the modification of the SM are often clustered with the backbone biosynthetic enzymes. Located within or in the flanking regions of these clusters are also genes encoding putative transcription regulators and membrane transporters. These putative transcription regulators are hypothesized to regulate the activity of the genes of the cluster while the transporters are presumed to be involved in the export of the SM. We have overexpressed ~60 genes encoding predicted transcription regulators that are located within or adjacent to the SM clusters in the hope of awakening the normally silent SM cluster genes, thereby resulting in the over-production of novel compounds. The overexpression of transcription regulator genes shows different types of results. In most cases, they do not result in the production of new SM compounds. In a few instances, SMs are overproduced. In two instances, the overexpression of a single transcription regulator results in the overproduction of multiple compounds. The implications of these results will be discussed.

DETERMINATION OF PLANT-GROWTH PROMOTING COMPOUND IN ENDOPHYTES, ISOLATED FROM *SOPHORA FLAVESCENS*

ADIYADOLGOR TURBAT[1], DÁVID RAKK[1], ENKH-AMGALAN JIGJID[2], CSABA VÁGVÖLGYI[1], ANDRÁS SZEKERES[1]

[1]Department of Microbiology, University of Szeged, Szeged, Hungary; [2]Institute of General and Experimental Biology, Mongolian Academy of Science, Ulaanbaatar, Mongolia

Genetic and external environmental factors, nutrients and chemical hormones play important role in the growth and development of a plant. Most of the time, hormones synthesized in one part of the plant, and transported to another location. In special cases these hormones could be also produced by symbiotic and pathogenic microorganisms. In these interactions fungi could take a part as well, in which the growth and development of the plants. Associations like that can result changes in the development in plant growth, such as increased biomass production, better health and better resistance against pathogenic organisms. It has been also reported that the fungal endophytes are able to produce indole-acetic acid. It is the most abundant naturally occurring auxin, forming a class of phytohormones implicated in the regulation of plant growth and development. In Mongolia, there are several plants which has never been examined in the case of endophytic content but in the country there are 845 medicinal and 150 endemic plants. Endophytic fungi in these plants can be a potential new source of new bioactive products. In our work, we isolated 8 endophytic fungi from the medicinal plant *Sophora flavescens*, Ait sampled from Mongolia, on the territory of Dornod province. The taxonomical characterization of the strains were based on molecular techniques with the PCR amplification and sequencing of ITS region of the genomic DNA. To investigate the plant growth promoting activity of these strains, indol-acetic acid, siderophore contents and phosphate solubilisation ability were tested by agar-diffusion methods. Furthermore, HPLC-UV-MS technique was used for determination of indole-acetic acid in the crude extracts.
Based on our results four fungal strains were able to produce indole-acetic acid and five strains showed siderophore-, and two strains phosphate solubilisation activity.
Supported by the project GINOP-2.3.2-15-2016-00012. AS was supported through the New National Excellence Program of the Ministry of Human Capacities (ÚNKP-16-4).

FIRST REPORT OF COMPUTATIONAL STRUCTURE ELUCIDATION OF TRIPLEURIN XIIC PRODUCED BY STRAIN *TRICHODERMA PLEUROTI* TPHU1

CHETNA TYAGI[1], TAMÁS MARIK[1], ANDRÁS SZEKERES[1], CSABA VÁGVÖLGYI[1], LÁSZLÓ KREDICS[1], FERENC ÖTVÖS[2]

[1]Department of Microbiology, Faculty of Science and Informatics, University of Szeged; [2]Institute of Biochemistry, Biological Research Centre, Szeged, Hungary

In our working group, new peptaibols named tripleurins were recently identified from a *Trichoderma pleuroti* strain known to cause green mould disease of oyster mushroom. In order to understand their mode of action, the knowledge of their three-dimensional structure is a pre-requisite. Peptaibols are a special class of fungal peptides with an acetylated N-terminal and a C-terminal 1,2-amino alcohol. Along with these, non-standard amino acid residues like D-isovaline (Div) and the most characterized residue aminoisobutyric acid (Aib) also constitute its primary structure. These non-standard residues were parametrized using RESP charges and their force field libraries were created to be used for molecular dynamics-based simulations. Tripleurin XIIc is an 18-mer peptide with five ambiguous positions. Div 6, Div 8 and Div 12, based on previous comparisons, are conserved and might have structural implications. Another simulation was set up after substituting these positions with valine (Val). The two 100 ns simulations were set up to compare structural implication of Val/Div substitution at respective positions and its effect on overall conformation. The first sequence variant,

named T1, shows a left-handed helical conformation at the N-terminal and a 3-10 helix formation at the C-terminal. The Div-Aib-Pro bond at the center unwinds the helix in a more linear conformation. Div 6, Div 8 and Div 12 show a coil secondary structure throughout the simulation. The second variant T2, without any Div, conforms into a strict right-handed alpha helix at the N-terminal while a 3-10 helix is at the C-terminal. The role of Div is probably to prevent the peptide to take a strict alpha-helix conformation while Val promotes a compact helix stability. Their respective positions in the sequence ensure the formation of a helical peptide which is not compactly packed as an alpha-helix. Another important observation is the presence of a glutamine residue at position 7 between Div 6 and Div 8, and at position 17. Glutamine (Gln) side-chains were observed to form inter-molecular interactions with other monomer units during trans-membrane pore formation. The overall conformation of these peptaibols should allow these side-chains to face outwards for easy interaction. The coil-like helical conformation of T1 is more likely to provide easy access to Gln7 than the tightly packed alpha helix of T2. The overall structure is a bent helix with a highly fluctuating C-terminal. The distance between the two terminals keeps fluctuating throughout the simulation for T1, while for T2 it is stable till 70,000 steps and then increases by two-folds till the end. This is a preliminary report on three-dimensional structure of tripleurin and the implication of Val/Div substitutions.

Supported by grant NKFI K-105972 (National Research, Development and Innovation Office, Hungary).

COMPARISON OF DIVERSITY AND ACTIVITY OF SOIL BACTERIA INHABITING MAIZE FIELD AND GRASSLAND WITH SIMILAR LOCATION AND SOIL PROPERTIES – A CASE STUDY

GERGELY UJVÁRI[1], JÚLIA MARGIT ASZALÓS[1], ANDREA K. BORSODI[1], TIBOR SZILI-KOVÁCS[2], MÁRTON MUCSI[2], GERGELY KRETT[2], ZOLTÁN SZALAI[3], KÁROLY MÁRIALIGETI[1]

[1]Department of Microbiology, Faculty of Science, ELTE Eötvös Loránd University; [2]Institute for Soil Sciences and Agricultural Chemistry, Centre for Agricultural Research, Hungarian Academy of Sciences; [4]Department of Environmental and Landscape Geography, Faculty of Science, ELTE Eötvös Loránd University, Budapest, Hungary

Soil - as environment - is a fundamental part of agricultural-ecosystems where microorganisms have essential roles in maintaining the processes of nutrient cycling and organic matter degradation. However, microbes may also interact with different plants in several ways. The revealing of the composition and function of soil microbial communities is of great importance to make tillage more effective with maintenance of soil fertility. The aim of this case study was to get insight into the genetic diversity and metabolic activity of bacterial communities living in different horizons of cultivated and uncultivated soils. Samples were collected from two distinct, non-fertilized maize fields and a fallow area as local control in Martonvásár, furthermore from a natural loessy grassland as absolute control, located in a Natura2000 protected area near Bicske (18 km away from the field experiment) in spring 2017. Soil samples were taken from A, AC and C horizons; soil depths varied because of their heterogeneity. 16S rRNA gene based diversity was studied by denaturing gradient gel electrophoresis (DGGE) and microbial activity using MicroResp method. DGGE resulted in variable patterns in all samples, and showed remarkable differences between samples according to depth and origin. Catabolic activities of microbial communities considering almost all substrates were lower from the maize fields than the control fallow and grassland areas.

The support provided from the National Research, Development and Innovation Fund of Hungary, financed under the GINOP-2.3.2-15-2016-00056 funding scheme.

CHARACTERIZATION OF NOVEL LYTIC BACTERIOPHAGES AGAINST *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES

SARSHAD KODERI VALAPPIL[1], ZOLTÁN DEIM[1], GABRIELLA TERHES[2], EDIT URBÁN[2], GYÖRGY SCHNEIDER[3], TAMÁS KOVÁCS[4], GÁBOR RÁKHELY[1, 5]

[1]Department of Biotechnology; [2]Institute of Clinical Microbiology and Diagnostics, University of Szeged, Szeged; [3]Department of Medical Microbiology and Immunology, University of Pécs; [4]Department of Biotechnology, Nanophagetherapy Center, Enviroinvest Corp. Pécs; [5]Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Bacteriophages naturally and widely occur in the environment and they are able to infect and kill bacteria. The most common mode of action of phages against bacteria is bacteriolysis, which occurs at the end of the phage lytic cycle. Lytic phages encode gene products which disrupt or destroy the bacterial cell wall in two different ways according to their bacteriolytic mechanism: the virolysin-holin system to hydrolyse bacterial cell wall or the single lytic factor to compromise the strength of the cell wall. Within the infected bacteria, phage DNA is self-replicated and then incorporated into a bacteria-infectious particles that are manufactured from chemical components stolen from the bacterial host. The production and further release of phage particles allows subsequent phage infection of additional bacteria in an exponential pattern, showing a faster killing effect than other therapeutic alternatives. *P. aeruginosa* is a Gram-negative opportunistic pathogenic bacterium occuring in diverse environments such as water, soil, and plants. The strains have multiple targets and are able to infect different organisms, including plants, animals, and humans. This pathogenic bacterium is a common causing agent of health care-associated diseases, including pneumonia, bloodstream infections, urinary tract infections, and surgical site infections. It is especially hazardous for patients with reduced immunological responses. Most of the infections caused by *P. aeruginosa* are strikingly difficult to treat using conventional antibiotic therapies due to the high intrinsic resistance of the strains to a wide range of antibiotics. In our study, we used various clinical samples for isolation of *Pseudomonas aeruginosa* strains and their lytic phages.
The serotypes of the strains were determined and the infectivity of the phages were tested on the various hosts. The morphology of the phages was analyzed by electron microscopy and the genomes of the phages have been sequenced and compared to eachother and the sequences available in the databanks. *P. aeruginosa* tends to form biofilm. The effectivity of the individual phages and phage coctails in disruption of biofilms is also presented.

SYNTHESIS AND FUNCTIONAL MAPPING OF THE *NEOSARTORYA FISCHERI* ANTI-YEAST PROTEIN (NFAP2)

GYÖRGYI VÁRADI[1], LILIÁNA TÓTH[2], KORNÉL NEDECZKY[2], ÁKOS VENDRINSZKY[1], ATTILA BORICS[3], ZOLTÁN KELE[1], GÁBOR K. TÓTH[1], CSABA VÁGVÖLGYI[2], FLORENTINE MARX[4], LÁSZLÓ GALGÓCZY[2]

[1]Department of Medical Chemistry, Faculty of Medicine; [2]Department of Microbiology, Faculty of Science and Informatics, University of Szeged; [3] Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary; [4]Division of Molecular Biology, Biocenter, Medical University of Innsbruck, Innsbruck, Austria

Due to the increasing number of antifungal drug-resistant *Candida* isolates, which can cause serious life-threatening infections there is an urgent need to develop new antifungal drugs and therapeutic strategies. The *Neosartorya fischeri* antifungal protein 2 (NFAP2) is a novel, extracellular, small,

cysteine-rich and cationic antifungal protein, which effectively inhibits the growth of different *Candida* spp. as it possibly disrupts the fungal membrane. One of the limiting factors of its future application, however, is the low-yield production by the native, non-generally recognized as safe organism *N. fischeri* NRRL181. In this study, solid-phase peptide synthesis and native chemical ligation were applied to produce NFAP2. This synthesis strategy allowed the functional mapping of NFAP2 to identify amino acid motifs, which might play a crucial role in anti-yeast activity. The average yield of synthetic NFAP2 was remarkably higher than in the native producer. Mass spectrometry measurements, reversed-phase high performance liquid chromatography analyses, and electronic circular dichroism spectroscopy verified the correct folding of synthetic NFAP2. The detected minimal inhibitory concentration values of synthetic NFAP2 against clinically relevant *Candida* spp. were comparable to the native protein in broth microdilution assays. Functional mapping revealed that the mid-N-terminal part of the protein influences the antifungal activity that rather depends on the net charge and hydrophilicity of this region than on the primary structure. The C-terminal part of NFAP2 contains the consensus γ-core motif [GXC]-[X3-9]-[C], which is important for the activity of antimicrobial peptides from animals, humans, and antifungal plant defensins. Interestingly, the peptide fragment containing the γ-core motif of synthetic NFAP2 was inactive against yeasts. Therefore, we conclude that this specific motif alone has no anti-yeast function, but presumably needs the structural-functional support from other parts of NFAP2.

Considering the structural similarity of native and synthetic NFAP2, the applied native chemical ligation method is suitable for the bulk production of correctly folded NFAP2, which will serve for further detailed studies on its structure-function relationships.

L.G. is financed from the Postdoctoral Excellence Programme (PD 120808) and the bilateral Austrian-Hungarian Joint Research Projects (ANN 122833) of the Hungarian National Research, Development and Innovation Office (NKFI Office). This work was supported from the Austrian Science Fund (FWF 25894-B20 and I1644-B20 to F.M.), projects GINOP-2.3.2-15-2016-00035 (to L.T.) and GINOP-2.3.2-15-2016-00014 (to G.K.T.). The work of A.B. was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

THE EVOLUTION OF AGARICOMYCETES: TRENDS IN DIVERSIFICATION RATES OF LINEAGES AND EVOLUTIONARY FAVOURABLE TRAITS OF FRUITING BODIES

TORDA VARGA[1], KRISZTINA KRIZSÁN[1], JÁNOS GERGŐ SZARKÁNDI[2], BÁLINT DIMA[3, 4], BRIGITTA KISS[1], CSABA VÁGVÖLGYI[2], TAMÁS PAPP[2], LÁSZLÓ G. NAGY[1]

[1]Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Science; [2]Department of Microbiology, University of Szeged, Szeged, Hungary; [3]Department of Plant Anatomy, Faculty of Science, ELTE Eötvös Loránd University, Budapest, Hungary; [4]Plant Biology, Department of Biosciences, University of Helsinki, Helsinki, Finland

A central question of biology is that what kind of evolutionary processes have led to the present pattern of species richness. The evolutionary history of lineages can be shaped by different mechanisms such as occurrence of key innovations or mass extinction events. Here we examine the tempo of the evolution (net diversification rate) of Agaricomycetes lineages and detect diversification rate shifts through the history of this class. Furthermore, we estimate the time and number of mass extinction events. To interpret the evolutionary history of clades we examined character evolution and estimated character dependent diversification rates using three character coding sets related to enclosed fruiting body development, hymenophore protection from above and increased spore bearing structures. We assembled the biggest multilocus dataset of the Agaricomycetes to date,

comprising 5284 species with three loci (4835 nrLSU; 1253 RPB2 and 721 ef1-a sequences), including 1386 newly sequenced taxa. We inferred 245 maximum likelihood phylogenies for a 5284-taxon dataset using a phylogenomic dataset of 103 species as a backbone constraint. Using eight fossil calibration points 20 chronograms were produced. Applying BAMM (Bayesian Analysis of Macroevolutionary Mixtures) model we explored the speciation and extinction rates among lineages through time and detected core diversification rate shifts. To evaluate the time and the number of mass extinction events CoMET (CPP on Mass-Extinction Times) model was used. Based on the literature, we assigned character states to the species according to the three coring stets. We analyzed the transition probabilities between character states using maximum likelihood and Bayesian comparative methods (BayesTraits). Furthermore, applying character dependent diversification models (BiSSE or MuSSE) we sought connection between the tempo of the evolution and character states. The detailed results of comparative phylogenetic analyses of Agaricomycetes will first be discussed here and shed light on the evolution of basidiomycete fruiting bodies.

INVESTIGATION OF THE ANTIMICROBIAL EFFICACY OF VARIOUS ANTISEPTIC AGENTS APPLIED IN PERI-IMPLANTITIS CLINICAL THERAPY ON THE *STREPTOCOCCUS SALIVARIUS* IN VITRO MODEL

ANNAMÁRIA VENKEI[1], KRISZTINA UNGVÁRI[2], KINGA TURZÓ[2], EDIT URBÁN[1]

[1]Institute of Clinical Microbiology, Faculty of Medicine; [2]Department of Oral Biology and Experimental Dental Research, Faculty of Dentistry, University of Szeged, Szeged, Hungary

Bacterial colonization and biofilm formation on the implant surface are the major etiological factors of peri-implant infections which can lead to bone resorption and loss of dental implant. Therefore, the reduction of biofilm is an essential step for the successful therapy of implant-related infections. Several antiseptic agents have been used, however further in vitro experiments are needed to help the dentists to chose the effective therapies to eliminate the biofilm. The aim of the present study was to investigate the antibacterial efficacy of three different antiseptics, which are used in clinical practice on *Streptococcus salivarius* in vitro model. Commercially pure (CP4) sand blasted, acid etched titanium sample discs (Denti® System Ltd., Hungary) and Ti discs with polished surface (Denti® System Ltd., Hungary) were used. These disks were incubated with a culture of *S. salivarius*, a first colonizer in titanium dental implant associated infections, for 48 hours than the bacterial biofilm was treated with three different antiseptics: clorhexidine (Curasept ADS 220, 0.2%, Switzerland), povidone-iodine (Betadine, 10 %, Switzerland) and chlorine dioxid (Solumium dental, 0.12%, Hungary) for 5 minutes than washed with ultrapure water. Control discs were treated with ultrapure water. The clinical isolate of *S. salivarius* was identified with MALDI-TOF (matrix assisted laser desorption ionisation- time of flight) mass spectrometer. The surfaces of the discs were analyzed using SEM (scanning electron microscopy) and AFM (atomic- force microscopy). The antibacterial effect of the antiseptics in each group was represented by the bacterial survival ratio after incubation, determined by MTT (dimethylthiazolyl-diphenyltetrazolium bromide) assay and CFU (colony-forming unit) method. Our results demonstrated that more bacterial cells attached to the surface of the control polished titanium discs than to the control sand blasted and acid etched titanium surfaces. According to MTT results the povidone- iodine and the chlorine dioxid were the two most effective antiseptics against bacterial biofilm on both type of surfaces.

We observed the least living bacterial cells on the surfaces of sand blasted and acid etched samples after the 5 minutes povidone -iodine treating. Our MTT assay results were confirmed by the CFU counting method. According to our results the povidone- iodine showed the most effective

antibacterial effect on the surfaces of sand blasted and acid etched sample discs. Therefore, we can conclude that the sand blasted and acid etched titanium implant and the using of povidone- iodine can be an effective choice in the therapy of peri-implant infections. Connecting to current results we plan to test the antifungal effect of these antiseptic agents against oral *Candida* species.

STUDY OF THE HUMAN *BUD*31 GENE AND ITS COUNTERPARTS

ILDIKÓ VIG, LAJOS ÁCS-SZABÓ, LÁSZLÓ ATTILA PAPP, MATTHIAS SIPICZKI, IDA MIKLÓS

Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen, Hungary

Human *BUD31* gene is involved in mRNA splicing via spliceosome, affecting many cell processes. According to our knowledge, *BUD31* gene is a positive regulator of the androgen receptor activity and transcription of many genes via promoter of RNA polymerase II. Therefore it is a potential target of cancer research, such as androgen receptor mediated prostate cancer or MYC-controlled cancer. Since the exact function of this gene is not known in detail, its study is advisable. In the present work, we wanted to study functional conservatism of the human *BUD31* and its counterpart genes. To find its counterpart genes bioinformatic analyses were carried out. They revealed that *Schizosaccharomyces pombe cwf14* and *Candida albicans BUD31* gene are its orthologues. Clustal Omega and domain analysis showed conserved amino acids and regions in the homologous proteins. To investigate functional homology, the human *BUD31* gene, the *C. albicans* and the *S. pombe* genes were amplified and cloned into a *S. pombe* specific expression vector. Transformation of the DNA constructs into *Δcwf14 S. pombe* mutant strain and investigation of the transformants are underway.

PREVALENCE OF LEPTOSPIROSIS, LYMPHOCYTIC CHORIOMENINGITIS AND HANTAVIRUS INFECTIONS IN ENDEMIC REGIONS OF THE CROATIAN MAINLAND, 2016-2017

TATJANA VILIBIC-CAVLEK[1, 2], ZDENKA PERSIC[1], VLADIMIR STEVANOVIC[3], TENA ORESKI[4], BRANKO KOLARIC[5], MISA KORVA[6], BOZANA MIKLAUSIC[7], IRENA TABAIN[1], LJUBO BARBIC[3], ANDREA BABIC-ERCEG[1], BERNARD KAIC[1], VLADIMIR SAVIC[8], MARIO SVIBEN[1], SUNCANICA LJUBIN-STERNAK[5], TATJANA AVSIC-ZUPANC[6]

[1]Croatian Institute of Public Health; [2]School of Medicine, University of Zagreb; [3]Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia; [4]Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; [5]Andrija Stampar Teaching Institute of Public Health, Zagreb, Croatia; [6]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; [7]General Hospital "Dr Josip Bencevic", Slavonski Brod, Croatia; [8]Croatian Veterinary Institute, Zagreb, Croatia

In the last decades, the number of zoonotic diseases has increased in many parts of the world. Leptospirosis and hantavirus infections are the most important rodent-borne zoonoses. Lymphocytic choriomeningitis is a neglected zoonosis with unknown prevalence. We analyzed the frequency of rodent-borne infections in endemic regions of continental Croatia. From January 2016 to July 2017, a total of 165 serum samples collected from febrile patients were tested for the presence of leptospira, lymphocytic choriomeningitis and hantavirus antibodies. All of them reported contact with rodents through occupational exposure (forest and agriculture workers) or reported frequent visiting forest areas, rodent infestation around house/resting house as well as cleaning rodent infested areas.

Antibodies to *Leptospira* spp. were confirmed using a microscopic agglutination test. Antibodies to lymphocytic choriomeningitis virus (LCMV) and hantaviruses: Hantaan (HTNV), Puumala (PUUV), Dobrava (DOBV), Seoul (SEOV) and Saaremaa virus (SAAV) were detected using an indirect immunofluorescence assay. In the tested group, there were 132/78.8% males and 33/21.2% females. Leptospirosis was confirmed in 6/3.6% (95%CI=1.7-8.5) patients and hantavirus infection in 76/46.1% (95%CI=38.9-54.6) patients. In one patient, dual leptospira and hantavirus infection was detected. Patients were 16-87 years of age and predominantly male (68/80.9%). Patients from rural areas were infected more common than patients from urban areas (53/63.8% vs. 30/36.2%). Although cases occurred throughout the year, the majority were reported from May to July (66/79.5%). Detected leptospira serovars were Patoc, Sejroe, Gryphotyphosa and Bratislava. PUUV was the most common hantavirus confirmed in 70/90.1% patients and DOBV in 7/9.9% patients. Acute LCMV infections were not detected during the tested period, however, in 8/4.8% (95%CI=2.1-9.3) patients LCMV IgG antibodies of low titers were detected indicating previous exposure to virus. In addition, four patients showed hantavirus IgG (two PUUV and two DOBV) antibodies. Our results indicate that continental Croatian regions are still endemic for rodent-borne diseases. Due to occupational exposure, males were infected more common than females. Cases showed seasonal distribution.

BIOINFORMATIC ANALYSIS OF *SALMONELLA ENTERICA* SUBSP. *ENTERICA* SEROVAR INFANTIS STRAINS ISOLATED FROM HUMANS AND BROILERS IN HUNGARY

TÍMEA WILK[1], AMA SZMOLKA[2], MÓNI SZABÓ[1], JÁNOS KISS[1], BÉLA NAGY[2], FERENC OLASZ[1]

[1]Agricultural Biotechnology Institute, National Agricultural Research and Innovation Centre, Gödöllő; [2]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

Salmonella enterica subsp. *enterica* serovar Infantis is an emerging serovar in poultry and humans in several countries in and outside Europe. It is most prevalent *Salmonella* serovar in broiler flocks in Hungary and certain multiresistance clones of *S.* Infantis are endemic in several countries. As a consequence both the number of analyzed isolates and the number of whole genome sequences of genomes has enormously increased. Thus we have sequenced 10 genomes in the interest of examination the variability of the whole genomes in different aspects. Our isolates show a significant tendency in time, representing two peak incidents. Our human and broiler isolates represented two peak incidents. The first peak incidence in Hungary (1980-1994) was represented by two pansensitive strains SI15023h and SI220h with origin from humans (GenBank MRUU00000000 and MRUV00000000) [1] and by the broiler strain SI59/94 (GenBank JRXB00000000) [2]. An unique chicken isolate 1326/28 from the United Kingdom from the 1980s (GenBank LN649235) was also analysed in comparison to the Hungarian strains. Representing the second, ongoing peak incidence in Hungary (2004-2016) we also sequenced broiler strains SI54/04 from 2004 (GenBank JRXC00000000) [3], SI3337/12 from 2012 (GenBank MIJS00000000), SI757/13 and SI786/13 from 2013 (GenBank MIJT00000000 and MIJR00000000) [4], and two multiresistant isolates SI240/16 and SI1070/16 from 2016 (GenBank MRUW00000000 and MRUX00000000). This second group of strains represented the multiresistant clone B endemic in Hungary. In this work we present a bioinformatic analysis of these genomes which will provide new insight into the genome evolution and recent spread of *S.* Infantis in poultry. To determine genetic relationships a phylogeny analysis of the above genomes was carried out. In order to have a broader perspective, and to be able to represent some associations (e.g. time and/or geographical), we have selected and analyzed further *S.* Infantis

genomes from NCBI database. Furthermore, we have chosen the *Salmonella enterica* subsp. *enterica* serovar Typhimurium LT2 strain (AE006468.2) as a reference genome for a baseline *Salmonella enterica* sequence and we defined its genomic islands. We performed a comparison of our genomic data to find out the differences relative to some important genetic loci. We focused on the analyses of the SPI1,2,3,5 islands which play a determining role in pathogenicity. We have also carried out a core genome analysis to find out the core and unique gene set of the genomes to pinpoint the most likely important steps in the evolution of *S.* Infantis strains. We expect that this sequence comparison should improve our understanding of the genome evolution and spread of *S.* Infantis in poultry.

[1] Wilk et al. (2017). DOI: 10.1128/genomeA.01735-16
[2] Olasz et al. (2015). DOI: 10.1128/genomeA.01468-14
[3] Szmolka et al. (2016). DOI: 10.1016/j.fm.2017.03.011
[4] Wilk et al. (2016). DOI: 10.1128/genomeA.01400-16

THE CORE MICROBIAL POPULATIONS AND CO-OCCURRENCE PATTERNS OF MICROALGA-FED BIOGAS DIGESTERS

ROLAND WIRTH[1], TAMÁS BÖJTI[1], GERGELY LAKATOS[2], GERGELY MARÓTI[2], ZOLTÁN BAGI[1], GÁBOR RÁKHELY[1, 3], KORNÉL L. KOVÁCS[1, 3, 4]

[1]Department of Biotechnology, University of Szeged; [2]Institute of Biochemistry; [3]Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences; [4]Department of Oral Biology and Experimental Dental Research, University of Szeged, Szeged, Hungary

Alternative bioenergy production is one of the hot topics in biotechnology. Microalgae based bioenergy production is a promising route with regard to the wide variety of algal species and inexpensive cultivation conditions. For many years the possibilities of using microalgae for biohydrogen, biodiesel and biogas production have been studied. Currently biogas generation from microalgal biomass appears the most sustainable because in this process the entire biomass is degraded by a complex microbial community and non-sterile growth conditions can be applied. The biogas from the microalgal biomass digestion is higher in CH_4 compared to the commonly used maize silage although the volumetric quantity is typically lower, since microalgae have low carbon to nitrogen (C/N) ratio. Co-digestion with higher C/N ratio substrates may solve this problem and makes the process stable as an additional valuable feature. Therefore, understanding core prokaryotic communities, their co-occurrence patterns, and their relationships to environmental factors is important to manage anaerobic digestion systems effectively. In this study Chlorella vulgaris microalgal dry biomass was tested as substrate. The laboratory scale CSTR biogas reactors were fed with microalgae, maize silage and 50-50% mixture of maize silage, and maize silage as control. Mesophilic fermentation temperature was maintained at 37^0C, the pH and redox potential in the reactors and volumetric gas production were continuously monitored. Other fermentation parameters like volatile organic acids, ammonium ion concentration, gas composition were measured regularly. The composition of the microbial community was established at frequent intervals through metagenomics analysis of datasets obtained by next generation DNA sequencing. Using powerful bioinformatics tools (DIAMOND, MEGAN, STAMP) microbial communities were identified in the CSTR reactors at high resolution. The community structures varied significantly depending on the applied substrates and were affected by environmental factors, such as ammonium and volatile fatty acids. Although, the communities were remarkably different, core prokaryotic communities could be established, which were independent of these fermentation parameters. Establishment of stable core

microbial communities in biogas digesters enhances the resistance against environmental stress factors and maintains digestion sustainability in the anaerobic digestion process.

Project no. PD 121085 has been implemented with the support provided from the National Research, Development and Innovation Fund (NRDIF) of Hungary, financed under the PD16 funding scheme. This study has been also supported by the NRDIF projects GINOP-2.2.1-15-2017-00065, GINOP-2.2.1-15-2017-00081. Z.B. is the Principal Investigator of the NRDIF-OTKA project FK 123902.

CARDIOVASCULAR RISK AND HIV INFECTION

ERZSÉBET IRINGÓ ZAHARIA KÉZDI[1], ANDREA FODOR[2], ZSUZSANNA SUCIU[3], ELŐD NAGY[4], CRISTINA GÎRBOVAN[5], ÁKOS VINCE ANDREJKOVITS[1]

[1]1st Infectious Disease Clinic, Mures County, Targu Mures; [2]Department of Laboratory Medicine, University of Medicine and Pharmacy Tîrgu-Mureş; [3]Center of Advanced Research in Multimodality Cardiac Imaging, Cardio Med Medical Center, Tîrgu Mureş; [4]Department of Pharmaceutical Biochemistry; [5]Department of Infectious Diseases, University of Medicine and Pharmacy Tîrgu-Mureş, Tîrgu Mureş, Romania

According to the projections of global mortality and burden of disease from 2002 to 2030 (by Mathers CD, Loncar D) the World Health Organization predicts that HIV/AIDS and ischemic heart disease will be in the top 3 causes for both global mortality and global disability-adjusted-life in the year 2030. The cardiovascular diseases (CVD) in HIV infected patients in the next time will became a public health challenge, these data motivated us to research this field. We performed a cross sectional retrospective study on 54 HIV positive patients, from this 34 male, with mean age 30,6 years (between 19-56 years), diagnosed with HIV average 12,4 years (extremes, 1-27 years), attending to the Mureş County Clinical Hospital,1stInfectious Diseases Clinic of Tîrgu Mureş. We followed demographics, datas about HIV infection, risk factors for cardiovascular diseases, immunological, virological and therapeutical parameters. We included in this study only patients who had all data we need for online calculators. Using obtained data, we assessed the cardiovascular risk with online calculators: D.A.D (Data Collection on Adverse Effects of Anti-HIV Drugs) CVD prediction tool/algorithm, based on a full model, PROCAM (Prospective Cardiovascular Munster), SCORE (European High Risk Chart), and Pooled Cohort Risk Assessment Equation. Because of the age restriction we not used Framingham score. Estimating CV risk with DAD (F) score, we found 14 patients (25,9%) with 1-5% risk and 3 patients (5,5%) with >5% risk for developing a cardiovascular disease (CVD) within the next 5 years. With Pooled Cohort Risk we found that only 3 patients (5,5%) had elevated 10-year risk for a first atherosclerotic cardiovascular event. With PROCAM score 6 patients had 5% risk to develop in the next 10 years an acute coronarian event and 3 patients had a 10% risk. 10-year risk of fatal cardiovascular disease was assessed with SCORE estimation and we found 3% risk at 8 patients. In summary 17 patients (31,4%) had one (3 patients) or more (14 patients) positive results based on these estimations and 37 patients had no elevated cardiovascular risk. In our young cohort of study 31,4% of the patients had a 10-year risk to develop different kind of cardiovascular events. We could not generalize these results, because of heterogenicity of our patients. These results suggest a higher risk than in general population. For real assessment we need better tools for estimation like biomarkers and good stratification of HIV infected patients.

This work is linked to the Semmelweis University (Budapest, Hungary) PhD Doctoral School SE 00049728, consulent: Prof. Dr. Károly Nagy.

AUTHOR INDEX

ÁBRÓK, M. 81
ÁCS-SZABÓ, L. 1, 19, 20, 73, 105, 117, 189
ADÁNYI, N. 7, 45
ADONYI, Á. 14
ÁG, N. 2, 106, 132
ALBERT, M. 114
ALMÁSI, E. 106, 139
AMARAL, L. 155
ÁMON, J. 2, 3, 13, 107, 113
ANDA, D. 3, 24, 107, 121
ANDERSEN, M.R. 108
ANDREJKOVITS, Á.V. 108, 192
ANTAL, K. 24
ANTUNOVICS, ZS. 1, 129
ARUNA, V.R. 162
ASZALÓS, J.M. 4, 59, 109, 185
ASZTALOS, L. 117
AVSIC-ZUPANC, T. 189
ÁY, É. 110
BABIC-ERCEG, A. 189
BACH, Á. 127
BAGI, Z. 113, 128, 169, 176, 191
BAKA, E. 5, 7, 47, 70, 88, 137
BALÁZS, A. 70
BALÁZS, B. 5, 28, 38, 52, 106, 110, 130, 181
BALÁZS, ZS. 60
BALI, D. 141
BALI, K. 6
BÁLIND, Á. 97
BÁLINT, Á. 27
BÁLINT, B. 79, 139, 144
BÁLINT, M. 53
BÁN, E. 63
BANKS, L. 111, 171
BÁNYAI, K. 6, 8, 27, 31, 37, 42, 52, 91, 124
BARABÁS, A. 8
BARABÁS, E.CS. 8
BARANYI, N. 9, 92
BARBIC, L. 189
BARCSAY, E. 18
BARTA HOLLÓ, B. 112
BARTA, ZS. 66
BARTAL, A. 13, 41
BÁRTFAI, GY. 120
BATA-VIDÁCS, I. 5, 7, 47, 88, 137, 144
BAYER-DANDÁR, E. 18
BEKŐ, K. 168
BELÁK, Á. 119, 145
BENCSIK, O. 9, 10, 97
BENDE, G. 33
BENEDEK, T. 11, 86
BENHALIMA, M. 157
BENYÓ, J. 88
BEREK-NAGY, P.J. 45
BÉTÉRI, T. 154
BIDIGA, L. 117
BIKSI, I. 168
BIRKÓ, ZS. 12, 57, 174
BÍRÓ, H. 32, 114
BIRÓ, S. 12, 57, 71, 174
BLUM, I. 110
BÓDAI, V. 25, 65, 76
BODOR, A. 112, 142
BODOR, P. 3, 121
BÓKA, B. 13, 41, 139
BOKOR, E. 2, 3, 13, 107, 113
BOLBA, N. 15
BOLDOGH, S. 42
BOLDOGKŐI, ZS. 60
BORICS, A. 30, 186
BOROS, Á. 14, 15, 32, 72, 114
BOROS, É. 180
BOROS, I.M. 163
BORSODI, A. 3, 4, 24, 49, 59, 62, 107, 109, 121, 151, 185
BOUCHELAGHEM, S. 140
BOUNEDJOUM, N. 112, 142
BOZÓ, A. 16, 47, 56, 145, 152
BÖJTI, T. 113, 191
BRATEK, Z. 96
BRLEK GORSKI, D. 128
BUDINSKI, I. 8, 42
BUJNA, E. 70, 84, 178
BUKTA, E. 80
BURIÁN, K. 16, 61, 95, 115, 135, 148, 160
BÜCS, SZ. 42
ČADEŽ, N. 88, 170
CERQUEIRA, G. 2, 106, 132

CHAKRABORTY, T. 170
CHEN, L. 139
CHIRIAC, C. 108
CRETU, C.M. 8
CZELECZ, ZS. 18
CZUNI, L. 20
CSABAI, ZS. 60
CSABÓK, D. 116
CSEPREGI, J.ZS. 99
CSERCSIK, R. 17
CSERESNYÉS, I. 85
CSIRE, M. 18
CSOMA, E. 18, 117
CSOMA, H. 19, 20, 21, 117
CSONKA, A. 118, 134
CSONKA, Á. 112
CSONKA, K. 99
CSONTOS, P. 62, 138, 151
CSŐSZ, I. 42
DALLOS, B. 55
DÁLYAI, L. 20, 21
DAMJANOVA, I. 17, 144, 182
DÁN, Á. 6
DANDÁR, E. 27
DARÓCZI, L. 152
DE VRIES, R.P. 119
DEÁK, Á. 93
DEÁK, B. 119
DEÁK, J. 22, 120
DECSI, G. 127
DEIM, Z. 186
DÉKÁNY, I. 93
DELWART, E. 14, 32, 72, 114
DEMCSÁK, A. 127
DENCS, Á. 22, 127
DIMA, B. 23, 100, 187
DLAUCHY, D. 88
DOBÁK, A. 17
DOBAY, O. 39
DOBOLYI, CS. 5, 132, 137, 165
DOBRÓNÉ TÓTH, M. 165
DOFFKAY, ZS. 160
DOMÍNGUEZ-ÁLVAREZ, E. 118, 122
DOWNES, D.J. 177
DRUZHININA, I.S. 91
DUC QUANG, N. 26, 27, 70
EMRI, T. 24, 36, 53
ENDRÉSZ, V. 61, 95, 115, 160
ENYEDI, N.T. 24, 121
ERDEINÉ KIS, Á. 112, 142
ERDÉLYI, B. 25, 45, 65, 76
ERDENEBILEG, S. 98
EREGA, A. 128
ESTÓK, P. 42
ESZIK, I. 95
FABRIS, A. 128
FALGENHAUER, L. 170
FARAGÓ, J. 154
FARKAS, Á. 56, 151
FARKAS, CS. 26, 27
FARKAS, M. 11, 86, 121, 162
FARKAS, SZ. 27, 91
FARKAS, T. 34
FATEMEH, E. 5, 38, 52
FEHÉR, Á. 182
FEHÉR, E. 6
FEKETE, CS. 20, 91, 140
FEKETE, E. 2, 51, 60, 68, 106, 132, 136
FELFÖLDI, T. 3, 49, 59, 65, 76, 83
FENYVESI, H. 14
FERENCZI, E. 63
FERREIRA, M-J.U. 134
FÉSŰS, A. 5, 28, 38, 52, 130
FICZE, H. 30
FIDLER, G. 71
FLIPPHI, M. 2, 51, 60, 106, 132
FODOR, A. 192
FODOR, K. 164
FORRÓ, B. 37, 124
FÓZER, D. 29
FÖLDES, F. 8, 42, 52, 100
FRIEDRICH, L. 66
FÜLÖP, L. 88
FÜSTÖS, Z. 145
FÜZI, M. 17, 29
FÜZY, A. 85, 138
GABALDON, T. 67
GÁCSER, A. 67, 99
GAJDÁCS, M. 112, 122
GÁL, B. 27
GALGÓCZY, L. 30, 90, 186
GÁLL, T. 117
GAZDAG, A. 160
GAZDAG, O. 31, 62, 123, 151
GAZDAG, Z. 20, 58, 140

GÉCZI, F. 20
GEORGESCU, A. 108
GERGELY, L. 18, 117
GERŐCS, A. 124
GÎRBOVAN, C. 192
GORÁCZ, O. 28, 38, 130
GORFER, M. 68
GÖCZŐ, I. 17
GÖNCZÖL, É. 164
GÖRFÖL, T. 42
GÖRFÖL-SULYOK, K.M. 31
GRÓZNER, D. 124
GULYÁS, M.,E. 125, 154
GYÖNGYÖSI, E. 126, 171
GYŐRI, Z. 110
GYURANECZ, M. 31, 124, 168
HADHÁZI, ZS. 1
HÁHN, J. 89
HAJDÚ, E. 166
HAMARI, ZS. 2, 3, 13, 107, 113, 127
HANDZLIK, J. 118, 122, 148
HARGITAI, R. 32
HATVANI, L. 91, 131
HEDERICS, D. 141
HEGEDŰS, B. 33
HEGYI, A. 82
HERCZEG, G. 45
HERCZEG, R. 55
HETTMANN, A. 22, 127
HILD, A. 34
HÓDI, B. 173
HOFFMANN, A. 180
HOHMANN, J. 135
HORNOK, L. 35
HORTON, R. 175
HORVÁTH, F. 154
HORVÁTH, GY. 55, 141
HORVÁTH, M. 20, 87
HORVÁTH, P. 97
HRIVNÁK, V. 168
HUBE, B. 36
HUNJAK, B. 128
HUNTER, C.C. 178
IGAZ, N. 76, 173
ILLYÉS, Z. 96
IMRE, CS. 35, 48, 157
IMRE, V. 139
IMREFI, I. 45
IMRI, Á. 35, 48
INCZE, A. 108
INOTAI, K. 137, 165
IVÁN, M. 78, 164
JACOB, C. 148
JAKAB, Á. 36
JAKAB, F. 8, 22, 27, 42, 52, 55, 100, 141
JÁMBOR, M. 35, 48
JANG, Y. 148
JANKOVICS, I. 63, 164
JANKOVICS, M. 164
JÁNOSI, SZ. 31, 124
JANOVÁK, L. 93
JÁNVÁRI, J. 17
JÉRE, CS. 42
JERZSELE, Á. 31
JEVERICA, S. 166
JIGJID, E-A. 183
JÓNÁS, G. 66
JUHÁSZ, E. 78
JUHÁSZ, R. 178
JUHÁSZ, V.O. 116
JURAK, B.A. 146
JURECSKA, L. 59, 65, 76, 107, 109
KAIC, B. 189
KAKUK, B. 113, 128
KÁLI, SZ. 53
KÁLLAI, Z. 21
KARAFFA, L. 2, 37, 51, 60, 68, 106, 132, 136
KARAI, A. 15
KARANYICZ, E. 1, 129
KARDOS, G. 5, 28, 37, 38, 52, 56, 64, 80, 110, 130, 145, 152, 153, 172, 181
KARDOS, SZ. 39
KARDOS, T. 16, 145
KARI, A. 40
KÁROLY, É. 15
KÁRPÁTI, É. 35, 40, 147, 157
KÁRPÁTI, S. 110
KARPÍŠKOVÁ, R. 175
KARTALI, T. 41, 131
KASZAB, E. 6
KASZAP, A. 132
KÁTAI, A. 15
KATONA, M. 18

KAVALECZ, N. 132
KAWASE, M. 43
KECSKEMÉTI, A. 13, 40
KECSKEMÉTI, S. 27
KEDVES, O. 139
KEISHAM, K. 2, 3, 13
KELE, Z. 30, 90, 186
KELLER, N.P. 68
KEMENESI, G. 8, 22, 42, 52, 100, 141
KEPNER, A. 52
KERÉNYI, Z. 133
KEREPESI, V. 27
KERESZTÉNY, T. 133
KERTÉSZ, A.M. 32
KINCSES, A. 43, 112, 118, 134, 135, 148, 155
KIRÁLY, R. 18
KIRICSI, M. 76, 163
KIS, I. 18
KIS, Z. 56, 144, 164
KISS, B. 29, 43, 106, 135, 139, 187
KISS, E. 44
KISS, G. 49
KISS, J. 82, 136, 190
KISS, K. 168
KISS, L. 68
KISS, T. 72
KISS, V. 95
KNAPP, D. 45
KNITLHOFFER, V. 49
KOCSIS, B. 20
KOCSIS, R. 133
KOCSUBÉ, S. 5, 9, 71, 92
KOHÁRI, SZ. 25, 45
KÓKAI, D. 115
KOLARIC, B. 189
KOLBL, S. 154
KOLLÁTH, I.S. 136
KOLPASZKY, D. 26
KÓNYA, J. 80, 126, 171
KÓNYA, Z. 76, 163
KORDA, B. 46
KORMOS, V. 156
KOROKNAI, A. 110
KORPONAI, K. 83
KORVA, M. 189
KÓS, P. 33
KOSZTIK, J. 47, 137
KOVÁCS, B. 136
KOVÁCS, D. 20, 58, 76, 163
KOVÁCS, G.M. 23, 45, 68, 93, 100
KOVÁCS, K.L. 113, 128, 169, 176, 191
KOVÁCS, R. 16, 35, 40, 47, 48, 56, 85, 138, 145, 152, 157
KOVÁCS, T. 160, 186
KOVÁCS, Z. 74, 157
KÖDÖBÖCZ, L. 31, 123
KÖMÜVES, J. 136
KRASZNAI, Z. 39
KRAVCHENKO, K. 42
KREDICS, L: 91, 97, 98, 131, 139, 184
KREIZINGER, ZS. 31, 124, 168
KRETT, G. 49, 62, 107, 151, 185
KRIFATON, CS. 5, 70
KRISHNA, G.M. 163
KRISTÓF, K. 50, 78, 164
KRISZT, B. 11, 70, 89, 121, 132, 162, 165
KRISZTALOVICS, K. 182
KRIZSÁN, K. 44, 97, 106, 139, 187
KRÖEL-DULAY, GY. 93
KRTINIC, B. 52
KUBICEK, C.P. 136
KUGLER, R. 91
KUKOLYA, J. 5, 7, 47, 88, 137, 144, 147
KULCSÁR, G. 51
KULCSÁR, L. 51
KUN, SZ. 84, 178
KUNGLER-GORÁCZ, O. 5, 52
KURNÁSZ, H.D. 140
KURUCZ, K. 8, 42, 52, 100, 141
KÚSZ, K. 135
KUTAS, A. 55
KUTASI, J. 35, 40, 48, 147, 157
KUTI, D. 164
KWAIK, Y.A. 146
LACZI, K. 33, 112
LAGZI, I. 45, 142
LAKATOS, B. 110
LAKATOS, G. 191
LAKATOS, L. 142
LÁNG, D. 154

László, B. 80, 125
Latkóczy, K. 78, 164
Laub, K. 39
Lázár, A. 54, 81
Leiter, É. 53, 71
Lengyel, Gy. 6, 91
Lengyel, Sz. 37
Libisch, B. 133
Lippai, A. 53, 143
Liptai, Z. 18
Lipták, Á. 54
Ljubin-Sternak, S. 189
Lueders, T. 86
Luzics, Sz. 7, 47, 88, 144
Lysnyansky, I. 31
Madai, M. 55, 141
Mádl-Szőnyi, J. 3, 24, 107, 121
Madureira, A.M. 134
Magyar, D. 165
Magyar, I. 156
Magyar, N. 56, 144
Magyari, B. 115
Magyari, J. 112
Májer, J. 34, 124
Major, T. 39
Majoros, L. 16, 47, 56, 145, 152
Makay, K. 6
Makk, J. 3, 24, 107, 121
Makrai, L. 31
Man, A. 8
Manczinger, L. 13, 91
Mándi, Y. 61
Maráz, A. 74, 145, 157
Marecic, V. 146
Márialigeti, K. 3, 4, 24, 40, 49, 59, 65, 74, 76, 83, 107, 121, 176, 180, 185
Marik, T. 184
Maróti, G. 79, 191
Marschalkó, M. 110
Márton, É. 57, 174
Marton, Sz. 8, 27, 31, 91, 124
Marx, F. 30, 186
Massimi, P. 171
Máté, G. 20, 58
Máté, R. 147, 157
Megyeri, A. 52
Megyes, M. 59
Melegh, Sz.Zs. 78, 164
Mentes, A. 59
Merényi, Zs. 96
Mészáros Szécsényi, K. 112
Mészáros, É. 65, 76
Meszéna, R. 140
Mezei, M. 110
Miao, Y. 91
Mićunović, J. 175
Migeon, P.A. 178
Mihelcic, M. 146
Mihucz, V.G. 88
Miklausic, B. 189
Miklós, I. 1, 20, 21, 73, 105, 189
Mináróvits, J. 93, 110, 127, 148
Miskei, M. 53
Miszti, C. 78, 164
Mizsei, E. 37
Mócsai, A. 99
Móga, J. 59
Mogavero, S. 36
Moldován, N. 60
Molnár, Á.P. 60, 68, 136
Molnár, J. 43, 155, 160
Momodu, N. 61
Mosolygó, T. 61, 95, 115, 118, 122, 135, 148
Mouwakeh, A. 149
Mózes, J. 5, 28, 38, 52, 110, 181
Mrša, V. 150
Mucsi, M. 31, 62, 123, 151, 185
Mulhovo, S. 134
Nagy, A. 63, 144, 151
Nagy, B. 4, 82, 109, 175, 190
Nagy, D. 140
Nagy, E. 78, 156, 166, 192
Nagy, F. 16, 47, 145, 152
Nagy, G. 172
Nagy, I. 5, 88, 97, 106, 139, 144, 180
Nagy, K. 17, 63, 127, 168
Nagy, L. 44, 106
Nagy, L.G. 90, 97, 139, 187
Nagy, N. 174
Nagy, O. 63, 151
Nagy, P.I. 132
Nagy, S.Á. 31
Nagy, Zs. 64, 153, 172

NAGYMÁTÉ, ZS. 40, 65, 74, 76
NASIM, M.J. 148
NEDECZKY, K. 186
NEMES-BARNÁS, K. 124
NÉMETH, A. 97, 98
NÉMETH, Á. 29, 43, 49, 66, 94, 135, 153
NÉMETH, CS. 66, 154
NÉMETH, M.Z. 68
NÉMETH, T. 67, 99, 154
NÉMETH, V. 42, 55
NÉMETH, Z. 68
NÉMETH, ZS. 154
NOVÉ, M. 112, 155
NYILASI, I. 40, 131
OLÁHNÉ HORVÁTH, B. 156
OLÁHNÉ SZABÓ, R. 69
OLASZ, F. 34, 82, 124, 133, 136, 190
OLDAL, M. 8, 52, 55, 100
ONDER, P. 46
ORBÁN, P. 70
ORCZÁN, Á.K. 96
ORESKI, T. 189
OROSZ, E. 24, 53
OROSZ, I. 70
ÓVÁRI, M. 121
OZANIC, M. 146
ÖNDER, K. 95
ÖRDÖG, K. 166
ÖTVÖS, F. 184
PAHOLCSEK, M. 71
PÁL, S. 124
PÁL, S.E. 121
PÁLINKÓ, D. 127
PALLINGER, É. 164
PÁLYI, B. 56, 144
PANKOVICS, P. 14, 15, 32, 72, 114
PAPP, G. 20, 140
PAPP, L.A. 1, 19, 73, 105, 189
PAPP, P. 133
PAPP, SZ. 160
PAPP, T. 9, 10, 40, 131, 172, 180, 187
PARÁDI, I. 138
PÁRDUCZ, Á. 160
PÁSZTI, E. 18
PÁSZTI, J. 17, 175, 182
PÁZMÁNDI, M. 74, 157
PEJNOVIĆ, A. 128
PÉK, N. 40, 157
PENCHEV, K. 175
PENYIGE, A. 158
PEREI, K. 33, 112, 142, 173
PERRY, J. 166
PERSIC, Z. 128, 189
PESAVENTO, P.A. 114
PESTI, M. 58
PÉTER, G. 88
PETHŐ, Z. 153
PETKOVIĆ, H. 170
PETROVIC, U. 159
PETROVSZKI, P. 112
PFEIFFER, I. 76, 163
PINTÉR, R. 55, 160
PITLIK, P. 96
PÓCSI, I. 24, 36, 53
POCSKAY, Á. 110
POHNER, ZS. 74
POMÁZI, A. 34
PÓTÓ, L. 87
PRASANNA, A. 44, 106, 139
PRUKNER-RADOVCIC, E. 175
PUSPÁN, I. 35, 40, 48, 157
RAÁB, M. 15
RAFFAI, T. 160
RAJKAI, K. 85
RÁKHELY, G. 33, 112, 113, 128, 142, 160, 169, 173, 176, 186, 191
RAKK, D. 183
RALOVICH, B. 161
RÉDEI, D. 135
RÉKÁSI, M. 40
REUTER, G. 14, 15, 32, 72, 114
RÉVÉSZ, F. 121, 162
REZESSY-SZABÓ, J.M. 26, 27, 70
RISA, A. 5
RIZÓ, B. 40
ROHDE, M. 170
RÓKUSZ, L. 75
ROMSICS, CS. 40, 65, 76
RÓNAI, ZS. 31, 124
RÓNAVÁRI, A. 76, 163
ROVÓ, L. 127
RÓZSAVÖLGYI, Z. 61

SAIJO, R. 43
SALAMON, B. 66
SALEH, Q. 56
SANCHA, S. 134
SÁNDOR, E. 60, 136
SANMARTÍN, C. 118, 122
ŠANTEK, B. 167
SANTIC, M. 146
SÁRI, E. 83, 176
SARKADI, J. 164
SÁRKÁNY, D. 144
SÁRKÁNY, E. 77
SÁRKÁNY, O. 176
SÁRVÁRI, K.P. 78, 81, 164
SÁTORHELYI, P. 25, 45, 65, 76
SÁTORI, G. 135
SAVIC, V. 189
SCAZZOCCHIO, C. 2, 106, 132
SCHNEIDER, GY. 87, 160, 186
SCHÖNHARDT, K. 91
SEBŐK, F. 5, 132, 165
SEIBOTH, B. 166
SERES, A. 132
SHAHAB, D. 131
SHEVCHUK, O. 146
SIPICZKI, M. 1, 19, 20, 21, 105, 117, 129, 189
SIPOS, GY. 139
SNYDER, M. 60
SÓKI, J. 54, 78, 164, 166
SOLTI. Á. 96
SOMFAY, A. 61
SOMOGYI, B. 59
SOMOGYVÁRI, F. 116
SONDEREGGER, C. 30
SOÓS, Á. 136
SÓS, E. 47
SÖRÖS, K. 84
SPENGLER, G. 43, 112, 118, 122, 134, 135, 148, 155
ŠTAFA, A. 167, 170
STAMMNÉ FELDE, O. 168
STEINERT, M. 146
STERZ, B. 168
STEVANOVIC, V. 189
STEYER, J.T. 178
STINGU, C-S. 166
STOIAN, A. 108
STRANG, O. 169
STRECKER, T. 56
STYEVKÓ, CS. 22
STYEVKÓ, G. 70
SUCIU, ZS. 192
SULYOK, K.M. 124, 168
SURÁNYI, J. 154
SVÁB, D. 79, 170
SVETEC MIKLENIĆ, M. 167, 170
SVETEC, I.K. 167, 170
SVIBEN, M. 189
SZABÓ TÖNKI, Á. 118
SZABÓ, A. 3, 49, 59, 65, 76, 107, 109, 143
SZABÓ, Á.M. 43
SZABÓ, B. 41
SZABÓ, D. 17
SZABÓ, I. 11, 162
SZABÓ, J. 39
SZABÓ, M. 180
SZABÓ, ZS. 53
SZAKÁCS, G. 45
SZAKÁCS, L. 80
SZALAI, B. 56
SZALAI, Z. 185
SZALAY, A.R. 86
SZALMÁS, A. 126, 171
SZÁNTÓ, Á. 58
SZARKA, K. 64, 80, 153, 172
SZARKÁNDI, J.G. 187
SZEBENYI, CS. 172
SZÉCSÉNYI, M. 81
SZÉKÁCS, A. 7
SZEKERES, A. 5, 9, 10, 13, 41, 97, 98, 184
SZENTGYÖRGYI, F. 11
SZENTMIHÁLYI, K. 132
SZERENCSÉS, B. 163
SZILÁGYI, A. 110
SZILÁGYI, Á. 173
SZILÁGYI, J. 82
SZILÁGYI, M. 174
SZILÁGYI-BÓNIZS, M. 12, 57, 158
SZILI-KOVÁCS, T. 31, 62, 123, 138, 151, 185
SZINAI, M. 153, 172
SZLÁVIK, J. 110
SZMOLKA, A. 82, 175, 190

Szoboszlay, S. 11, 86, 89, 121, 132, 165
Szodoray-Parádi, A. 42
Szodoray-Parádi, F. 42
Szőke, B. 34, 124
Szuhaj, M. 128, 176
Szuróczki, S. 53, 83, 176
Szűcs, A. 18, 64, 117, 153, 172
Szűcs, D. 160
Szűcs, I. 18
Ta Phuong, L. 84, 178
Tabain, I. 189
Takács, M. 18, 63, 110, 127, 144, 151, 177
Takács, T. 31, 85, 123, 138
Takó, M. 90
Tamaskó, G. 96
Táncsics, A. 11, 70, 86, 121, 162
Tarcsai, K. 151
Terényi, M. 51
Terhes, G. 127, 186
Thuer, E. 67
Tierney, D. 166
Tigyi, Z. 87
Tóbiás, A. 88
Todd, R.B. 178
Tomaić, V. 171, 179
Tombácz, D. 60
Tóth, A. 66, 99, 154
Tóth, Á. 7, 17, 47, 88, 144, 147, 182
Tóth, B. 110
Tóth, E. 53, 83, 88, 143, 172, 176
Tóth, E.J. 180
Tóth, F. 65, 76, 180
Tóth, G. 89
Tóth, G.K. 186
Tóth, H. 38, 117, 130, 181
Tóth, I. 79, 170
Tóth, K. 182
Tóth, K.F. 5, 52
Tóth, L. 90, 186
Tóth, V. 155
Tóth, Z. 16, 47, 56, 145, 152
Tóth-Ihász, K. 91
Tóthpál, A. 39
Tsang, A. 183
Tugyi, N. 59
Tung, G.P. 14, 32, 114
Turbat, A. 183
Turcsányi, I. 31
Turzó, K. 93, 188
Tyagi, C. 184
Ujvári, G. 93, 185
Ulger, N. 166
Unger, V. 155
Ungvári, K. 188
Urbán, E. 54, 78, 81, 93, 122, 127, 164, 166, 186, 188
Urbán, P. 20, 42, 91, 100, 140
Uzinger, N. 40
Vági, P. 68
Vágvölgyi, Cs. 2, 3, 9, 10, 13, 20, 41, 90, 91, 92, 97, 98, 107, 113, 131, 139, 163, 172, 180, 183, 184, 186, 187
Vajna, B. 93, 180
Valappil, S.K. 160, 186
Valasek, A. 91
Váradi, Gy. 30, 186
Varga, B. 112, 134
Varga, I. 160
Varga, J. 5. 9
Varga, Sz. 134
Varga, T. 96, 97, 100, 187
Varga-Kugler, R. 8, 27
Vásárhelyi, B.M. 144
Vásárhelyi, O. 155
Vaz Amanda, G. 172
Vendrinszky, Á. 186
Venkei, A. 93, 188
Vera Cruz, C.M. 160
Veress, A. 136
Veress, Gy. 126
Vidra, A. 94
Vig, I. 189
Vigneshwari, A. 97, 98
Vikor, J. 96
Vilibic-Cavlek, T. 189
Villányi, I. 31
Virágh, M. 30, 90
Virók, D. 61, 95, 115, 160
Visontai, I. 164

VITÁNYI, B. 133
VIZI, T. 160
VLASCHENKO, A. 42
VOLFORD, B. 97
VÖLCZ, G. 96
VÖRÖS, L. 59
WATANABE, G. 43
WEHMANN, E. 31
WEISER, D. 25, 45
WEISZBURG, T.G. 88
WILK, T. 190
WIRTH, R. 113, 191
WYBO, I. 166
YOSHIMURA, K. 168
ZAHARIA KÉZDI, E.I. 108, 192
ZAJTA, E. 99, 100
ZANA, B. 8, 42, 52, 100
ZANDONA, A. 170
ZEGHBIB, S. 100
ZOLTAI, A. 154
ŽUNAR, B. 167, 170