ABSTRACTS

of the

Annual Meeting
of the
Hungarian Society for Microbiology

Guest-Editors

K. MÁRIALIGETI and O. DOBAY

October 19–21, 2016
Keszthely, Hungary

Acta Microbiologica et Immunologica Hungarica, 64 (Suppl), pp. 1–101 (2017)
DOI: 10.1556/030.64.2017.101

ASSEMBLY OF THE GENOME OF SCHIZOSACCHAROMYCES CRYOPHILUS USING CONSERVATIVE GENOMIC FEATURES

LAJOS ÁCS-SZABÓ, LÁSZLÓ ATTILA PAPP, ZSUZSA ANTUNOVICS, EDINA KARANYICZ, ZSUZSANNA HADHÁZI, MATTHIAS SIPICZKI, IDA MIKLÓS

Department of Genetics and Applied Microbiology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

Comparative genomic studies enable us to better understand how genomes function and evolve. Although the sequencing processes become more and more accurate and fast, assembling of draft genomic sequences remains a serious challenge in many cases. However, this task is worth the effort, because it allows us to perform extensive and thorough comparative evolutionary studies, as more complete understanding can be obtained by comparing entire genomes. The fission yeasts (*Schizosaccharomyces*) provide an interesting model for genome evolution as they belong to one of the basal groups of Ascomycota (Taphrinomycotina) and have a distinct life history from other yeasts. If we compare them to the genus *Saccharomyces*, they are as different from each other as they are from animals. *Schizosaccharomyces cryophilus* is a relatively new, recently described fission yeast species so named for its preference for growth at lower temperatures compared with its other relatives. Although *S. cryophilus* is morphologically similar to *S. octosporus*, analysis of several rapidly evolving sequences revealed significant divergence from any previously characterized *Schizosaccharomyces* strains. The genome of the fission yeast species have been sequenced and assembled recently, except the *S. cryophilus* genome, as the scaffolds of its sequence was not assembled into complete chromosomes. It has been also reported that fission yeasts could have unusually stable genome structures because conservation of the gene content in their genomes is significantly high. Here we suggest a hypothetic genome assembly of *S. cryophilus* depending on synteny relationships and common genomic features. We created pairwise whole genome alignments with the Mauve aligner using either the sequence of *S. pombe* or *S. octosporus* as reference genomes. Furthermore, we identified the subtelomeric and centromeric contig ends of *S. cryophilus* and we used them as anchor points for the assembly. Thereafter we validated our hypothesis with PCR using sequence specific primers annealed to contig ends and karyotypic analysis combined with Southern-hybridization. Finally, we introduced some new genome characteristics of *S. cryophilus* by comparing the assembled genome to the other related genomes. Our analysis clearly pointed out that *S. cryophilus* is more similar to *S. octosporus* than to *S. pombe* not only in sequence identity but also in gene order. We observed that the genome of *S. cryophilus* underwent approximately equal number of rearrangement events as *S. octosporus* compared with *S. pombe*. The alignments suggested that the main chromosomal rearrangements occurred between the species were interchromosomal translocations. Surprisingly, examples of large-scale inversions between the genomes of *S. cryophilus* and *S. octosporus* seemed to be very rare. Considering the extent/scale of the sequence divergence and the number of rearrangements found in the fission yeasts, we supposed that their structural and sequence evolution are correlated at least in the case of the above three species.

1217-8950/$ 20.00

FUNGAL SPLICEOSOMAL TWIN INTRONS (STWINTRONS): EVOLUTION AND RELEVANCE

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

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

Spliceosomal twin introns ("stwintrons") are unconventional intervening sequences in nuclear transcripts where a standard "internal" U2 intron interrupts a canonical splicing motif of a second, "external" U2 intron. The generation of a full-length ORF thus necessitates two successive U2 splicing reactions. The stwintron hypothesis predicts in each case a specific splicing intermediate. We have demonstrated experimentally the proposed intermediates and thus the presence of internal introns in donor and/or acceptor sequences of external introns for transcripts of *Fusarium verticillioides*, *Trichoderma reesei* (Sordariomycetes), *Helminthosporium solani* (Dothideomycetes) and *Aspergillus nidulans* (Eurotiomycetes). In one instance, we have shown that the excision of an internal intron from a split external intron donor is mutually exclusive with an alternative splicing pathway, which yields a nonsensical RNA. This may be at the root of a post-transcriptional regulatory mechanism, akin to intron retention where the resulting faulty RNA would be degraded by nonsense-mediated mRNA decay. In two examples of experimentally confirmed stwintrons, which position is conserved in entire classes of Pezizomycotina, the cognate phylogenies suggest that intron/exon structure displays a remarkable evolutionary plasticity, where either the internal intron or indeed the whole stwintron can be gained or lost within related clades.

THE INTRACELLULAR LOCALIZATION OF THE TRANSCRIPTION FACTOR HXNR IN *ASPERGILLUS NIDULANS*

JUDIT ÁMON, ESZTER BOKOR, KABICHANDRA KEISHAM, CSABA VÁGVÖLGYI, ZSUZSANNA HAMARI

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

Many microorganisms utilize nicotinic acid as sole nitrogen-source, even so only the prokaryotic catabolic pathways were studied so far. Because of the industrial potential of the pathway enzymes, in prokaryotes the research topic of nicotinic acid catabolism is popular, since these enzymes can serve as bioconversion tools for processing heterocyclic precursors to obtain biological active drugs and compounds for pharmaceutical- and agricultural chemicals. The investigation of the first eukaryotic nicotinic acid catabolic pathway has begun in our laboratory. We elucidated the genetic background of the pathway and identified the pathway-specific regulator, HxnR, a binuclear Zn2Cys6 transcription factor. In order to study the intracellular localization of HxnR we constructed *hxnR-gfp* expression vectors, where the fusion-cassette was driven by the proline inducible prnD promoter. We have transformed the *hxnR-gfp* expressing vector and also the empty gfp vectors as controls to *hxnR* deletion strain, which expresses red fluorescent protein labelled H1 histone (HhoA::mRFP). The transformed vector was integrated in trans of the hxnR::zeo allele at random chromosomal loci in different copy numbers. The copy number of *hxnR-gfp* in the transformants was determined by qPCR using *hxnR* and *acnA* (gamma-actin) specific primer pairs and applying ΔΔCt analysis. Selected transformants were tested in growth tests and protein assay to check whether the fusion protein complements the deletion phenotype and single copy transformants were used for fluorescence microscopy. We studied the localization of HxnR-Gfp under non-induced (on neutral nitrogen

source), induced (induction with nicotinate) and induced-repressed (induction with nicotinate followed by ammonium repression) conditions. Clear nuclear localization was observed under all conditions (non-induced, induced and induced-repressed), in which HxnR differs from many other metabolic pathway-specific transcription factors, where shuffling of localization between cytoplasm and nucleus is usually observed at least in one of the conditions.
Supported by the National Research, Development and Innovation Office (NKFI-K16 119516).

OBTAINING AND CHARACTERIZATION OF CONSTITUTIVE HXNR MUTANTS – STRUCTURE-AND-FUNCTION ANALYSIS

JUDIT ÁMON, ESZTER BOKOR, KABICHANDRA KEISHAM, CSABA VÁGVÖLGYI, ZSUZSANNA HAMARI

Department of Microbiology, University of Szeged, Szeged, Hungary

In eukaryotes the nicotinic acid catabolic pathway is unkown, despite of many prokaryotic pathways had been studied so far. Our workgroup is the first to reveal the genetic background of the nicotinate catabolic pathway of an eukaryotic model organism, *Aspergillus nidulans*. We revealed six *hxn* genes on chromosome VI *(hxnP/Z/T/Y/R/S*), which are organized in a cluster (NDC – Nicotinic acid Degradation Cluster) and are regulated by the Cys6Zn2 transcription factor, HxnR. We also showed that activation of gene expression occurs through the induction of nicotinic acid or the downstream metabolite, 6-hydroxynicotinic acid and we also showed that ammonium acts as a strong repressor when neutral (non-inductive or non-repressive) N-source is appplied. Here we present an UV mutagenesis coupled selection method for the obtaining of HxnR constitutive mutants, in which the nicotinic acid catabolic pathway is activated in the absence of the metabolic inducer. By mapping the mutations on the consurf and 3D model of the transcription factor we revealed details about the mode-of-action of HxnR. We carried out UV mutagenesis on conidia of hxA+, hxA18 and hxAΔ strains and isolated more than 30 nicotinate catabolic pathway constitutive mutants on selective minimal media where hypoxanthine was used as sole nitrogen-source and in case of hxA+ parental strain it was supplemented with allopurinol. On this media only those strains were able to grow which constitutively expressed and produced the purine hydroxylase II enzyme (HxnS), which hydroxylate hypoxanthine to xanthine in the absence or upon the inhibition (by allopurinol) of HxA. We identified the constitutive mutations by sequence analysis and provided experimental evidence for the constitutivity (growth test and protein assay). As some mutational changes were detected several times in separate mutation runs, we have probably near-saturated the *hxnR* gene with the class of constitutive mutations. We constructed a CONSURF profile of the protein, using putative orthologues from 123 species of the Pezizomycotina, and observed that all mutations map in highly conserved regions, most of them exposed. We could propose that in order for the protein to be in its physiological inactive state, subject to the action of the co-inducer, it is necessary to have aromatic residues at position 226 and 228 and a basic residue at position 605.
Supported by the National Research, Development and Innovation Office (NKFI-K16 119516).

IN SITU EXPERIMENTAL MODEL SYSTEM FOR STUDYING THE BIOFILM DEVELOPMENT IN A HYDROTHERMAL SPRING CAVE

DÓRA ANDA[1], ATTILA SZABÓ[1], TAMÁS FELFÖLDI[1], PETRA BODOR[2], JUDIT MAKK[1], JUDIT MÁDL-SZŐNYI[2], KÁROLY MÁRIALIGETI[1], ANDREA BORSODI[1]

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

The Buda Thermal Karst System is one of the modern analogues of hypogenic karsts, associated with the discharge regimes of intermediate flow systems, where springs and caves can be observed directly. The microbial composition of biofilms found on the rock surfaces of the caves belonging to the Buda Thermal Karst System have already led to several new results recently. Nevertheless, the phases of biofilm development and the factors influencing it have not been extensively studied. Therefore, the aim of this study was to monitor the biofilm development in the Rudas-Török spring cave using an in situ experimental model system which offered optimal conditions for the analysis. In parallel with the experiment, physical and chemical parameters of the water were also measured. During the 30 weeks of the monitoring phase, glass sides were immersed into the water-filled cave, and samples were taken at three weekly intervals. High-throughput next generating sequencing method, light microscopy and FIB-SEM techniques were applied parallel to each other to observe the microbial communities and analyze the 16S rRNA gene of bacteria.

The experiment resulted in an increase in the taxonomic diversity of bacterial communities which reached the maximum at phylum level in the third week. The members of phyla Proteobacteria, Plantomycetes, OD1, Nitrospirae, Chloroflexi and Bacteroidetes proved to be the most abundant but their relative abundance changed at different rates during the studied period.

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

ECOLOGICAL TOLERANCE OF PSYCHROPHILIC BACTERIA INHABITING THE HIGH-ALTITUDE AQUATIC ENVIRONMENTS OF OJOS DEL SALADO VOLCANO, DRY-ANDES

JÚLIA MARGIT ASZALÓS[1], BALÁZS NAGY[2], KÁROLY MÁRIALIGETI[1], ANDREA BORSODI[1]

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

The highest volcano on Earth, Ojos del Salado (6893 m) lies in a special mountain desert caused by the presence of the South American Dry Diagonal. This is a harsh environment owing to its extreme aridity, great daily temperature range, permafrost from 5000 meters above sea level and intense UV radiation. Due to the volcanic activity and the presence of permafrost, high-altitude aquatic environments of this remote site (such as fumaroles, hot springs, saline lakes, permafrost derived shallow freshwater lakes) are characterised by various physical and chemical features (water temperature from 9 to 60°C; pH between 2 and 9.5). From a series of aquatic environments, found between 3770 and 6500 meters above sea level, sediment samples were taken in February, 2016. More than 400 bacterial strains were isolated from the 28 samples using four different types of media (R2A and PYG supplemented with and without NaCl). Following the ARDRA grouping, the taxonomic affiliation of the strains were identified based on their 16S rRNA gene sequences. The ecological tolerance of the isolates for salt and pH was examined by measuring their growth in liquid media characterized with NaCl between 0 and 10% (w/V) and pH values from 2 to 7. The estimation of the average germ counts revealed that in most cases the oligotrophic R2A media yielded one order of magnitude higher amount of CFU/g than the rich PYG media, therefore strains from the studied environments prefer lower concentration of organic compounds. Most of the strains are well adapted to cold temperatures, some isolates showed tolerance for high concentrations of NaCl. Members of the genera *Arthrobacter*, *Glaciimonas*, *Flavobacterium*, *Cryobacterium* and *Massilia* were identified.

PRELIMINARY RESULTS OF INVESTIGATION OF STERIGMATOCYSTIN PRODUCING MOULDS BY MOLECULAR AND BIOMONITORING METHODS AND BIODEGRADATION POSSIBILITES

ERZSÉBET BAKA[1], CSILLA KRIFATON[2], ANITA RISA[2], ANDRÁS SZEKERES[3], JÁNOS VARGA[3], SÁNDOR KOCSUBÉ[3], ILDIKÓ BATA-VIDÁCS[1], FLÓRA SEBŐK[2], CSABA DOBOLYI[1], ISTVÁN NAGY[4], JÓZSEF KUKOLYA[1]

[1]Department of Environmental and Applied Microbiology, Agro-Enviromental Research Institute, National Agricultural Research and Innovation Center, Budapest; [2]Department of Environmental Safety and Ecotoxicology, Institute of Aquaculture and Environmental Safety, Faculty of Agricultural and Environmental Sciences, Szent István University, Gödöllő; [3]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary; [4]Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Martinsried, Germany

The genotoxic sterigmatocystin (ST) is produced by the members of *Aspergillus* genus during the biosynthesis of B-group and G-group aflatoxins. Though, this mycotoxin is less genotoxic than the aflatoxinB1, since its spread is considered much wider than the aflatoxin-producers, investigation of this mycotoxin is of great importance. The aims of the current work were to develop reliable and rapid methods for detection of ST producing moulds containing molecular methods and biomonitoring, to investigate its spread in Hungary and to explore biodegradation possibilities. Probable ST-producing moulds have been isolated from soil sample of cornfields and obtained from culture collections. All samples have been identified by traditional culturing technique and molecular methods (calmodulin gene sequencing). All identified mould samples have been targeted to PCR reactions specific for the ST metabolic pathway. Parallel ST-production was monitored by biomonitoring analysis (SOS-Chormotest and SWITCH test) and analytical methods. Several mould samples, member of *Aspergillus* genus have been proven to be capable of ST-production. The development of a novel, enhanced for mycotoxins E. coli-based biomonitoring strain is in progress that carries the mycotoxin specific P450 enzyme gene from turkey catalysing the internal activation of ST. Additionally, biodegradation experiments have been performed with good aflatoxin-B1 degrader *Rhodococcus* strains. So far no excellent ST-degrader stain has been identified.

Acknowledgement: This research was supported by the Hungarian Scientific Research Fund (OTKA-K116631)

DYNAMICS OF CARBAPENEMASE AND AMINOGLYCOSIDE RESISTANCE GENES IN *ACINETOBACTER BAUMANNII*

BENCE BALÁZS[1], JULIANNA MÓZES[1], EBRAHIMI FATEMEH[1], KINGA FANNI TÓTH[1], ORSOLYA KUNGLER-GORÁCZ[1, 2], ADINA FÉSŰS[1, 2], GÁBOR KARDOS[1]

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

Earlier work from our group has reported a high prevalence rate (125/160) of *blaOXA*-23-like carbapenemase gene in *Acinetobacter baumannii* clinical isolates in 2010-2011 together with high prevalence of aminoglycoside resistance genes *aac(6')*-Ib (91/160) and *aph(3')*-VIa (144/160) and appearance of the *armA* aminoglycoside resistance methylase gene (19/160). Carbapenem resistance appeared to be linked to carbapenem, especially meropenem consumption. The present work investigates the dynamics of carbapenem and aminoglycoside resistance in more recent *Acinetobacter baumannii* isolates and its relationship with antibiotic consumption. From 2012 and 2014, 131 and

128 *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 five carbapenem-hydrolysing oxacillinase genes (*blaOXA*-23-like, *blaOXA*-24-like, *blaOXA*-48-like, *blaOXA*-51-like, *blaOXA*-58-like), six aminoglycoside-modifying enzyme genes (*aac(6')*-Ib, *aac(3")*-Ia, *ant(2')*-Iia, *ant(3")*-Ia, *aph(3')*-Ia, *aph(3')*-VIa) as well as the 16S rRNA methylase genes (*arm-A*, *rmtA* and *rmtB*) using PCR assays. Monthly antibiotic consumption was measured in DDD/100 beddays between October 2004 and February 2015. Aminoglycoside consumption decreased slightly (0,46 vs 0,37 DDD/100 beddays), while carbapenem consumption showed a gradual increase (0,34 to 1,67 DDD/100 beddays). In parallel, occurrence of *blaOXA*-23-like continued to be high in 2012 (69.5%, 91/131; $p>0.05$), but showed further increase in 2014 (94.5%, 121/128; $p<0.001$). All isolates tested carried the *blaOXA*-51-like carbapenemase gene; prevalence of *blaOXA*-24-like remained low in 2012 (1.3%, 2/160 vs. 2.3%, 3/131) and became undetected in 2014.
The other two tested genes continued to be absent in this setting. The dominant aminoglycoside modifying enzyme genes behaved differently. The gene *aac(6')*-Ib remained comparable (56.9%, 91/160 vs. 59.6%, 78/131) in 2012 but showed increasing occurrence in 2014 (85.9%, 110/128; $p<0.001$), while prevalence of *aph*(3')-VIa decreased significantly in 2012 (90.0%, 144/160 vs. 77.9%, 102/131; $p<0.001$), remaining comparable in 2014 (73.4%, 94/128). The gene *arm*A behaved similarly to *blaOXA*-23-like carbapenemase gene (11.9%, 19/160 vs. 42.8%, 56/131 vs. 73.44%, 94/128 in 2010/11, 2012 and 2014, respectively; $p<0.001$ in all comparisons). In conclusion, gene prevalences were increasing steadily in case of genes *blaOXA*-23-like, *armA* and *aac*(6')-Ib, suggesting that aminoglycoside resistance genes may be linked to carbapenemase genes and their prevalence is influenced by carbapenem consumption rather than by aminoglycoside consumption.
G. Kardos was supported by a János Bolyai research scholarship of the Hungarian Academy of Sciences.

SEARCH FOR GOOSE ORIGIN POLYOMAVIRUSES AND CIRCOVIRUSES IN PUTATIVE WILD BIRD RESERVOIRS

KRISZTINA BALI[1], ESZTER KASZAB[1], KOLOS MAKAY[1], GYÖRGY LENGYEL[2], ÁDÁM DÁN[3], KRISZTIÁN BÁNYAI[1], ENIKŐ FEHÉR[1]

[1]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences; [2]Hungarian Defence Forces, Military Medical Centre; [3]Veterinary Diagnostic Directorate, National Food Chain Safety Office, Budapest, Hungary

Members of the Polyomaviridae and Circoviridae virus family may cause serious infection of immunocompetent birds. Anser anser polyomavirus 1 is responsible for hemorrhagic nephritis and enteritis of geese and may replicate without any symptoms in domesticated duck as well. The avian circoviruses, e.g. Beak and feather disease virus or Goose circovirus cause lymphocytic depletion, developmental abnormalities and multisystemic disorders in the infected hosts. Recently, a number of novel polyomavirus and circovirus strains have been described in domesticated, pet and wild birds. However, the reservoirs of these viruses have not been thoroughly explored. The aim of this study was the assessment of polyomavirus and circovirus infection in wild birds, including waterfowls. DNA was extracted from 90 cloacal swab samples and was tested negative by broad-spectrum polyomavirus specific nested PCR system. The DNA samples were used also for circovirus detection and amplification of rep sequences was successful from one third of the specimens. The partial rep sequences of specimens collected near Sárbogárd (from *Anser anser*, *Ardea cinerea*, *Buteo buteo*) showed high similarity (95%≤) with goose circoviruses, while those from Mezőberény (from *Anas platyrhynchos*, *Anser erythropus*, *Podiceps cristatus*) and Köröstarcsa (from *Anser erythropus*)

matched (98%≤) to partial rep sequences amplified from human stool sample. The partial rep sequence of a sample collected near Sárbogárd (from *Anas crecca*) showed moderate similarity (76%) with bat circoviruses. Biases in the sequencing results suggested multiple infections of variable circovirus strains in other specimens (from *Anser albifrons*, *Anser erythropus*, *Anas platyrhynchos*, *Ciconia ciconia*, *Corvus frugilegus*, *Garrulus glandarius*, *Haliaeetus albicilla*, *Phalacrocorax carbo*, *Pica pica*, *Podiceps cristatus*). The significance of circovirus infection in different bird species needs to be further investigated. To reveal the host spectrum and reservoirs of polyomaviruses we plan to examine the polyomavirus prevalence in waterfowls.

DIFFERENCES IN THE DOMINANT MICROFLORA OF SPICE PAPRIKA POWDER OF DIFFERENT CLIMATE ORIGINS

ILDIKÓ BATA-VIDÁCS[1], ERZSÉBET BAKA[1], ÁKOS TÓTH[1], SZABINA LUZICS[1], NÓRA ADÁNYI[2], ANDRÁS SZÉKÁCS[3], JÓZSEF KUKOLYA[1]

[1]Department of Environmental and Applied Microbiology, Agro-Enviromental Research Institute; [2]Food Research Institute; [3]Department of Environmental Analysis, Agro-Environmental Research Institute, National Agricultural Research and Innovation Centre, Budapest, Hungary

As presence of mycotoxins or pesticide residues in food products of plant origins from countries outside the EU may represent health hazards to the consumers, there have been attempts to find methods that make determination of the place of origin possible. For spice paprika, a method based on the measurement of colour was tried, and also the δ18O fruit water and corresponding source water and compound specific stable hydrogen isotope data were analyzed. As microbial contamination of spices comes primarily from the soil, and soil microbiota is dependent on climate, geography, and agriculture, qualitative aiming at the dominant microflora of spices instead of the quantitative determination of commonly studied spoilage and pathogenic microflora might give a better picture on the geological origin of the sample. The aim of our investigations was to identify with molecular microbiological methods the dominant bacteria of spice paprika produced at different countries and to look for species that can be characteristic to spices from the given regions. Our results, in agreement with the literature data, have shown that no differentiation can be made among spice paprika samples of different geographical origin by the total bacterial count or extent of mould contamination. However, when the dominant microflora is examined, bacterial species could be identified in the spice paprika samples that are characteristic to climate. According to our study, the presence of *Bacillus mycoides* and *Bacillus licheniformis* was characteristic of the microflora of spice paprika grown in Middle-Europe; *Bacillus safensis* was the bacterium that could be detected in all four paprika samples examined of the tropical monsoon climate; the species common in all three samples of the tropical climate group were *B. amyloliquefaciens* subsp. *plantarum* and subsp. *amyloliquefaciens*, and *Bacillus mojavensis* was detected as characteristic species of Spain. No common species were found in the paprika samples originating from China, probably due to its vast geographical expanse and a variety of climate regions. Though the experiments done in our investigation were only of a limited number of samples, the obtained results clearly show that the dominant microflora of spice paprika samples is dependent on the climate of cultivation, and characteristic species can be identified that relate to their geographical origin.

Acknowledgement: This work was supported by the EU-FP7-SEC-2012-1-312631 SPICED project.

NOVEL ROTAVIRUS SPECIES IN SCHREIBER'S BENT-WINGED BATS, SERBIA

KRISZTIÁN BÁNYAI[1], GÁBOR KEMENESI[2, 3], IVANA BUDINSKI[4], FANNI FÖLDES[2, 3], BRIGITTA ZANA[2, 3], SZILVIA MARTON[1], RENÁTA VARGA-KUGLER[1], MIKLÓS OLDAL[2, 3], KORNÉLIA KURUCZ[2], FERENC JAKAB[2, 3]

[1]Lendület Pathogen Discovery Research Group, Institute for Veterinary Medical Research, Centre for Agricultural Research, HAS-Centre for Agricultural Research, Budapest; [2]Virological Research Group, Szentágothai Research Center; [3]Institute of Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary; [4]Department of Genetic Research, Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia

Rotaviruses (RVs, family Reoviridae, genus *Rotavirus*) are a major cause of acute diarrhea in mammals and birds. The genus *Rotavirus* comprises nine species, designated *Rotavirus A* to *I*. Among these, RVA to RVC, RVE, RVH and RVI are known to infect mammals and RVA is the most widespread species in most, if not all, mammalian hosts. To explore the viral diversity six fecal specimens collected form apparently healthy *M. schreibersii* bats were processed for viral metagenomics. In these samples various amounts of sequence reads mapped onto known eukaryotic viral sequences (range, <<0.1% to 0.9%). Retrovirus specific reads were detected in all samples. Herpesvirus, astrovirus and coronavirus sequences were detected in at least three samples. Rotavirus and gemycircularvirus sequences were found in two and one samples, respectively. In one sample RV sequences were the most abundant genomic traits (98.5%); however, these sequence reads were distributed among various RV species (incl. RVB, RVG, RVH and RVI). Whole genome sequencing and phylogenetic analysis classified a representative strain (BO4351/Ms/2014) into a tentative tenth rotavirus species, we provisionally called *Rotavirus J*. Once the consensus rotavirus gene sequences were assembled for all 11 genomic segments, the 5' and 3' ends of each segment were validated by an independent method. The resulting genome was 18,135 bp in length (range, 3533 bp for segment 1 and 620 bp for segment 11). The novel virus shared a maximum of 50% amino acid sequence identity within the VP6 gene to currently known members of the genus. This study extends our understanding of the genetic diversity of rotaviruses in bats.

CASE REPORT OF A RARE HUMAN PARASITIC INFECTION WITH *HYMENOLEPIS DIMINUTA* IN ROMANIA

ENIKŐ CSILLA BARABÁS[1], ANNAMÁRIA BARABÁS[2], CARMEN MICHAELA CRETU[3], ADRIAN MAN[4]

[1]Department of Cell Biology and Microbiology, University of Medicine and Pharmacy Targu Mures; [2]Pediatric Clinic, County Hospital Mures; [3]Department of Parasitology, University of Medicine and Pharmacy Carol Davila, Bucharest; [4]Department of Microbiology, University of Medicine and Pharmacy Targu Mures, Targu Mures, Romania

Hymenolepiasis is caused by two species of cestodes called *Hymenolepis nana* and *Hymenolepis diminuta*. The first one is more frequent and spreads among humans and rodents. It does not need an intermediate host and infection caused by it has a familiar character. Therefore, it is found mainly in institutionalised children like in orphanages, childcare centres, etc. *Hymenolepis diminuta*, also called the rat tapeworm, is very rare and circulates among rodents and arthropodes, such as cockroaches, moth, etc. Throughout the world the number of human cases is very low (less then 500 reported cases before 2004). During accidental ingestion of the intermediate host by humans cysticercoids penetrate the gut-wall and adult worms develop in the small intestine. We report the case of a 3-year-old girl

from a rural area of Mures County who underwent a routine physical examination without having any complaints. She was breastfed by her mother who needed to attend the hospital with her elder daughter having a recurrent urinary tract infection. The girl was examined physically and no alterations were found. Blood and urinary samples were also taken and porved to be negative. During the stool examination by the wet preparation method charcteristic *Hymenolepis diminuta* eggs were discovered in the feces. They were oval-round shaped, had 70-90 μm diameter and contained six-hooked oncosheres inside. The movement of the hooklets could be detected clearly marking that the oncospheres were alive. The two outer thick layers were separated by clear zones missing the polar filaments; the morphology and the size of the eggs were good methods to differentiate between the Hymenolepis nana and *Hymenolepis diminuta* eggs. No other member of the family had the infection. Oral administration of albendazole 15mg/kg/day for 5 days, proved to be inefficient, the eggs were passed even after finishing the treatment. The patient was put on 25 mg of praziquantel per kg/day and then we carried out the parasitological examination of the stool one months after the completion of the medication. The result was negative. We plan to test again the child after another 5 month. In conclusion, *Hymenolepis diminuta* infection is very rare in our region, it does not cause a severe change in the patient's health and treatment depending on the medication has a good response.

SIGNIFICANT MYCOTOXIN PRODUCING ASPERGILLI

NIKOLETT BARANYI, SÁNDOR KOCSUBÉ, CSABA VÁGVÖLGYI, JÁNOS VARGA†

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

Species assigned to the *Aspergillus* genus are able to cause serious diseases which can negatively impacting human and animal health. *Aspergillus* species can also contaminate foods and feeds at different stages including pre- and postharvest, processing, and handling. The most important aspect of food and feed spoilage is the formation of mycotoxins. Mycotoxins are secondary metabolites of fungi which may have harmful effects on human and animal health. Several *Aspergillus* mycotoxins have been identified as contaminants in foods and feeds. The economically most important are the aflatoxins, ochratoxins, patulin, and fumonisins. Aflatoxins are proven carcinogenic to humans and the primary target is the liver. They are produced by at least 20 species assigned to three sections of the genus *Aspergillus*: sections *Flavi*, *Nidulantes* and *Ochraceorosei*. Ochratoxins were proved to exhibit nephrotoxic, immunosuppressive, teratogenic and carcinogenic properties. Regarding Aspergilli, species assigned to sections *Circumdati, Nigri* and *Flavi* are able to produce ochratoxins. Patulin provokes congestion and edema of pulmonary, hepatic, and gastrointestinal blood vessels and tissues. The economically most important patulin-producing *Aspergillus* species *A. clavatus* is frequently isolated from various cereals. Fumonisins are possibly carcinogenic to humans and can cause various disorders. Among Aspergilli, *A. niger* and *A. welwitschiae* (formerly named as *A. awamori*) are fumonisin producers. In this study, we wish to give an overview of the *Aspergillus* species which are able to produce important mycotoxins.

We would like to recommend this presentation for the memory of our colleague Prof. Dr. János Varga (died on June 16, 2016) and his remarkable work in the topic.

Acknowledgement. This work was supported by OTKA grant K115690.

OPHIOBOLINS: OVERVIEW OF A RARE MYCOTOXIN GROUP

OTTÓ BENCSIK, TAMÁS PAPP, CSABA VÁGVÖLGYI, ANDRÁS SZEKERES

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

Ophiobolins are a naturally occurring tricyclic (C25) sesterterpenoids. They are produced by numerous filamentous fungi belonging to the genus *Bipolaris* and their teleomorph pairs in the genus *Cochliobolus*, however, several ophiobolin analogues have been reported from other genera, such as *Drechslera*, *Cephalosporium*, *Ulocladium* and *Aspergillus*. Ophiobolin A, the firstly described member of this group of secondary metabolites, was reported in 1957. Since then more than 40 ophiobolin derivates have been reported, 18 of them in the last five years, which was anticipated by Au et al (2000). Interestingly most of the newly discovered ophiobolin analogues were isolated from marine microorganisms, which have become preferable sources of new bioactive compounds in the last decade. These structurally related compounds are composed of a tricyclic ring system (5-8-5) named as A-, B- and C-ring, respectively. Their molecular weights range from 358 to 430. Ophiobolins exhibit a broad spectrum of biological activities, such as calmodulin antagonist, antimicrobial, nematocid, anticancer or HIV-1 integrase inhibitory effects. The best-known member of the ophiobolin family, the ophiobolin A was extensively studied during the past decades and its biological activities has been reported in numerous papers. The purification of these secondary fungal metabolites from the ferment broth contained usually comparable isolation and separation steps regarding their similar structures and chemical properties. Furthermore, the elucidations of the ophiobolin structures were generally undertaken using NMR methods due to their high similarity. The aim of this review is to summarize the significant amount of information about the purifications, structural elucidations and biological activities of ophiobolins.

Connected to the project GINOP-2.3.3-15-2016-00006 and supported by GINOP-2.3.2-15 C113410.

STABILITY OF OPHIOBOLIN A IN VARIOUS ORGANIC SOLVENTS

OTTÓ BENCSIK, TAMÁS PAPP, CSABA VÁGVÖLGYI, ANDRÁS SZEKERES

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

Filamentous fungi can produce a large number of bioactive secondary metabolites, amongst them the ophiobolins are promising compounds produced mainly by *Bipolaris* and *Cochliobolus* species, though several analogues have been reported from the genera *Drechslera*, *Aspergillus* and *Ulocladium*. Recently, more than 45 different natural ophiobolin analogues has been reported and characterized belonging to the the sesterterpene-type molecules, and up to 40% of them were discovered in the past 5 years. Ophiobolins show multifaceted biological activities including antimicrobial, nematocid, cytotoxic or calmodulin antagonistic effects. The first described member of this family, the ophiobolin A is the most extensively studied ophiobolin derivate displaying high in vitro growth-inhibitory effects in mammalian cells and showing high in vivo antitumor activity. For the purpose of the future pharmaceutical formulation of ophiobolin A as well as for the undertaken of comprehensive bioassay, it is essential to possess information about the stability and degradation characteristics of a potential bioactive compound. Until now there are only few reports about the solvent stability and the degradation patterns of ophiobolin A. In our study, the stability of ophiobolin A in 6 different organic solvents (acetonitrile, methanol, ethanol, ethyl-acetate, dimethyl sulfoxide and isopropyl alcohol) was investigated. Equal amount of purified ophiobolin A was dissolved in 500 µl of each organic solvent and stored at three different temperatures (-20^{o}C, 4^{o}C and 25^{o}C) for a 10 days long period. During the storage, each sample was analyzed with a stability indicated HPLC method on a daily basis. Our results indicated that ophiobolin A generally degrades into two ophiobolin analogues, 6-epi-ophiobolin A and 3-anhydro-6-epi-ophionolin A in the presence of

alcohols. The degradation rate of ophiobolin A in dimethyl sulfoxide one of the most popular organic solvent in bioassays was found to be the highest amongst the examined solvents, The observed degradation rates of the ophiobolin A were increased with the increasing storage temperature in the presence of all examined organic solvents. This rate could increase in certain cases up to 50% at 25°C after 10 days. The results of our investigation of the solvent stability of ophiobolin A can provide an important data for design of further bioactivity assays of this potential bioactive compound.
Connected to the project GINOP-2.3.3-15-2016-00006 and supported by GINOP-2.3.2-15 C113410.

SELECTIVE ENRICHMENT AND POPULATION DYNAMICS OF MICROAEROBIC AND AEROBIC BTEX DEGRADING BIOFILM BACTERIA

TIBOR BENEDEK[1], FLÓRA SZENTGYÖRGYI[2], ISTVÁN SZABÓ[2], MILÁN FARKAS[2], SÁNDOR SZOBOSZLAY[2], BALÁZS KRISZT[2], ANDRÁS TÁNCSICS[1]

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

The aim of the study on the one hand is to selectively enrich and obtain pure cultures of good aerobic and microaerobic BTEX degrading bacteria from a biofilm developed in a hypoxic (microaerobic) BTEX contaminated groundwater. On the other hand it was also aimed to identify the active BTEX degrading biofilm community members by tracking the phylogenetic and functional dynamics of the initial biofilm community throughout the aerobic and hypoxic BTEX amended enrichments (microcosm experiments for 5 weeks). Cultivation dependent microbiological- and cultivation independent molecular microbiological techniques such as 16S rDNA and I.2.C *C23O* functional gene based T-RFLP, molecular cloning and sequencing of I.2.C *C23O* genes, as well as Illumina 16S amplicon sequencing were used. Results indicated that the aerobic enrichments differed in a large extent in both phylogenetic and functional points of view from the microaerobic ones. In fact, the latter were characterized with higher bacterial diversities based on results obtained with the aid of the applied techniques. On basis of high throughput metagenomic analysis aerobic microcosms after 5 weeks of consecutive enrichments were overwhelmingly dominated by the genus *Malikia* (61%) and by the genera *Macromonas* (6%), Corynebacterineae (5%). Microaerobic microcosms were dominated mainly by genera *Acidovorax* (18%), *Pseudomonas* (14%), *Giesbergeria* (11%), *Zoogloea* (5%), *Rhodoferax* (4%) and *Achromobacter* (3%). Molecular cloning of I.2.C *C23O* genes unveiled that by the end of the enrichment 66% of microaerobic microcosms affiliated *C23O*s belonged to the *C23O* of *P. putida* UCC22, 3% to *P. veronii/extremaustralis* lineage *C23O*s and 3% belonged to *Variovorax boronicumulans*/w11Tn2R6F clade related *C23O*s. In turn, 94% of aerobic microcosms derived *C23O* clones related to the *C23O* clone X3.13 obtained previously from the same biofilm. 6% showed the closest relatedness to the previously mentioned *V. boronicumulans*/w11Tn2R6F clade. The X3.13 *C23O* clone sequence had shown 91.1% amino acid sequence similarity with one of the C23O proteins of *P. spadix* BUG14 isolated also previously from the same biofilm. The *C23O* clone w11Tn2R6F was obtained for the first time from a microbial community of a soil highly contaminated with jet fuel subjected to air sparging bioremediation. Interestingly, *P. putida* related *C23O*s as revealed by functional gene based T-RFLP analysis were present only in microaerobic enrichments. In contrast, the presumably *Pseudoxanthomonas* related *C23O* affiliated T-RFs were present overwhelmingly with increasing abundances throughout the enrichments only in aerobic microcosms (>60% abundance). In microaerobic microcosms or in the bulk groundwater the abundance of this *C23O* T-RF was as low as 4.2 % or zero. Moreover, *P. veronii/extremaustralis*

lineage related *C23O*s by the end of the fifth week gradually disappeared from the aerobic enrichments, while in microaerobic ones its abundance was considerable throughout the experiment.
Supported by the Research Centre of Excellence -8526-5/2014/TUDPOL and 9878/2015/FEKUT.

ANALYSIS OS THE ROLE OF FACTOR C PROTEIN IN MORPHOLOGICAL DIFFERENTIATION AND ANTIBIOTIC PRODUCTION IN THE A-FACTOR PRODUCING *STREPTOMYCES GRISEUS* NRRL B-2682/AFP BALD MUTANT STRAIN

ZSUZSANNA BIRKÓ, MELINDA SZILÁGYI-BÓNIZS, SÁNDOR BIRÓ

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

Streptomycetes are filamentous, spore forming Gram positive bacteria that constitute a major part of the soil bacterial communities. Their complex life cycle is connected with production of secondary metabolites. Members of the genus *Streptomyces* produce over 60% of all known antibiotics and a wide range of industrial enzymes. The variation of onset of morphological differentiation and antibiotic production imply the presence of complex regulatory network consisting of multiple signal transfer systems acting independently or having complex interactions. Analysis of mutant strains showing different aberrations of differentiation are really important in identification of those genes that are involved in a pathway or connect different pathways. In *Streptomyces griseus* both morphological development and secondary metabolism are triggered by A-factor, a small microbial hormon-like molecule. A-factor binds to and removes ArpA (A-factor receptor protein), a repressor of *adpA*. This switches on the transcription of *adpA* that encodes a transcriptional activator. AdpA activates a number of genes required for morphological development and secondary metabolite formation. Another interesting autoregulator is the secreted signalling protein Factor C that plays a key role in cellular communication and cytodifferentiation. Factor C originally was isolated in our department from the liquid culture of *Streptomyces albidoflavus* 45H. The aim of our study was to analyse the effect of Factor C protein in the A-factor producing *Streptomyces griseus* NRRL B-2682/AFP mutant strain that is not able to produce aerial mycelium on solid medium therefore the colonies appear as bald. In this experiment the *facC* gene was amplified by PCR reaction using the *S. albidoflavus* genomic DNA as a temlate and ligated into the pHJL401 low copy number and the pWHM3 high copy number shuttle vector. The transformants were selected on the basis of thiostrepton resistance. One our particularly interesting finding was that when we transformed the mutant strain with *facC* gene, the expression of the secreted regulatory protein from both low and high copy plasmid restored aerial mycelium, mature spore formation and even increased antibiotic production on solid medium in the transformants. In the wild type *S. griseus* NRRL B-2682 we failed to identify a *facC* homologue. Despite the absence of *facC*, response elements of its signal transduction pathway are present in *S. griseus* NRRL B-2682 and its bald mutant. On the basis of these results we can suppose that there is a connection between two divergent extracellular regulatory substances- the protein factor C and the small butyrolactone A-factor- and their pathways in *Streptomyces griseus*. In order to find out if the *adpA* is the mutant gene that lead to the morphological aberration in the analysed mutant strain, we plan to amplifie *adpA* by PCR from the genomic DNA of the *S. griseus* NRRL B-2682/AFP strain and sequence it.

VARIATIONS IN THE YIELDS OF SURFACTIN ISOMERS INFLUENCED BY CULTIVATION PARAMETERS

BETTINA BÓKA, ANITA KECSKEMÉTI, ATTILA BARTAL, LÁSZLÓ MANCZINGER, CSABA VÁGVÖLGYI, ANDRÁS SZEKERES

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

Surfactins are biosurfactant molecules with high importance. They are produced by the gram-positive bacteria belonging to the *Bacillus* genus. These compounds possess strong anti-tumor, anti-viral and anti-inflammatory properties, therefore, they are considered for scientific and industrial usability in the past 50 years. According to their wide scale biological effects, different therapeutic and environmental applications of these molecules are also within the scope of research activities around a word. Surfactins are lipopeptid-type molecules, which may vary in the length of the fatty acid chain (12-15) and in the sequence of the seven amino acids of the peptide loop, generating a wide spectrum of different homologues and isomers. In our study, a liquid chromatography mass spectrometry (HPLC-MS) method was developed for separation of different type of surfactins with ESI ionization and a single quadrupole mass analyzator. The sodium adducts of these molecules were identified in our previous work with ion trap mass spectrometry and some new types were also described. Therefore, the knowledge of the retention time data, the elution orders and the sodiated molecule ions leaded to transfer our method to the single quadrupole MS system, which allowed analyzing the composition of surfactin isomers among various cultivation parameters. In the present work, the effects of various carbon and nitrogen sources in the culture medium were determined on the surfactin profile. The amounts of the different surfactin isoforms were influenced by the medium composition and showed characteristic patterns related to the carbon and nitrogen sources.

Connected to the project GINOP-2.3.3-15-2016-00006 and supported by GINOP-2.3.2-15 C113410. A. Sz. is a grantee of ÚNKP-16-4 Scholarship of the Ministry of Human Capacities of Hungarian Goverment.

IDENTIFICATION OF *HXNS* GENE PRODUCT AS THE EARLIER CHARACTERIZED PURINE HYDROXYLASE II

ESZTER BOKOR, JUDIT ÁMON, KABICHANDRA KEISHAM, CSABA VÁGVÖLGYI, ZSUZSANNA HAMARI

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

Even though many microorganisms can utilize nicotinic acid as a sole nitrogen source, its degradation was studied in only a few prokaryotes so far. In eukaryotes only the first step of a nicotinate catabolic pathway was revealed by describing the biochemical properties of the Purine Hydroxylase II enzyme (PHII), which proved to be a very special enzyme. It is able to hydroxylate hypoxanthine to xanthine similarly to its putative paralogue, the Purine Hydroxylase I (belongs to the purine degradation pathway), although PHII cannot catalyse the subsequent hydroxylation step, by which xanthine is converted to uric acid. Importantly, nicotinic acid proved to be an additional substrate for the PHII, which substrate is hydroxylated to 6-OH nicotinic acid. It was also shown that PHII is regulated by a transcription factor other than UaY, the positive regulator of the purine degradation pathway. For achieving PHII activity on neutral N-sources, addition of nicotinic acid or 6-OH nicotinic acid to the neutral N-source at a low concentration (1 mM) was required. During our work we identified a cluster in the genome of *Aspergillus nidulans* which contains the *hxnR*, the gene of the common transcription factor of the degradation pathway, the *hxnS*, which is the paralogue of the gene that codes the Purine Hydoxylase I, thus most probably the product of *hxnS* is the Purine Hydroxylase II and four other

genes, which show coregulation with *hxnS*. We named this cluster Nicotinic acid Degradation Cluster (NDC). In this presentation we provide experimental evidence, that Purine Hydroxylase II is identical with the gene product of hxnS. In our study we used Purine Hydroxylase II loss-of-function mutants isolated in the 1960s, and *hxnS* deletion mutant we obtained by applying the gene-deletion method of Chaveroce et al. (2000). Loss-of-function mutants were sequenced and the *hxnS* mutations were determined. *hxnS*41 and *hxnS*35 alleles carry chain termination mutations (1162stop and 98stop, respectively), while *hxnS*29 allele carries a missense mutation (F1213S). The latter has a leaky in vivo phenotype on hypoxanthine N-source and loss-of-function phenotype on nicotinic acid. In vitro, enzyme activity with both hypoxanthine and nicotinic acid substrate was showed. Transformation and expression of *hxnS*+ in these mutants reultedin the full recovery of wild-type phenotype with wild type Purine Hydroxylase II functions. This together with the growth tests and protein assays on Purine Hydroxylase II mutants and *hxnS* deletion mutant proves that the *hxnS* gene product is identical with the earlier characterized Purine Hydroxylase II

Supported by the National Research, Development and Innovation Office (NKFI-K16 119516).

OVERVIEW OF THE ENTERIC RNA VIRUS LANDSCAPE OF A SINGLE DIARRHEIC CHICKEN

ÁKOS BOROS[1], PÉTER PANKOVICS[1], ÁDÁM ADONYI[1], HAJNALKA FENYVESI[1], GIA PHAN TUNG[2], ERIC DELWART[2], GÁBOR REUTER[3]

[1]Government Office of Baranya County, Public Health's Organization, Pécs, Hungary; [2]Blood Systems Research Institute, San Francisco, US; [3]Department of Medical Microbiology and Immunology, Faculty of Medical Sciences, University of Pécs, Pécs, Hungary

In general, the distinct and board-range of genetically different viruses presented in the gut microbiome of healthy and diseased (e.g. gastroenteritis) vertebrates including birds is a widely accepted statement, although despite the continuous efforts the exact range of genetic diversity of enteric viruses is far from the full understanding. To demonstrate that an enteric virome of a diseased bird is how diverse could be, the genomes of several viruses, particularly RNA viruses of family Picornaviridae, identified from a single cloacal specimen of a diarrhoeic chicken is investigated and discussed. The analysis of RNA virus sequences acquired by viral metagenomics and RT-PCR methods showed that beside multiple viruses of family Astroviridae, Caliciviridae and Picobirnaviridae all of the currently known chicken picornaviruses including a novel one (provisionally named as chicken phacovirus 1) were also presented. The complete genomes of seven of the eight picornaviruses originated from a same specimen were determined and analyzed which are belonged to the genera *Sicinivirus*, *Gallivirus*, *Tremovirus*, *Avisivirus*, "*Orivirus*" (two genotypes) and "*Phacovirus*" (novel tentative genus). The genomes of identified picornaviruses are ranged between 7031-nt of avian encephalomyelitis virus 1 (AEV-1) to 9883-nt of *Sicinivirus* A1 which has the longest known genome among picornaviruses. Furthermore the studied viruses showed considerable variations in phylogenetic positions, genomic organizations, IRES types and 3'UTR structures, although the presence of common sequence motifs of the 3'UTR of phylogenetically distant picornaviruses could suggests a currently unknown mechanism of lateral transfer of genomic motifs. Among the analysed picornaviruses members of genetically more stable viruses like the AEV with a single known genotype (AEV-1) and genetically more variable ones, like chicken avisivirus or oriviruses with multiple genotypes and slightly different genome plans are also identifiable. The analysed sample was collected from a 4-week-old diarrheic chicken introduced to a new location of a family "back-yard" farm with lack of routine disinfection a few days before the onset of the

symptoms. The well known long-lasting environmental stability of (picorna)viruses and the continuous supply of susceptible hosts most likely contributed to the persistence and continuous enrichment of species-rich local environmental virome. These viruses could cause simultaneous infections in a recently introduced susceptible host.
The presence of unexpectedly high number of viruses co-infected the same animal could promote and facilitate the recombination and evolution of (picorna)viruses and eventually could contribute to the severity of the diarrhea in chicken, in one of the most important food sources of humans.

SALIVIRUS (PICORNAVIRIDAE) OUTBREAK AMONG NEWBORN BABIES WITH ACUTE GASTROENTERITIS IN A NEONATAL HOSPITAL UNIT IN HUNGARY

ÁKOS BOROS[1], MARGIT RAÁB[2], ÉVA KÁROLY[3], ADRIENN KARAI[3], ANDREA KÁTAI[4], NÓRA BOLBA[1], PÉTER PANKOVICS[1], GÁBOR REUTER[5]

[1]Government Office of Baranya County, Public Health's Organization, Pécs; [2]Department of Epidemiology, Government Office of Bács-Kiskun County, Kecskemét; [3]Department of Pediatrics, Neonatal Care Unit, Town Hospital of Baja, Baja; [4]Laboratory of Virology, Government Office of Csongrád County, Szeged; [5]Department of Medical Microbiology and Immunology, Faculty of Medical Sciences, University of Pécs, Pécs, Hungary

Gastrointestinal infection related diarrhea causes the death of 1.5 million people each year and approximately 40% of the cases still remain unknown. *Salivirus* is a small, non-enveloped virus with a positive-sense, single-stranded RNA genome, a novel member of the family Picornaviridae was discovered in 2009. *Salivirus* infection may be associated with acute gastroenteritis among humans. Retrospective investigation with RT-PCR method and complete genome characterization of salivirus in faecal samples from a hospital gastroenteritis outbreak with unknown etiology among newborn babies. Between July 13 and 17, 2013, five (26.3 %) of 19 exposed newborns aged between 1.5 and 5 days, had symptoms of acute gastroenteritis in a neonatal care unit in a town hospital in Hungary. The apparent symptoms were diarrhea (N = 5, 100 %), fever (N = 2, 40 %), vomiting (N = 2, 40 %) and lack of appetite (N = 2, 40 %). Clinical microbiological and epidemiological investigations of the faecal specimens for common bacteria (*Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *E. coli*, *Y. enterocolitica*, *S. aureus*), viruses (rotavirus, adenovirus, norovirus, sapovirus, astrovirus, Aichi virus, human parechovirus) and parasites were negative in all cases. Two years later from all three of the archived faecal samples highly similar saliviruses (99.7% nucleotide sequence identity in the immundominant VP1 capsid region) have been detected. The complete 8021 nucleotide long genome of one salivirus strain (KT240115) was determined at the first time in the literature. Quantitative RT-PCR analysis revealed 2.1-2.6x109 genomic copies/g faeces of salivirus which indicate strong viral replication. A newly discovered virus was detected in 2-year-old archived faecal samples from a nosocomial gastroenteritis outbreak with unknown etiology. Proof of mono-causality, high viral loads and almost identical viral nucleotide sequences are supporting the possibility of salivirus can be an etiological factor of gastroenteritis at least in newborns.
This is the first report of salivirus outbreak in humans.

IN VITRO INTERACTION BETWEEN FARNESOL AND CASPOFUNGIN OR MICAFUNGIN AGAINST *CANDIDA PARAPSILOSIS* BIOFILMS

ALIZ BOZÓ, FRUZSINA NAGY, ZOLTÁN TÓTH, TAMÁS KARDOS, LÁSZLÓ MAJOROS, RENÁTÓ KOVÁCS

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

Farnesol is a quorum-sensing molecule, which blocks the yeast-hypha transition and biofilm formation for *Candida* species. We have limited data about interactions of traditional antifungal agents and farnesol in case of *Candida* biofilms. Therefore, the in vitro activity of caspofungin and micafungin was determined with and without farnesol in RPMI-1640 against *C. parapsilosis* biofilms. Drug interactions were examined using the XTT colorimetric assay-based broth microdilution checkerboard method. Drug-drug interactions were assessed utilizing a fractional inhibitory concentration index (FICI) and time-kill experiments. ΣFIC = FICA+FICB = CAcomb/MICAalone + CBcomb/MICBalone, where MICAalone and MICBalone are the MIC values of agents A and B using alone and CAcomb and CBcomb are the MICs of agents A and B when acting in combination, respectively. FICI was defined as the lowest ΣFIC. Synergy was specified if the FICI was ≤0,5, between >0,5 and 4 was indifferent and as antagonistic if the FICI was >4. MICs alone and at all of the isoeffective combinations were determined as at least 50% reduction of metabolic activity compared with control.

Based on the results of XTT assay, three caspofungin and micafungin concentrations were chosen (4, 8 and 16 mg/L) and their anti-biofilm effect alone and in combination with 75 μM farnesol was examined. The median sessile MICs of five *C. parapsilosis* clinical isolates ranged between 32-256 mg/L, 16-512 mg/L and >300 μM for caspofungin, micafungin and farnesol, respectively. The median MICs for caspofungin and micafungin in combination with farnesol showed 8-64- and 4-64-fold decrease, respectively. Based on FICIs, synergism was observed for caspofungin (range of median FICIs: 0.155-0.5) and micafungin (range of median FICIs: 0.093-0.5). In accordance with FICI findings, synergistic interactions were confirmed by time-kill experiments. The metabolic activity of fungal cells was significantly inhibited by caspofungin+farnesol at all three tested combinations (4mg/L+75μM, 8mg/L+75μM, 16mg/L+75μM) between 3 and 24 hours compared with control ($P<0.05$-0.001). Significant inhibition was observed for micafungin+farnesol between 3 and 12 hours ($P<0.001$) but not at 24 hours. Despite the favorable effect of farnesol in combination with echinocandins, further in vivo studies are needed to confirm its therapeutic advantage in catheter-associated infections caused by *C. parapsilosis*.

CHLAMYDIA MURIDARUM INFECTION CAN INDUCE INDOLEAMINE 2,3-DIOXYGENASE IN BALB/C MOUSE LUNGS IN CONTRAST TO THE EARLIER IN VITRO RESULTS

KATALIN BURIÁN

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

Members of the genus *Chlamydia* are characterized by an obligatory intracellular lifestyle and biphasic developmental cycle. The elimination of the intracellular form of *Chlamydia* is mediated by the interferon-gamma (IFN-γ) produced principally by CD4+ T cells. The mechanism of human IFN-γ activity against *Chlamydia* is the depletion of the host tryptophan pool via the induction of the host

indoleamine 2,3-dioxygenase (IDO). In mouse cells, however, IDO was not inducible by IFN-γ and/or *Chlamydia* infection, instead a set of novel anti-chlamydial host genes, the p47 GTPases were highly induced. Based on the in vitro non-inducibility of murine IDO, its role in murine anti-chlamydial defense was considered negligible. Since mouse models are important to study the immune response against *Chlamydia*, the exact murine in vivo elimination mechanisms should be clarified. Our aim was to investigate the expression of the above described anti-chlamydial host genes in an in vivo mouse model of *C. pneumoniae* and *C. muridarum* infections. Semipurified *C. muridarum* and *C. pneumoniae* elementary bodies were used to infect 6-week old female Balb/c mice intranasally. Infected and non-infected, control mice were sacrificed on day seventh. The number of recoverable chlamydiae was determined from the supernatant of the lungs' suspensions using an indirect immunofluorescence method. Total RNA content of the lungs were extracted and the expressions of the *IDO1* and *IDO2* genes were detected by qRT-PCR. The quantity of the tryptophan and its degradation product kynurenine were measured in the homogenized lungs by HPLC. Expression of the *IDO1* and *IDO2* on protein level were proved by immunohystochemistry. Infections with both pathogens were successful. The number of the recoverable pathogens after *C. pneumoniae* and *C. muridarum* infections was 5,56 X105 ±7,8X104 and 2,8 X105 ±1,2 X104 IFU/ lung, respectively on day seventh. Surprisingly, qPCR data showed, that both forms of murine IDO, *IDO1* and *IDO2* were highly induced by *Chlamydia* infection. *C. pneumoniae* infection induced the *IDO1* and *IDO2* gene expressions at an average of 15.47 fold and 88.91 fold respectively. *C. muridarum* infection induced the *IDO1* and *IDO2* gene expressions at an average of 20.38 fold and 38.21 fold respectively. Also, the kynurenine/ tryptophan ratio which is a marker of IDO activity was markedly higher in the *C. pneumoniae* and *C. muridarum* infected lung homogenates than in the uninfected controls. In this study, we proved that both the human *Chlamydia* strain *C. pneumoniae* and the mouse *Chlamydia* strain *C. muridarum* can highly induce the production of murine *IDO1* and *IDO2* in vivo, in contrast to the earlier in vitro results. Our data strongly indicates that murine IDO could have an anti-chlamydial defence role in vivo.
In the future, we will attempt to identify the cytokine(s) which can induce murine IDOs in vivo.

DIVERSE FITNESS COST ASSOCIATED WITH RESISTANCE TO FLUOROQUINOLONES MIGHT HAVE IMPACTED THE CLONAL DYNAMICS OF MULTIRESISTANT *ACINETOBACTER BAUMANNII*

RITA CSERCSIK[1], ÁKOS TÓTH[2], JUDIT PÁSZTI[2], IVELINA DAMJANOVA[2], JUDIT JÁNVÁRI[2], ISTVÁN GÖCZŐ[1], DÓRA SZABÓ[1], ANDRÁS DOBÁK[3], KÁROLY NAGY[1], MIKLÓS FÜZI[1]

[1]Institute of Medical Microbiology, Semmelweis University; [2]Department of Bacteriology, National Center for Epidemiology; [3]Corden International Ltd., Budapest, Hungary

Our group recently demonstrated that dissimilar fitness cost associated with resistance to fluoroquinolones strongly influenced the clonal dynamics of methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase(ESBL)-producing *Klebsiella pneumoniae* in the healthcare setting. Since, similarly to MRSA and ESBL-producing *K. pneumoniae*, the dissemination of multiresistant *Acinetobacter baumannii* is linked to the spread of a few major international clones we have tested whether or not an analogous mechanism could have impacted the clonality of this pathogen. The fitness of major clone multiresistant *A. baumannii* complex strains was compared with those of fluoroquinolone susceptible minor clone isolates and their mutants in which ciprofloxacin resistance was induced in vitro. Major clone strains retained much greater fitness than fluoroquinolone resistant minor clone mutants which strongly suggests that fitness cost associated

with resistance to fluoroquinolones could have contributed to the emergence of the big international clones of multiresistant *A. baumannii*. Major clone strains proved capable of developing favorable mutations in the gyrase and topoisomerase IV genes in greater numbers than minor clone isolates a trait certainly crucial for preserving fitness.

LABORATORY AND CLINICAL ASPECTS OF HUMAN HERPESVIRUS 6A AND 6B INFECTIONS

MÁRTA CSIRE[1], ZOLTÁN LIPTAI[2], ESZTER BAYER-DANDÁR[2], ERZSÉBET BARCSAY[1], MÁRIA TAKÁCS[1], ILDIKÓ KIS[3], ZSUZSANNA CZELECZ[3], ROXÁNA KIRÁLY[3], IVÁN SZŰCS[3]

[1]Department for Hepatitis and Herpesvirus, Division of Virology, National Center for Epidemiology; [2]Department of Paediatric Infectology, United St. László and St. István Hospital, Budapest; [3]Department of Paediatrics, St. Borbála Hospital, Tatabánya, Hungary

Human herpesvirus 6 (HHV-6) A and B belongs to the group of Betaherpesvirinae together with Human cytomegalovirus (CMV) and Human herpesvirus 7 (HHV-7). HHV-6A may cause roseola, and it can cause some neurological disorders, but the majority of asymptomatic infections are followed by persistent viraemia. HHV-6B is well-known for causing the majority of exanthema subitum cases, a childhood disease, but the primary HHV-6 infection can cause acute encephalopathy without exanthema subitum. After primary infection, the viruses may also persist in the body causing latent infection, but fever, diarrhoea, rash, neurological symptoms and hepatitis are caused occasionally, as well. Following transplantation, reactvivation of HHV-6A and 6B may occur with encephalopathy, pneumonitis, delayed platelet engraftment or thrombotic microangiopathy. Pediatric acute encephalopathy was sometimes attributed to virus infection. Certain virus families, especially herpesviruses such as HHV-6, can cause encephalitis. Authors present cases of children under two years old of age, and draw attention to the difficulties of clinical and laboratory diagnostics. One of the children died within 24 hours after hospitalisation. When hospitalized her general conditions was satisfactory, but she was pale, apathetic, had nasal secretion, fever and 14 hours later she had repeated convulsions with loss of consciousness. HHV-6, respiratory pathogens, entero-, hepatitis A- and E-, rota-, adeno- and calicivirus (as well as other pathogens *Toxoplasma gondii* and *Acanthamoeba* sp.) appear to play role in clinical course of the disease.
Blood, cerebrospinal fluid, ascites, stool, pericardial fluid and tissue-samples were tested. The other child recovered with residual symptoms. HHV-6 encephalitis must be treated with ganciclovir and foscarnet alone or in combination. Cidofovir is recommended as a second-line therapy. Other antiviral compounds potentially effective against HHV-6 are cyclopropavir, H2G, brincidofovir, maribavir, CMV423, 3-Deaza-HPMPA, Artesunate. In conclusion, primary HHV-6 infection can cause acute encephalopathy with and without exanthema subitum. The etiological diagnosis is possible only by testing both cerebrospinal fluid and blood. This condition should always be included in the differential diagnosis of acute encephalopathy even in patients older than 3 years, because HHV-6 viral infection might result in a severe acute encephalopathy.

STUDY OF HUMAN POLYOMAVIRUS 3, 4 AND 9 IN RESPIRATORY AND CEREBROSPINAL FLUID SAMPLES

ESZTER CSOMA[1], MELINDA KATONA[1], ATTILA SZŰCS[2], ERIKA PÁSZTI[2], LAJOS GERGELY[1]

[1]Department of Medical Microbiology; [2]Department of Otolaryngology and Head and Neck Surgery, University of Debrecen, Debrecen, Hungary

Respiratory transmission of human polyomavirus 3 and 4 is suggested, although the site, cell of replication is not yet known. These viruses may replicate themselves locally in the respiratory tract or may spread in the body. Since both the well known, "old" BK and JC polyomaviruses are able to infect the central nervous system, it is suggested that the novel polyomaviruses also might be. Little or hardly anything is known about human polyomavirus 9, not even the way of transmission. The aim of our study to examine the way of the transmission, the site of the replication, the possibility of latency and the clinical consequences of human polyomavirus 3,4 and 9. Adenoids, tonsils, upper respiratory and ear discharge samples from 129 patients (age range: 2.5-39.2 years; median: 5.3 years) were collected and analyzed for the presence of human polyomavirus 3,4 and 9 by different PCR methods. All the studied viruses were detected, and human polyomavirus 4 shows the highest prevalence. Examinations, analyzes are in progress, but the data refers to that the viruses may replicate in the respiratory tract. The presence of the novel human polyomaviruses is also examined in 97 cerebrospinal fluids from 95 patients (age range: 1 day-92.8 years; median: 19.4 years) suffering from encephalitis. Samples were collected for the routine diagnosis of human herpesvirus 1 and 2, however altogether 5 samples for positive for human herpesvirus 1.
There is no probability that these novel polyomaviruses are causative agents of encephalitis, since none of the studied polyomaviruses was detected from the samples.

EXAMINATION OF THE GENETIC DIVERSITY OF *CANDIDA ZEMPLININA* WINE YEAST

HAJNALKA CSOMA, LAJOS ÁCS-SZABÓ, LÁSZLÓ ATTILA PAPP, MÁTYÁS SIPICZKI

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

Candida zemplinina is one of the most studied wine-associated yeast in the last decade. It could be used in winemaking as co-starter with *Saccharomyces cerevisiae* due to its favourable oenological properties. The mtDNA restriction - or microsatellite analysis are commonly used in the examination of the yeast ecology of spontaneous fermentation, biogeography and biodiversity. Both methods are applied in the investigation of the intraspecific diversity of *Saccharomyces* and non-*Saccharomyces* species strains. To investigate the intraspecific mtDNA variations of *C. zemplinina* yeast, we analyzed the RFLP patterns of the mitochondrial genomes from a set of 41 *C. zemplinina* isolates. These strains were isolated in various countries worldwide from ecological sources of various kinds (grape/wine, insect guts, rotten fruits, and soil). 7 restriction endonucleases were used to digest the mtDNA. To gain greater insight into the biodiversity of this species, we compared the results of the mtDNA analysis with specific microsatellites markers developed for *C. zemplinina* by Masneuf-Pomarede and co-workers (2015). The band patterns of the mtDNAs and microsatellites were used to create a dendrogram using the UPGMA method. Our results showed that populations isolated from wines and vineyards are quite diverse at the genetic level. Strains isolated from the same vineyard or wineries usually displayed quite different genotypes and were frequently distributed throughout the dendrogram trees. The strain isolated in the USA from *Drosophila pinicola* not of eonological origin, was found to be the most distantly related to the type strain of the species and formed the outermost group on the dendrogram. We suppose a link between the substrate origin of the strains (fruit/eonology versus wild origins) and genetic diversity of the mtDNA.

SEARCHING FOR INTRASPECIFIC POLYMORPHISM OF *ZYGOSACCHAROMYCES LENTUS* WINE SPOLAGE YEAST STRAINS

HAJNALKA CSOMA, LÍVIA DÁLYAI, LAJOS ÁCS-SZABÓ, MÁTYÁS SIPICZKI, IDA MIKLÓS

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

The *Zygosaccharomyces* genus is polyphyletic and consists of two groups. The first group is indicated as *Zygosaccharomyces sensu stricto* group and includes several species, among them *Z. lentus*. Species of the *Zygosaccharomyces* genus are often regarded as synonymous with food spoilage. Namely, this genus includes some of the most osmotolerant organisms known, yeast that are able to resist concentrations of food preservatives vastly in excess of those normally encountered. Thus, products with low pH or high sugar concentration, such as soft drinks and fruit juices or wine are at risk of deterioration caused by *Zygosaccharomyces*. We isolated *Z. lentus* strains from three sweet wines in Hungary (Wines-A, B, C). Macro- and microscopic differences were observed among the strains. Thus their karyotypes and the mtDNAs were investigated to detect possible intraspecific polymorphism. Chromosomal profiles of 12 Hungarian, one French (CBS 3014) and one Swiss (CBS 2009) wine strains as well as two food spoilage isolates (CBS 8574T, CBS 8517) were determined by pulsed field gel electrophoresis using CHEF-Mapper apparatus. We observed 9 karyotype-patterns among the 16 strains. The size of the chromosomes ranging from above 2,2 to 1,0 Kb. Some isolates have 5, other have 6, 7 or 8 chromosomes. The electrophoretic karyotyping showed a high level of variability in the case of *Z. lentus* strains isolated from wines. The characterization of our isolates was also done using RFLP's of the mitochondrial DNA. MtDNA restriction patterns generated with HinfI and RsaI were used to create dendrogram with the UPGMA method. Our results showed that, most strains isolated from Wine-A belonged to one group, while isolates of Wine-B, C and the strains isolated in Switzerland and France from wine showed similar features and belonged to another group. The examined strains do not form groups based on their geographical origin. Instead, the source could affect the molecular characteristics of the *Z. lentus* strains.
The knowledge of the biodiversity of this spoilage species could contribute to the understanding of their adaptation to alcoholic beverages such as wine.

INVESTIGATION OF ANTIBIOTIC SUSCEPTIBILITY, BIOFILM FORMING ABILITY AND OXIDO-REDUCTIVE CONDITION OF CLINICAL ISOLATES OF *CANDIDA* SPECIES

LILLA CZUNI[1], PÉTER URBÁN[1], GÁBOR PAPP[2], FANNI GÉCZI[3], DOMINIKA KOVÁCS[2], CSABA FEKETE[1], GÁBOR MÁTÉ[1], MARIANNA HORVÁTH[3], CSABA VÁGVÖLGYI[4], BÉLA KOCSIS[3], ZOLTÁN GAZDAG[1]

[1]Department of General and Environmental Microbiology, Faculty of Sciences; [2]Microbial Biotechnology Research Group, János Szentágothai Research Centre; [3]Department of Medical Microbiology and Immunology, Medical School, University of Pécs, Pécs; [4]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Candida species are opportunistic human pathogen yeasts, causing invasive mycoses, and also candidaemia. *C. albicans* is able to form biofilm on almost all of the medical devices, e.g., catheters and prostheses. These biofilms show high resistance against antibiotics used in clinical practice. The principal aim of our study is to investigate whether the cell antioxidant protector system contributes to the azole resistance in normal state of the cell, or the protecting system of the cell responds better to the azole treatment. Some components of the oxidative stress and stress responses were

investigated earlier, however, comprehensive study has not been carried out yet. Our aims were also to investigate the antibiotic susceptibility and biofilm forming ability of 54 human *Candida* isolates; germ tube forming ability (an important virulence factor) of 11 *C. albicans* isolates; investigate the mixed biofilm forming ability of two *Candida* species. For the investigation of oxidative stress, resistant and sensitive *C. albicans* strains were selected and determined their thiol-, superoxide anion radical and total reactive oxygen species (ROS) content. *C. albicans* ATCC 44829 have been used as a control strain. Fluconazole (FLU) and voriconazole (VOR) resistant (FLU: MIC≥64μg ml^{-1}, VOR: MIC≥4μg ml^{-1}) isolates of *C. albicans* (FLU or VOR resistant number of species (n.s.): 3;4), *C. tropicalis* (n.s.: 7;3) and *C. krusei* (n.s.: 1;0) strains have been found. Most of the resistant isolates (82%) also revealed biofilm forming ability on polystyrene microtiter plates. Significant relationship between the germ tube formation and resistance, and between the germ tube formation and biofilm forming ability in the case of *C. albicans* isolates have not been found, however, the high proportion of resistant and biofilm-forming isolates form also germ tube. *C. tropicalis* (SZMC 1404) strain is significantly higher biofilm former in dual species biofilm with *C. dubliniensis* (SZMC 1471) and *C. krusei* (SZMC 779), compared to single biofilms of these isolates. To determine the concentrations for further oxidative stress analysis, growth rate of selected *C. albicans* strains have been investigated during 4 h VOR treatment in shaking cultures. VOR concentrations selected which allow 50% growth compared to the control. These concentrations for sensitive, control and resistant strains were 4, 8 and 64 μg ml^{-1}, respectively; however, 4 μg ml^{-1} VOR treatment has also been tested for all these strains. VOR treatments with above mentioned concentrations have caused a 3.1-to 6.3-fold increase in thiol content of the tested *C. albicans* strains. However, 4 μg ml^{-1} VOR treatments have increased the total ROS level of all the tested strains, but the superoxide anion radical have increased only in the non-biofilm forming azole resistant strain, almost sevenfold.

According to our opinion, better understanding of the relationship of oxidative stress and azole resistance can contribute to the clinical treatment of resistant *Candida* infections.

This work was connected to the project GINOP-2.3.3-15-2016-00006.

OENOLOGICAL CHARACTERISATION OF *ZYGOSACCHAROMYCES LENTUS* WINE SPOILAGE YEAST STRAINS

LÍVIA DÁLYAI[1], HAJNALKA CSOMA[1], ZOLTÁN KÁLLAI[2], MÁTYÁS SIPICZKI[1], IDA MIKLÓS[1]

[1]Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen; [2]Research Institute of Viticulture and Oenology Tokaj, Tarcal, Hungary

Special attention is paid to the yeast species of the genus *Zygosaccharomyces* as spoilage yeasts within the food industry. Their ability to grow in high sugar, high acid and ethanol content environments in the presence of preservatives creates difficulty for the food industry. Spoilage results in yeast clouds, particulates, off-flavours, odours and excessive gas production. We isolated *Zygosaccharomyces lentus* strains from three sweet wines in Hungary (Wines -A, B and C). The wines were aged in wooden barrels in cold wine cellars. On the basis of previous studies, the strains of the species have a very wide pH range (1.9-7.0) for growth, are osmotolerant (60%) and grow well at 4°C. Strong gas production is shown during sugar fermentation. The literature does not cover the oenological properties of *Z. lentus* wine spoilage yeasts; therefore we examined some of these features. The growth of 12 own and 4 CBS strains at 10°C-37°C, 1% acetic acid resistance, H_2S-production on Nickerson medium, acid production on $CaCO_3$ agar and qualitative extracellular enzyme activity were determined. Examination of the fermentation power, determination of the physical-chemical parameters of the must samples and the qualitative identification of the secondary

metabolites by GC-MS are in progress. All of our isolates were grown at 10°C and were unable to grow at 37°C. They tolerated 1% acetic acid; produced organic acids and hydrogen-sulfide. The examined *Z. lentus* strains did not secrete extracellular cellulase, protease, β-glucosidase or amylase enzymes. 99% of the strains isolated from Wine-A creates star-shaped multicellular clumps. Their oenological properties are quite the same: low sulphate reduction, delayed growth on 1% acetic acid. The cells of the strains isolated from Wines-B, C and CBS 2900 (isolated in Switzerland) as well as CBS 3014 (isolated in France) occur singly, in pairs or forms pseudomycelia.
The examined oenological properties of this morphological group were not uniform. We detected differences both in the amount of H_2S and organic acid production.

SCREENING OF EMERGING VIRAL INFECTIONS AMONG HUNTERS, FISHERS, GARDENERS AND KEEPERS

JUDIT DEÁK[1], GÁBOR KEMENESI[2], FERENC JAKAB[2]

[1]Institute of Clinical Microbiology, University of Szeged, Szeged; [2]Virological Research Group, János Szentágothai Research Center, University of Pécs, Pécs, Hungary

Among viral infections, Hantaviruses and West Nile viruses (WNV) have been detected. Chikungunya virus, which can cause outbreaks in the temperate region, has been found in Italy. The members of Sandfly fever viruses (Genus: *Phlebovirus*): Sicilian, Naples, Toscana and Cyprus types have been detected in Italy, Portugal, Spain, France, Greece, Austria, Croatia and Turkey. Crimean-Congo hemorrhagic fever has been detected in the Balkan states, and dengue fever in Croatia, France and Norway. *Aedes albopictus*, the vector of yellow fever, is widespread among the European coastal regions and islands. The history of yellow fever and dengue fever in temperate regions confirms that the transmission of both diseases could recur. PCR and RT-PCR (TIB Molbiol, Berlin, Germany and Roche, Indianapolis, USA) methods have been introduced for the screening of the nucleic acids of Chikungunya, Crimean-Congo hemorrhagic fever, Dengue, Hanta, Sandfly fever, and West Nile viruses in healthy risk groups (hunters, fishers, gardeners and keepers in zoological gardens) and blood donors in South Hungary. Indirect immunofluorescence (IIF) methods were performed with BIOCHIP slides (Euroimmun Med. Lab. AG., Lübeck, Germany). PCR examinations proved negative both in risk groups and controls. Hantavirus Seul, Dobrava, and Puumala IgG antibodies proved positive in the cases of 5, 4 and 1 individuals, respectively. Sandfly fever viruses: Sicilian, Naples, Toscana and Cypres IgM were positive in 6, 2, 5 and 1, and IgG antibodies in 21, 17, 16 and 11 individuals. WNV IgM was positive in 3, and IgG in 22 cases. Chikungunya and Crimean-Congo IgM and IgG were negative. Dengue virus IgM was positive in 10 cases, while IgG was negative. The intensification of migration, the growth in the density of the population, the susceptibility to infectious diseases, the decline of human immunity in consequence of insolation (UV effect), and malnutrition all tend to make humanity sensitive to infectious illnesses. Prevention, recognition, early diagnosis and treatment are very important to counter local endemics and epidemics.
Funding from the Hungarian National Development Agency (TÁMOP-4.2.2.A-11/1/KONV-2012-0035)

DRUG RESISTANCE IN THE TREATMENT OF CHRONIC VIRAL HEPATITIS

ÁGNES DENCS, CSILLA STYEVKÓ, ANDREA HETTMANN

Division of Virology, National Center for Epidemiology, Budapest, Hungary

Treatment of chronic viral hepatitis is still complicated by the emergence of drug resistant virus variants. Until recently all approved regimens for treating hepatitis C virus (HCV) infection included pegylated interferon-α combined with ribavirin with or without additional protease inhibitor. Interferon based therapy is effective in about 40-80% of the patients and is associated with serious side effects. Interferon free treatment regiments combining direct acting antivirals (DAA) have been a major advancement in the therapy of chronic hepatitis C virus infection. Even though most of the patients achieve sustained viral response, treatment still fails in 1-15% of the cases. Most of these patients experience a relapse after completion of therapy. The most important factors affecting treatment success are the patients' therapy adherence, their genetic background, their level of liver damage, but most commonly the presence or the emergence of viral strains resistant to DAAs. A second treatment is an option, but it is more important to optimize the first-line regime to effectively prevent therapy failure due to drug resistance. Besides pegylated interferon, nucleot(s)ide analogs (NAs) have been widely used in the treatment of chronic hepatitis B virus (HBV) infection for years. They effectively inhibit the viral polymerase, but after years of treatment viral variants may appear, which are partially or completely resistant to these compounds. Monotherapy with earlier introduced NAs lamivudine and adefovir often resulted in the selection of resistant HBV strains. Today the preferred first choices for therapy are entecavir and tenofovir, which have a high genetic barrier, thus resistance rarely develops in treatment naive patients. However, the presence of certain mutations in the viral population due to previous treatment with lamivudine or adefovir greatly increase the risk of treatment failure. We detected amino acid sequence changes in the polymerase of hepatitis B viruses of patients receiving nucleot(s)ide analog treatment and previously untreated patients. We used databases and software available online to genotype the these viruses and to predict the effects of the mutations to antiviral treatment. We identified mutations conferring NA resistance, compensatory mutations and several changes without knows effects. These mutations were common in both treated and treatment naive patients. Based on these results we suggest resistance testing should be considered before treatment of chronic HBV carriers with nucleot(s)ide analogs.

NEW SPECIES AND LINEAGES DISCOVERED IN THE GENUS *CORTINARIUS* (BASIDIOMYCOTA) FROM AFRICA AND MADAGASCAR

BÁLINT DIMA, GÁBOR M. KOVÁCS

Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, Budapest, Hungary

Cortinarius is the most species rich genus of the Agaricales with more than 2500 species distributed worldwide. As ectomycorrhizal fungi in wide range of habitats they play important role in ecosystem functioning. Our knowledge on the taxonomy, ecology and distribution of the genus is limited to the species of the Northern Hemisphere (mainly from Europe and less from North America), while studies of species growing in Southern Hemisphere almost completely lacking and those done have concentrated on Australia, New Zealand and South America. Our aims were to study specimens originated from Madagascar and tropical Africa with the aid of molecular methods and to test our hypothesis whether *Cortinarius* species associated with endemic plants belonging to the genera *Uapaca*, *Brachystegia* or families Asteropeiaceae and Sarcolaenaceae represent new species and/or even form new evolutionary lineages within the genus. Genomic DNA was extracted successfully from 25 specimens collected in Madagascar and Tanzania deposited in the National Museum of

Natural History, Paris (PC). We amplified the nrDNA ITS and LSU regions and phylogenetic analyses were performed with RAxML for both loci separately as well as for the concatenated dataset. Our preliminary phylogenetic analyses revealed at least 15 new *Cortinarius* species belonging to major clades/sections such as Anomali, Cortinarius, Delibuti, Dermocybe and Obtusi.
Furthermore a distinct clade formed by at least four different species appears to represent a new lineage within subgenus *Telamonia* which was previously known as exclusively distributed in the Northern Hemisphere. Our results indicate that the African continent including Madagascar potentially hosts many undescribed *Cortinarius* species and these taxa could help us to complete our knowledge of the evolutionary history of the genus.
Acknowledgements: We are grateful to Bart Buyck (PC) for managing the loans of the specimens.

FUNCTIONAL ANALYSIS OF GENOME WIDE TRANSCRIPTIONAL CHANGES UNDER OXIDATIVE STRESS IN *ASPERGILLUS NIDULANS*

TAMÁS EMRI[1], ERZSÉBET OROSZ[1], KÁROLY ANTAL[2], ISTVÁN PÓCSI[1]

[1]Department of Biotechnology and Microbiology, University of Debrecen, Debrecen; [2]Department of Zoology, Eszterházy Károly College, Eger, Hungary

Genome wide expression changes caused by three different oxidative agents - menadione sodium bisulfite (MSB), t-butylhydroperoxide (tBOOH) and diamide - were examined in a wild type and in an oxidative stress sensitive, delta-*atfA* mutant *Aspergillus nidulans* strain. Gene enrichment analysis of stress responsive genes suggested significant alterations in several biological processes including down-regulation of "ribosome biogenesis", "mitotic cell cycle" process, "ergosterol biosynthesis" and "nitrate utilization" as well as up-regulation of "antioxidative enzyme production" in all the three stress conditions. Up-regulation of "siderophore biosynthesis" genes and down-regulation of endoplasmatic reticulum related processes were characteristic for tBOOH and MSB stresses, respectively. "peroxisome organization" genes as well as "fatty acid catabolic process" genes were up-regulated both in tBOOH and diamide stresses, while "FeS cluster assembly" genes were up-regulated after MSB or diamide stress treatments. Deletion of the *atfA* gene - which encodes a true functional orthologue of fission yeast's Atf1 transcription factor - caused significant changes in the transcriptome. It prevented the proper up- or down-regulation of several biological processes (e.g. "FeS cluster assembly", "mitotic cell cycle", "ER to Golgi vesicle-mediated transport" and "nitrate utilization") in some but interestingly not necessarily all the three stress conditions. It modified the composition and the size of the co-regulated gene sets (containing genes showing unidirectional transcriptional changes under the three stress conditions) in many functionally related gene groups (e.g. "ribosome biogenesis" and "fatty acid catabolic process"). Deletion of *atfA* also made the three stress responses (at the level of regulated biological processes) more similar to one and other than it was found in the control strain. A simple model based on the networking nature of signal transduction was created and will be summarized in the presentation to explain the background of these changes.
This work was supported by the Hungarian Scientific Research Fund (K112181)

CULTIVATION OF RADIORESISTANT BACTERIA FROM THE BIOFILM DEVELOPED IN THE DIANA-HYGIEIA THERMAL SPRING

NÓRA TÜNDE ENYEDI[1], DÓRA ANDA[1], ANDREA BORSODI[1], JUDIT MÁDL-SZŐNYI[2], KÁROLY MÁRIALIGETI[1], JUDIT MAKK[1]

[1]Department of Microbiology; [2]Department of Physical and Applied Geology, Faculty of Science, Eötvös Loránd University, Budapest, Hungary

The Buda Thermal Karst System is a hypogenic karst area where thermal and lukewarm springs and spring caves can be found. The Diana-Hygieia spring cave (DHSC) is completely filled with thermal water where intensive bacterial colonization can be observed. The subaqueous iron-rich biogeochemical layer forming on the rock surfaces is efficient in absorbing radium which causes elevated (concentrations up to 1005,8 Bq/l) radon activity (by alpha decay of radium). The aim of this study was to isolate and identify radioresistant bacteria from the intact biogeochemical layer of the DHSC and following the irradiation of the samples taken from two places. The irradiation was performed by gamma radiation at various doses (5, 10, 15 kGy) using NORATOM equipment with a 60 Co source. The most abundant bacterial 16S rRNA gene sequences were different between the irradiated and non-irradiated biofilm samples. Members of the phyla Actinobacteria, Firmicutes, Alpha-, Gammaproteobacteria, Deinococcus-Thermus and Bacteriodetes were represented in irradiated sample, while Actinobactera, Firmicutes, Alpha- and Betaproteobacteria and Deinococcus-Thermus could be detected in non-irradiated biofilms. Isolates belonging to the genera *Bacillus*, *Micrococcus*, *Mycobacterium*, *Paracoccus*, *Kytococcus*, *Dermacoccus* and *Marmoricola* were cultivated from the highest dose (15 kGy) irradiated biofilm sample.
In accordance with the literature data, representatives of radioresistant extremophiles could be identified from the DHSC following the irradiating experiments.
This research was supported by the Hungarian Scientific Research Fund (OTKA) Grant NK101356.

DETAILS FROM THE LIFE OF A HUNGARIAN SME IN INDUSTRIAL MICROBIOLOGY: FERMENTIA'S 10TH ANNIVERSARY

BALÁZS ERDÉLYI, DIANA WEISER, SZABOLCS KOHÁRI, PÉTER SÁTORHELYI, VIKTÓRIA BÓDAI

Fermentia Microbiological Ltd, Budapest, Hungary

The Institute for Drug Research (GYKI) was involved in industrial microbiological projects for more than 50 years. The production of antibiotics by industrial fermentations was a key task in the life of GYKI, thus a separate Department of Microbiology and Fermentation Pilot Plant were established. By the time of termination of GYKI in 2006 several successful process adaptations were implemented in Hungarian pharmaceutical companies. The initial project of freshly established Fermentia Ltd. was to maintain the fermentation processes and keep the staff. Five members from the working team of GYKI are being involved in the current staff of Fermentia's 15 employees. Currently Fermentia has three main activities. One of the significant financial incomings base on the contract manufacturing. Basically, different bacterial soil inoculant fertilizers are produced, but the production of valuable proteins, enzymes or the microbial synthesis of optically pure alcohols as drug intermediers are also dynamically growing areas. In recent years, the production of dietary supplements containing probiotics by biotechnological methods became more and more important part of Fermentia's life. Our costumers network build from pharmaceutical-, food-, or agricultural companies from Hungary and other countries mainly of the EU area. We have invested and are currently investing considerable amounts of energy on the development of our own enzyme products and services. The immobilized lipase and ketoreductase enzyme-preparates can be used in syntheses of various chiral centers. Our shared project with the Department of Microbiology, Faculty of Science, Eötvös L. University is an effort to solve the biodegradation of polychlorinated hydrocarbons accumulated in ground-water. This bioremediation project is supported by the Norway Grants. In recent years we have successfully

closed 4 projects supported by various Hungarian and EU grants. There are currently two ongoing projects supported by the Norway Grants. Our third field of interest is the education, in which all of our researchers take part. Many BSc and MSc theses were written with the supervision of Fermentia Ltd., we have done guest lectures, several universities bring their bioengineer students for an educational visit in our plant, furthermore our colleagues also participate in the work of different examination boards of Hungarian universities. Every summer 3-5 students can do their summer practice in our laboratories. In the past ten years Fermentia Ltd. has upgraded most of its equipments. Both the power network and the auxiliary power network have been replaced, and an up-to-date process control system was put in place for the supervision of our plant. The controllers of the fermentors meet every requirement. New equipments have been acquired for the support of the downstream processes (homogenizer, lyophilizer, centrifuges, photometers, FPLC system, etc.).

MODELING LIGNOCELLULOSIC MATERIAL BIODEGRADATION WITH FUNGAL MULTICULTURES

CSILLA FARKAS, DÁNIEL KOLPASZKY, JUDIT REZESSY-SZABÓ, NGUYEN DUC QUANG

Department of Brewing and Distilling, Szent István University, Budapest, Hungary

Biological pre-treatment of lignocellulose-based agricultural by-products is an environmentally friendly and economical approach to produce sustainable green energy source. A variety of organisms, including different fungal and bacterial strains, efficiently degrade lignocellulolytic biomass even in solid state. Due to the structure and heterogeneity of these materials, bioconversion requires multiple enzyme activities. The beneficial effects of synergism between several organisms in multi-cultures enhance the released sugar yields and result in lower production costs compared with monocultures. In this study, we used 30 different lignocellulose-degrading filamentous fungal strains that were obtained from NCAIM (Budapest, Hungary). The strains were incubated on malt agar slants at 30°C and stored at 4°C until use. Under solid state conditions, wheat bran was inoculated with 106 conidia.10 g^{-1} substrate, which was supplemented with citrate-phosphate buffer to reach desired initial moisture content. The microbial-based process was carried out at 30°C for 168 hours. The amounts of carbohydrates and degradation products were monitored by the HPLC (Thermo Corporation USA). Several combinations of fungal strains were investigated and found that application of the multi-cultures of three strains resulted about 2.5-4-folds increase in saccharification efficiency compared to each mono-cultures. The soluble carbohydrate content reached 195 gL^{-1} level at 48 hours when wheat-bran was treated by fungal consortia of *Aspergillus niger* F.00632, *Penicillium chrysogenum* F.00814 and *Trichoderma viride* F.00795 strains.

The optimal parameters for fungal consortia were obtained using the following conditions: ratio of conidia of individual strains was 6:2.5:1.5 in 10^6 conidia.10 g^{-1} substrate; 5:1 ratio of initial moisture content to substrate, pH 5.5 and at 30°C. In this case, 28.5 gL^{-1} DP2, 35.2 gL^{-1} glucose, 13.7 gL^{-1} xylose were obtained at 24-48 hours of treatment. In each microbial pre-treatment, a decline tendency of the microbial growth was observed after 48-72 hours. To enhance the effect of multi-cultural pre-treatment, two step inoculation technique was used. In the first step *Phanerochaete chrysosporium* F.00740 strain was inoculated. The second step will follow it after 168 hours by inoculation of triple combination. In this case up to 25-30 gL^{-1} even more released carbohydrates were observed at 24-48 hours. Overall, fungal multi-cultures were able to convert efficiently lignocellulolytic biomass, as low-cost substrate into valuable products.

FED-BATCH AND CONTINUOUS CHEESE-WHEY FERMENTATION WITH MIXED CULTURE OF *SACCHAROMYCES CEREVISIAE* AND *KLUYVEROMYCES MARXIANUS*

CSILLA FARKAS[1], JUDIT REZESSY-SZABÓ[2], VERONIKA KEREPESI[2], NGUYEN DUC QUANG[2]

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

Sustainable bio-utilization of whey or whey permeate is a challenge for environmental problems of its high BOD value mainly caused by lactose. An alternative economical utilization of whey could be production of ethanol for biofuel industry. *Saccharomyces cerevisiae* yeast that is generally used in alcoholic fermentation efficiently converts some sugars mainly glucose into ethanol, but due to lack of β-galactosidase activity it does not able to ferment lactose directly. In contrast to it, some strains of *Kluyveromyces* yeast are able to hydrolyze and convert lactose to ethanol without any extragenous enzymes, thus combination of two yeast species can be exploited to enhance ethanol production and decrease residual amounts of lactose in whey. In this study, investigation of the effects of mixed culture at fed-batch and continuous fermentation process were focused. *Kluyveromyces marxianus* Y00963 facultative anaerobic yeast strain was obtained from NCAIM (Budapest, Hungary) and incubated on malt agar slants at 30°C. The culture medium contained 20 gL^{-1} glucose, 10 gL^{-1} yeast extract and 20 gL^{-1} peptone and sterilized at 121°C for 15 min. *Saccharomyces cerevisiae* dried yeast (Levuline FB) was rehydrated in 10% (w/v) glucose solution supplemented with complex manure (UVAVITAL, 25-30 ghL^{-1}). The concentration of cheese-whey powder (CWP) was varying between 10-30 % (w/v). The pH was adjusted to 4.0 with adding 21% (v/v) phosphoric acid. The fed-batch fermentation was operated for 7 days at 30°C with two times feeds per 24 hours and 48 hours. The continuous fermentation bioreactor filled with immobilized yeast cells on SIRAN Sikug® 035/xx/300/A beads was operated at 25°C for 28 days. The amounts of carbohydrates were monitored by the HPLC (Thermo Corporation USA). In the fed-batch fermentation, increased ethanol yield and reduced time demand was observed at supplementation of inoculum with 0.5% (w/v) CWP and two times feeds of 15% (w/v) CWP solution at 24 hours. The higher presence of thermotolerant yeast in mixed cultures showed significantly enhanced the rate of lactose utilization thus ethanol production. To improve efficiency of conversion of lactose to ethanol, different co-culture inoculation procedures was investigated at initial 15-20% (w/v) CWP solution. The highest ethanol yield was about 52.8-70.2 gL^{-1} and lactose utilization was about 69-88% by adding three-quarter at 24 hours and one-quarter at 48 hours. In the continuous fermentation, the maximum productivity (7.46 $gL^{-1}h^{-1}$) was gained from 10% (w/v) substrate in the continuous feed at a dilution rate of 0.14 h^{-1}. The ethanol concentration and efficiency were 51.8 gL^{-1} and 67.7%. Our results are very promising and can serve bases for development of environmental and value-added technology for treatment of cheese-whey.

ANALYSIS OF EVOLUTIONARY MECHANISMS IN HUNGARIAN CHICKEN *ORTHOREOVIRUS* STRAINS

SZILVIA FARKAS[1], SZILVIA MARTON[1], RENÁTA VARGA-KUGLER[1], ESZTER DANDÁR[2], BENCE GÁL[1], FERENC JAKAB[3], ÁDÁM BÁLINT[4], SÁNDOR KECSKEMÉTI[4], KRISZTIÁN BÁNYAI[1]

[1]Institute for Veterinary Medical Research, Centre of Agricultural Research, Hungarian Academy of Sciences, Budapest; [2]United Szent István and Szent László Hospital, Budapest; [3]János Szentágothai Research Centre, University of Pécs, Pécs; [4]Veterinary Diagnostic Directorate, National Food Chain Safety Office, Budapest, Hungary

Avian orthoreovirus (ARV) infections are widespread and had been associated with various clinical manifestations in domestic poultry. The majority of the strains, nearly 80% are non-pathogenic, but the remaining 20% causes considerable economic losses. Sequence data collected in the past few years suggests that the genetic material of ARV strains is continously changing due to the well-known evolutionary mechanisms of reoviruses such as point mutation and reassortment. In our study we determined the near complete genome sequences of ten ARV field strains collected from young chicken between 2002 and 2011 in Hungary to gain genetic data about circulating strains and to explore the genetic diversity and evolutionary mechanisms affecting avian orthoreoviruses in this region. Sequence analyses and phylogenetic calculations revealed that eight out of ten Hungarian strains showed the highest homology with the recently described Hungarian ARV strain T1781. The remaining two strains showed the highest similarity with the US origin reference strain AVS-B. Phylogenetic analysis of the 10 genomic segments resulted in different tree topologies, indicating the common evolutionary origin of these strains and also suggested that certain segments may have been constituted by distinct evolutionary mechanisms. According to our calculations numerous reassortment events might occurred recently between Hungarian, US, and Asian strains affecting mainly the outer capsid and non-structural protein coding genes. When analysing the μB gene a possible heterologous reassortment event was identified in three Hungarian strains. Recombination analysis revealed twelve putative recombination events, implying that beside point mutations and reassorment this mechanism also plays an important role in the diversification of ARVs.

CHANGES IN PERIOPERATIVE ANTIBIOTIC CHEMOPROPHYLAXIS AFTER ESTABLISHMENT OF CLINICAL PHARMACIST CONSULTATION IN AN ORTHOPAEDIC SURGERY WARD

ADINA FÉSÜS[1], ORSOLYA GORÁCZ[1], BÁLINT BALÁZS[2], JULIANNA MÓZES[2], GÁBOR KARDOS[2]

[1]Clinical Pharmacy; [2]Department of Medical Microbiology, University of Debrecen, Debrecen, Hungary

Systemic perioperative antibiotic prophylaxis is the standard practice to reduce surgical site infection risk in joint replacement surgery. An up to 24 hours prophylaxis, preferably with 1st or 2nd generation cefalosporins is recommended for noncomplicated cases. Our aim was to compare antibiotic prophylaxis in an orthopaedic surgery ward in 2010 and after five years of clinical pharmacist consultation in 2015-16. In 2010, 466 cases (250, 122, 31 and 63 undergoing total hip replacement, total knee replacement, implant revision and other operations including fracture fixation or scoliosis correction, respectively) were studied, while in 2015-16 193 cases (93, 79, 5, 12 and 4 undergoing total hip replacement, total knee replacement, total shoulder replacement, implant revision and other operations, respectively). All patients received perioperative antibiotic prophylaxis, defined as antibiotic administration started as part of preparation for the operation. Drug name, dose, prophylaxis duration (number of days receiving the same regimen as in the operation room), number of hospital days and deviations from recommended prophylaxis schemes were recorded. Chi-square test or Kaplan-Meier test was used for statistical analysis as appropriate. Cefuroxim was the most frequently used prophylactic drug in case of all operation types in both periods. Though prophylaxis with unrecommended regimens remained similar (13.9%, 65/466 vs. 13.4%, 26/193), median

prophylaxis duration decreased markedly (from 4 to 1 day, p<0.001). This decrease was also seen with cefuroxim or ciprofloxacin prophylaxis, but not with prophylaxis with other drugs or drug combinations. Cefuroxim was overdosed in both periods, but overdosing increased in 2015-16 (33/423 vs. 175/179; p=0.009). Median length of hospital stay decreased significantly in all patients (10 vs. 7, p<0.001); considering operation types, this was marked in patients undergoing hip replacement (10 vs. 7, p<0.001), knee replacement (11 vs. 7, p<0.001), but not in patients with implant revision (14 vs. 11.5, p=0.11) or other operation types (9 vs. 10.5, p=0.93). Similar decrease was found in patients under ciprofloxacin (11.5 vs 7, p<0.001) or cefuroxim (10 vs. 7, p<0.001) prophylaxis, but not in patients with unrecommended prophylaxis (14 vs. 13.5, p=0.67). Further antibiotic administration was necessary significantly less frequently with cefuroxim prophylaxis (7/277) than with ciprofloxacin (11/27, p<0.001) or unrecommended regimens (20/38, p<0.001) in 2010, these differences were present, but were not statistically significant (11/165 vs. 2/13 vs. 3/15, respectively). Clinical pharmacist consultation improved the adherence to prophylaxis guidelines, which resulted in shorter hospital stays and decreased the need for administration of antibiotics.

This work was supported by a János Bolyai research scholarship of the Hungarian Academy of Sciences.

DETERMINATION OF IDEAL GROWTH CONDITIONS FOR *CHLORELLA VULGARIS*

DÁNIEL FÓZER, BERNADETT KISS, ÁRON NÉMETH

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

Microalgae has gained great attention in recent decades because of the potential to produce valuable components such as high energy density containing biofuels (e.g., biodiesel, bio-char, biogas), proteins, fertilizers, pharmaceuticals and animal feeds. In order to enhance the microlagae's growth rate and production, the ideal levels of the growth parameters have to be determined. We chose *Chlorella vulgaris* as a commonly known microalgae species to examine it's growth characteristics. In our investigation a 24-well microplate have been applied that allows the examination of six different cultivation adjustment at the same time.

Various cultivation media, irradiation colors and light intensities are investigated. Based on our experimental data cultivation scale-up becomes available. Scaled-up fermentations are also carried out with a payload capacity of 2 L, where optimal aeration rate and light intensities are determined and evaluated for the sake of production maximalization.

DISSIMILAR FITNESS ASSOCIATED WITH RESISTANCE TO FLUOROQUINOLONES INFLUENCED THE CLONAL DYNAMICS OF VARIOUS MULTIRESISTANT BACTERIA

MIKLÓS FÜZI

Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary

Fitness cost associated with resistance to fluoroquinolones was recently shown to vary across clones of methicillin-resistant *Staphylococcus aureus* and extended-spectrum β-lactamase-producing *Klebsiella pneumoniae*. The resulting dissimilar fitness should have influenced the clonal dynamics

and thereby the rates of resistance for these pathogens. Moreover, a similar mechanism was recently proposed for the emergence of the H30 and H30R lineages of ESBL-producing *E. coli* and the major international clone (ribotype 027) of *Clostridium difficile*. An ability to develop favorable mutations in the gyrase and topoisomerase IV genes seems to be a prerequisite for pathogens to retain fitness while showing high-level resistance to fluoroquinolones. The findings suggest that the use of fluoroquinolones as a function of clonal distribution could ameliorate the state of antibiotic resistance. When the proportion of multiresistant clones influenced by fluoroquinolones are low the use of these antibiotics remains highly beneficial. However when their proportion increases the consumption of fluoroquinolones should transiently be reduced.

STRUCTURAL AND FUNCTIONAL DETERMINANTS OF *NEOSARTORYA FISCHERI* ANTIFUNGAL PROTEIN (NFAP)

LÁSZLÓ GALGÓCZY[1], ATTILA BORICS[2], MÁTÉ VIRÁGH[3], CHRISTOPH SONDEREGGER[1], HARGITA FICZE[3], GYÖRGYI VÁRADI[4], ZOLTÁN KELE[4], FLORENTINE MARX[1]

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

The increased incidence of severe fungal infections and the fast development of drug resistant filamentous fungi causing mycoses, plant infections or damage to cultural heritages strongly demand for the development of new antifungal strategies. The NFAP from *Neosartorya fischeri* NRRL 181 is a member of the ascomyceteous, small, cysteine-rich, cationic, highly stable antifungal proteins. NFAP shows high antifungal activity on numerous filamentous Ascomycetes. Features of NFAP render it exceptionally suitable as potential commercial preservative, bio-pesticide and drug against moulds. However, the structure-function relationship of NFAP has not been investigated in detail so far. Our in silico homology modelling experiments and analyses revealed that the disulphide bridges, the hydrophobic core, and the N-terminal β-strand of NFAP are responsible for the stability and coordinate the folding and structural formation.

Rational amino acid substitutions destroy these structural elements without affecting the net charge and the grand average of hydropathy value of the protein. To prove our in silico hypotheses we expressed the NFAP and its structural mutant variants in *Pichia pastoris* and investigated their folding property, stability and antimicrobial activity. Electronic circular dichroism (ECD) spectroscopic measurements revealed that the structure of NFAP is folded. In contrast to this, its hydrophobic core (NFAPΔh) and N-terminal β-strand (NFAPΔN) variants are unordered. The disulphide bridge mutant variant (NFAPΔC) proved to be unstable; it was degraded during the expression. Compared to the NFAP, NFAPΔh and NFAPΔN showed no or reduced growth inhibition activity, heat, pH and salt tolerance in antifungal activity bioassays against *Aspergillus nidulans*. The role of the loop regions of NFAP in the antifungal activity was also investigated. We tested the antifungal effect of synthetic N-terminal loop peptide on NFAP-sensitive *Neurospora crassa*. The less hydrophilic and negatively charged N-terminal loop peptide caused morphogenetic changes on the hyphae, but did not destruct them, while the more hydrophilic and positively charged full-length NFAP was able to kill the germinating conidia. Based on these results, we can conclude that the investigated structural elements play a key role in the proper folding, stability and antifungal activity of NFAP and could serve as potential bases to improve the antifungal features.

L.G. holds a Lise Meitner fellowship from Austrian Science Fund (FWF): M1776-B20. Research of A. B. has been supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

COMPARING ORGANIC AND CONVENTIONAL LAND USE HISTORY AT THREE DIFFERENT HUNGARIAN SOILS BY PHYSIOLOGICAL PROFILES OF THE SOIL MICROBIAL ASSEMBLAGE

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

Institute for Soil Sciences and Agricultural Chemistry, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

Conservation and sustainable use of soil biodiversity is very important because soil microorganisms have an essential role in biogeochemical cycling, soil fertility, productivity and ecological stability. The metabolic activity of soil microbes can be measured on the basis of CO_2 production. The level of microbial activity is influenced by the soil moisture, temperature, and the amount of biodegradable organics in the soil. Respiratory activity of the soil microbial community was compared with three Hungarian locations (Martonvásár, Karcag and Nyíregyháza). Three soils differing in texture (a loam, a clay, and a sandy) with organic and conventional land use history and two different sampling time (fall and spring) were selected. Soil physical, chemical and biological properties were determined. Basal and substrate-induced respiration (SIR) were measured by gas chromatography equipment. Microrespiration method (MicroRespTM) were tested on the soils with 23 different substrates (simple sugars, amino acids and carboxylic acids).

MicroResp method were used to carry out colorimetric detection of short-term CO_2 evolved from substrate utilisation in soils. According to the current microrespiration statistical analyses (discriminant analysis) separated the samples to their own group in 100% according to the different substrate utilization pattern soil types and land uses as well are clearly separated from each other. Humus content, soil salinity, pH and the vegetation can affect the rate of CO_2 evolution (μg CO_2-C/g soil/hour) and the respiration pattern of samples by added substrates.

This research was financially supported by OTKA (K108572) and Research Institute of Organic Agriculture.

IDENTIFICATION OF MARKERS RELATED TO ANTIBIOTIC RESISTANCE AND DEVELOPMENT OF MOLECULAR METHODS FOR THE DETECTION OF ANTIBIOTIC SUSCEPTIBILITY OF *MYCOPLASMA BOVIS*

KINGA MÁRIA GÖRFÖL-SULYOK[1], ZSUZSA KREIZINGER[1], ENIKŐ WEHMANN[1], SZILVIA MARTON[1], KRISZTIÁN BÁNYAI[1], ÁKOS JERZSELE[2], INNA LYSNYANSKY[3], ZSUZSANNA RÓNAI[4], IBOLYA TURCSÁNYI[4], LÁSZLÓ MAKRAI[5], SZILÁRD JÁNOSI[4], SÁRA ÁGNES NAGY[1], MIKLÓS GYURANECZ[1]

[1]Zoonotic bacteriology and mycoplasmatology, Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, HUNGARY; [2]Department of Pharmacology and Toxicology, University of Veterinary Medicine, Budapest, Hungary; [3]Division of Avian and Aquatic Diseases, Kimron Veterinary Institute, Bet Dagan, Israel; [4]Veterinary Diagnostic Directorate, National Food Chain Safety Office, Budapest; [5]Department of Microbiology and Infectious Diseases, University of Veterinary Medicine, Budapest, Hungary

Surveillance for antibiotic resistance of *Mycoplasma bovis* isolates is essential for effective treatment. In vitro susceptibility testing of Mycoplasmas is a time-consuming and labor-intensive procedure, therefore not routinely performed in diagnostic laboratories. The aims of the current study were to determine mutations associated with antibiotic resistance in *M. bovis* isolates and the development of rapid and reliable genetic assays for the determination of antibiotic resistant *M. bovis* in clinical samples. For the identification of molecular markers of antibiotic resistance, whole-genome sequencing of 35 Hungarian *M. bovis* field isolates and 36 laboratory-derived antibiotic resistant mutants was performed. Mutations related to fluoroquinolone resistance were found on *gyrA* and *parC* genes of QRDR (Quinolone Resistance Determining Regions). Markers associated with tetracycline resistance were identified on genes of 16S rRNA forming tetracycline binding site. Alteration of the nucleotide 1192 of *rrs3* gene (according to *Escherichia coli* numbering) resulted in spectinomycin resistance. Mutations responsible for macrolide, lincosamide, phenicol and pleuromutilin resistance were identified on genes encoding the 23S rRNA. Current study reports 12 MAMA (Mismatch Amplification Mutation Assay) and 6 HRM (High Resolution Melt) tests designed and evaluated for the simultaneous detection of markers responsible for antibiotic resistance in *M. bovis*. Real-time PCR based genetic methods for the detection of fluoroquinolone resistance could be applied directly on DNA obtained from the clinical samples. For the detection of antibiotic resistance to 30S or 50S inhibitors previous identification of *M. bovis* and separation of gene duplicates by PCR are necessary prior the application of assays. The use of present method is highly cost-efficient and can provide antibiotic susceptibility profile to 13 antibiotics of 7 antimicrobial groups within 9 hours. The assays developed in the present study can be carried out as routine tests in diagnostic laboratories and allow clinicians to promptly select appropriate treatment options.

DETECTION AND GENETIC CHARACTERISATION OF A NOVEL PARVOVIRUS DISTANTLY RELATED TO HUMAN BUFAVIRUS IN DOMESTIC PIGS

RENÁTA HARGITAI[1], PÉTER PANKOVICS[1], ATTILA MIHÁLY KERTÉSZ[2], HUNOR BÍRÓ[2], ÁKOS BOROS[1], GIA PHAN TUNG[3], ERIC DELWART[3], GÁBOR REUTER[4]

[1]Government Office of Baranya County, Public Health's Organization, Pécs; [2]SHP Ltd., Kaposvár, Hungary; [3]Blood Systems Research Institute, San Francisco, US; [4]Department of Medical Microbiology and Immunology, Faculty of Medical Sciences, University of Pécs, Pécs, Hungary

Human bufavirus is a novel parvovirus detected in the faecal samples of children with acute diarrhea in Burkina Faso in 2012. The pathogenic role of human bufavirus is not confirmed – but distantly related parvoviruses were reported from shrews, non human primates and bats. Viral metagenomic analysis of faecal samples of domestic pigs suffering from posterior paralysis with unknown etiology and detection of human bufavirus from faecal samples of children with gastroenteritis. A randomly selected porcine sample was tested with viral metagenomics and next generation sequencing (NGS) methods. The bufavirus-like parvovirus genom was determined by primer walking method. Faecal

samples of domestic pigs from 5 affected farms with cases of posterior paralysis and 1 control farm were tested by PCR method. Faecal samples (N=65) from children under the age of 10 years hospitalised with gastroenteritis were also tested by PCR for human bufavirus. More than 8 virus families were present in the porcine faecal sample by NGS. The nearly complete genome of the bufavirus-like sequence (swine/Zsana3/2013/HUN; KT965075) was determined. Swine/Zsana3/2013/HUN had 53-60% and 46-69% amino acid sequence identity in the non-structural (NS1) and the structural protein (SP) proteins to human bufaviruses and animal bufavirus-like viruses. Swine/Zsana3/2013/HUN may represents a novel parvovirus species. A total of 19 (90%) of the 21 and 5 (33%) of the 15 faecal samples were positive for bufavirus-like parvovirus in the 5 affected and a control farms, respectively. Human bufavirus could not be detected in human samples.
This study reports a taxonomically novel parvovirus species related to human bufavirus from domestic pigs. The classification of the recently reported novel animal parvoviruses should be reviewed, since they may each form novel parvovirus species. Further study needed to investigate the etiological role of the novel swine parvovirus in posterior paralysis.

CHARACTERIZATION OF THE BIODEGRADATION PROCESSES IN *NOVOSPHINGOBIUM SUBARCTICUM* SA1 GROWN ON SULFONATED AROMATIC COMPOUND IN BATCH FERMENTER

BOTOND HEGEDŰS[1, 2], PÉTER KÓS[1, 3], GÁBOR BENDE[1], KRISZTIÁN LACZI[1], KATALIN PEREI[1], GÁBOR RÁKHELY[1, 2, 4]

[1]Department of Biotechnology, University of Szeged; [2]Institute of Biophysics, Biological Research Center, HAS; [3]Institute of Biophysics, Biological Research Center, HAS; [4]Institute of Environmental and Technological Science, University of Szeged, Szeged, Hungary

Sulfanilic acid, being a bi-functional sulfonated aromatic amine, is widely used for the synthesis of various chemical products such as synthetic organic dyes and sulfa drugs. Due to its diverse and large scale industrial applications, substantial amount of sulfanilic acid is released into the environment. Its microbial uptake is not easy due to the zwitterionic character of the molecule, but within the cells, it might be toxic via the inhibition of the folic acid biosynthesis. There are very few strains capable to degrade sulfanilic acids which include the co-cultures of *Hydrogenophage intermedia* S1 and *Agrobacterium radiobacter* S2; *Hydrogenophaga* sp. PBC and *Ralstonia* sp. PBA; the first single isolate, *Novosphingobium subarcticum* SA1, as well as several other strains such as *Pannonibacter* sp W1, *Agrobacterium* sp. PNS-1 and *Ochrobacrtrum anthrop*. *N. subarcticum* SA1 was isolated from industrial wastewater in our lab. The characterization of the substrate specificity of the strain revealed that the bacterium has versatile metabolic routes including the catabolism of 4-aminobenzenesulfonate (sulfanilic acid), 4-aminobenzoate, 4-hydroxybenzoate, 3,4-dihydroxy benzenesulfonate, 3,4-dihydroxy benzoate, 3,4-dihydroxy benzoate and 2,3-dihydroxybenzoate. The genome of the strain has been sequenced by new generation sequencing systems. The analysis of the genetic material reveled a chromosome of around 3,8 Mbp and four extrachromosomal elements in a size range of 67 – 1 759,8 kb. The presence of various extrachomosomal elements might ensure the versatile biodegradation routes in this microbe. *N. subarcticum* SA1 could be cultivated on various substituted aromatic compounds (see above), glucose or LB. The protein patterns of the cells could be clearly distinguished for the various types of substrates. By applying proteomic tools, most components of the peripherical and central pathways could be identified. A comparative study on gene expression profiles of *N. subarcticum* SA1 grown on sulfanilic acid and glucose revealed the sulfanilic acid induced up-regulation of the genes of a peripheral and a central pathway as well as

other accessory genes encoding proteins involved in cellular processes likely assisting in the bioconversion of sulfanilic acid. The detailed whole cell transcriptomic analyses might provide a deeper insight into the self-protecting mechanisms against xenobiotics as well as the connection of biodegradation routes to other cellular processes, such as nitrogen or sulfur metabolsims.

Supported by the European Union and co-financed by the European Social Fund (grant agreement no. TÁMOP-4.2.2.B-15/1/KONV-2015-0006) and by the Norway Grant (grant agreement no. HU09-0044-A1-2013).

ISOLATION AND CHARACTERIZATION OF *SACCHAROMYCES CEREVISIAE* STRAINS FROM SPONTANEOUSLY FERMENTED WINES PRODUCED IN THE BADACSONY WINE REGION

ANNA HILD[1], BARNA SZŐKE[3], ANDREA POMÁZI[1], JÁNOS MÁJER[3], FERENC OLASZ[2], TIBOR FARKAS[2]

[1]Department of Microbiology and Biotechnology, Faculty of Food Science, Szent István University, Budapest; [2]Agricultural Biotechnology Institute, National Agricultural Research and Innovation Centre, Gödöllő; [3]Research Institute for Viticulture and Enology, National Agricultural Research and Innovation Centre, Badacsonytomaj, Hungary

In traditional wine making must is fermented spontaneously and different yeast species take part in the process. In modern industrial wineries, in order to avoid the potential risks of spontaneous fermentation (e.g. stuck fermentation, emergence of off-flavours), must fermentation is directed, which means that the must is inoculated with selected commercial starter cultures that supress the native microbiota and take over the fermentation. However, recently there is a growing demand for wines with more individual characters and this is often achieved by using indigenous yeast strains instead of commercial starter cultures. The aim of our study is to isolate indigenous wine yeast strains from the Badacsony wine region that can be used as starter culture in directed fermentation. In the first pilot experiment a must from a family vineyard in Szigliget (also part of the Badacsony wine region) was fermented spontaneously under laboratory conditions. Culture conditions were optimized, and we also tried to explore the microbiota of the must. Bacterial and yeast strains have been identified by determining the sequence of 16S RNA and ITS regions, respectively. In the spontaneously fermented must 8 yeast (*Candida*, *Lachancea*, *Saccharomyces*, *Rhodotorula*, *Hanseniaspora*, *Issatchenkia*, *Torulaspora*, *Cryptococcus*) and 2 bacterial genera (*Gluconobacter*, *Bacillus*) have been detected. For the isolation of indigenous wine yeast strains 13 spontaneously fermented wine samples, produced at a traditional winery of Badacsony and at the aforementioned family vineyard, were investigated. Saccharomyces strains were identified by genus specific colony PCR. The isolated strains were divided into 14 groups on the basis of the electrophoretic patterns of the interdelta PCR. A dendrogram representing the relationship between the 14 groups was set up from the patterns. The interdelta PCR was also carried out with 27 commercial starter cultures to exclude re-isolated starters. None of our isolates were identical to the investigated starter cultures. It was concluded that the two wineries have their own microbiota, as no isolate has been detected at both locations. On the other hand, in each spontaneously fermented wine several *Saccharomyces cerevisiae* strains were detected. Microbiological characterization (ethanol-, sulfur dioxide-, osmo-tolerance, acid production, killer activity) of the *Saccharomyces cerevisiae* isolates has been carried out. The evaluation of the oenological potential of the isolates is currently carried out in microvinification tests. After microvinification the analytical and sensory properties of the wines will be investigated. In the future we are planning to explore the microbiota of the spontaneously fermented wines by using metagenomic analysis.

GENETICS OF FUNGICIDE RESISTANCE IN PLANT PATHOGENIC FUNGI

LÁSZLÓ HORNOK

Plant Protection Institute, Szent István University, Gödöllő, Hungary

Since the introduction of systemic fungicides with highly specific mechanisms of action, numerous phytopathogenic fungi and oomycetes have developed resistance to these compounds leading to control failures in crop production. The main mechanisms of fungicide resistance are (i) a reduced binding of the antifungal agent to its target site, (ii) overproduction of the target molecule, (iii) reduced uptake of the fungicide, (iv) an increased excretion of the toxic material, and (v) enzymatic inactivation of the antifungal compound. Fungicide groups posing medium to high risks of resistance include compounds that inhibit or disturb (i) mitosis and cell division (methyl benzimidazole carbamates), (ii) sterol biosynthesis (mainly imidazoles and triazoles), (iii) respiration (strobilurins, as the major group), (iv) signal transduction (phenylpyrrols) and (v) nucleic acid synthesis (phenylamides). Extensive molecular studies allowed a better understanding of the genetic background of fungicide resistance and our improved knowledge on this subject contributed greatly to the development of efficient resistance management strategies. The objective of the present review is to summarize recent advances on this field of research. Resistance may result from single or multiple genetic changes in a fungus population. Qualitative (or major gene) resistance is caused by a single amino acid change in the target protein of the antifungal compound, whereas quantitative (or polygenic) resistance involves mutation of a number of different genes with additive or synergistic interactions. An interesting subclass of fungicide resistance is the multidrug resistance (MDR) mediated by two groups of transporter proteins: ABC (ATP binding cassette) transporters, that utilize ATP to translocate toxic compounds and MFS (major facilitator superfamily) transporters, that excrete small molecules using the chemiosmotic ion gradient route. Depending on fungicide classes and fungal populations, resistance may be associated with fitness costs owing to the pleiotropic nature of resistance causing mutations. If such mutations adversely affect fitness traits, like growth, sporulation or infection structures, the proportion of resistant lineages in a population will decline in the absence of the fungicide in question. An important output of genetic studies on fungicide resistance is the development of molecular diagnostic techniques that provide rapid and sensitive detection of resistant strains, allowing efficient monitoring for fungicide resistance.
Various molecular techniques based on hybridization, DNA-amplification and sequencing have been developed for the detection of resistant genotypes. The closing section of this review discusses advantages and limitations of these techniques.

COLONY MORPHOLOGY AND COMPARATIVE ANALYSIS OF SOIL INOCULANT *AZOSPIRILLUM* STRAINS AND DIFFERENTIAL STUDIES IN COCULTURE WITH *BACILLUS*, *ARTHROBACTER*, *PSEUDOMONAS* AND *KOCURIA* SOIL INOCULANT STRAINS

CSILLA IMRE[1], RITA KOVÁCS[1], MIHÁLY JÁMBOR[1], ILDIKÓ PUSPÁN[1], ÁDÁM IMRI[1], ÉVA KÁRPÁTI[2], JÓZSEF KUTASI[1]

[1]Biofil Ltd.; [2]Saniplant Ltd., Budapest, Hungary

The associative symbiont diazothroph *Azospirillum* strains are the most effective and well known Plant Growth Promoting Rhizobacteria (PGPR). Nitrogen fixing bacteria which are applied to soil inoculant. Use of effective *Azospirillum brasilense* strain is long known in agricultural methods. The beneficial effects are due to the indole-3-acetic acid production and nitrogen fixing. Primary effect of *Azospirillum* strains are more intense root growth an increase in the number of the lateral roots. The different colonies of *Azospirillum* strains' morphology are similar on agar plates which makes identification difficult. Their growth are slower so the colonies are small and translucent after overnight on the agar plates. Tend to show recognizable morphology only after 5 days of incubation. They suppressed and inseperable comparing to each other next to the faster growth bacteria strains. Two *Azospirillum brasilense*, one *Azospirillum irakense* and one *Azospirillum largimobile* strains can be found in the SBSS (Soil Bacteria Screening System, Biofil Ltd) collection of soil bacteria strains, as an effectione of PGPR. These were the bacteria's colony morphology as that were detected. Through our work, the *Azospirillum* strains were grown separetly and together with other bacterial groups (*Arthrobacter*, *Bacillus*, *Pseudomonas* and *Kocuria* strains from SBSS strain collection by Biofil Ltd.). In accordance with current to the recent literature we investigated the separatibility of strains on five different agar plates. The Nutrient agar (Scharlau) medium and malic acid contains medium were used which are applicable for *Azospirillum* strain detection. The agar plate medium contains vitamin B (biotin and pyridoxin) and indicator dyes (bromtimol blue and congo red) for an easier identification. The change of colony morphology was observed in different osmotic conditions under alcaline, neutral and acidic pH. Furthermore the morphology changes were detected during the induction of exopolysacharide (EPS) production contained under the sucrose Nutrient agar plate. The photo documentation and comparative assessment were made by these results.

Supported by Norway and EEA grants (HU09-0029-A1-2013).

EFFECT OF THE GLUCOCORTICOSTEROID BETAMETHASONE ON THE VIRULENCE OF *CANDIDA ALBICANS*

ÁGNES JAKAB[1], SELENE MOGAVERO[2], TAMÁS EMRI[1], BERNHARD HUBE[3, 4], ISTVÁN PÓCSI[1]

[1]Department of Biotechnology and Microbiology, University of Debrecen, Debrecen, Hungary; [2]Department of Microbial Pathogenicity Mechanisms, Hans Knoell Institute; [3]Friedrich Schiller University; [4]Center for Sepsis Control and Care, University Hospital, Jena, Germany

Glucocorticosteroids are widely used in medicine for their anti-inflammatory and immunosuppressive effects. A known important side effect of such corticoid therapy is the development of invasive fungal infections including candidaemia. This is probably due to the immunosuppressive effects of the steroids, but these drugs may also have a direct effect on the virulence of *Candida* species. In our research, we found out that high-dose betamethasone (BM) affected the physiology of the opportunistic human pathogenic fungus *Candida albicans*: (1) BM stimulated the virulence attributes, the extracellular phospholipase production and hypha formation of *C. albicans*. (2) BM treatments decreased the efficiency of the polyene antimycotics amphotericin B and nystatin against *C. albicans* in a dose-dependent manner. (3) Importantly, BM increased the anti-*Candida* efficiency of the oxidative stress generating agent menadione. Moreover, we determined the effect of BM on the dynamics of *C. albicans* infections in a human oral epithelial cell model. (4) BM stimulated the adherence of *C. albicans*, (5) facilitated the invasion of epithelia, (6) initiated cell damage of oral cells and (7) decreased the expression of proinflammatory cytokines and chemokines. These

observations suggest that high-dose BM may predispose patients to superficial and mucosal *C. albicans* infections. The corticosteroid-polyene drug interactions may be of clinical importance when *C. albicans* infections are treated. The combination of BM with oxidants may be a valid option for preventing infections after steroid treatment.

Supported by Hungarian Scientific Research Fund (K 108989), Campus Hungary and NTP-NFTÖ-16 scholarship.

BIOLOGY AND BIOTECHNOLOGY OF FUNGAL LACTOSE CATABOLISM

LEVENTE KARAFFA

Department of Biochemical Engineering, University of Debrecen, Debrecen, Hungary

The dairy industry generates huge amounts of liquid waste. Some 85 % of the milk destined for cheese manufacture is discarded in the form of a yellowish, watery lactose-rich by-product called whey. Generated at almost 2 million tons per year worldwide, untreated cheese whey poses environmental challenges, as wastewater treatment technologies are expensive. A better alternative is subjecting the whey to processes through which value-added products can be manufactured. A prime example is industrial-scale fermentation biotechnology where whey has traditionally been used as a cheap growth substrate for the (over)production of valuable fungal metabolites, such as penicillin by *Penicillium chrysogenum* or cellulase, hemicellulase and heterologous proteins by *Trichoderma reesei*. For those – mostly saprophytic- or plant pathogenic – filamentous fungi capable of hydrolyzing it into its two monomer hexoses D-glucose and D-galactose, lactose (1,4-O-β-D-galactopyranosyl-D-glucose; milk sugar) is a poor, slowly assimilated growth substrate never encountered in their natural habitats (in nature, mammalian milk is the sole source of lactose). Growth on lactose results in low specific growth rates, a condition that greatly facilitates the production of secondary metabolites (a term for products of metabolism that are believed not to be absolutely required for the survival of the organism) and hydrolytic enzymes. Regulation of lactose utilization by means of the lac operon in *Escherichia coli* is a paradigm in prokaryotic genetics, while the LAC regulon of the yeast *Kluyveromyces lactis* is a model system for transcriptional control in lower eukaryotes. However, several key aspects of fungal lactose metabolism in less substrate-adapted ascomycete filamentous fungi – including some widely employed cell factories – are still poorly understood. To optimize fermentation processes that use whey residue, to advance its use in second-generation biofuel generation, and to facilitate the biodegradation of lactose-heavy wastewaters and soils, our research group has been investigating the mechanisms of lactose and D-galactose assimilation in several filamentous fungi. In my Manninger Lecture, I will summerize our results on the characteristics and regulation of lactose catabolism in four Pezizomycotina sps: the model fungus *Aspergillus nidulans*, the penicillin producer *P. chrysogenum*, the cellulase producer *T. reesei* and the citric acid and hydrolytic enzyme producer *Aspergillus niger*.

FAECAL METAGENOME ANALYSIS OF *VIPERA URSINII GRAECA* NILSON&ANDRÉN 1988: A PILOT STUDY

GÁBOR KARDOS[1], BARBARA FORRÓ[2], EDVÁRD MIZSEI[3], KRISZTIÁN BÁNYAI[2], SZABOLCS LENGYEL[4]

[1]Department of Medical Microbiology, University of Debrecen, Debrecen; [2]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest; [3]Department of

Evolutionary Zoology and Human Biology, Faculty of Science and Technology, University of Debrecen, Debrecen; [4]Department of Tisza Research, Institute for Danube Research of the Hungarian Academy of Sciences, Debrecen, Hungary

Recent studies suggest that the composition and diversity of the intestinal microbiota are linked to the health of the host individual and is influenced by the environment as well, thus may have conservation implications in endangered species. The aim of the study was to carry out a pilot investigation on the bacterial metagenome of the faecal microbiota of *Vipera ursinii graeca*, Nilson and Andrén 1988; a potentially endangered snake taxon that has one of the smallest distribution range among European snakes. The snake lives in subalpine meadows of the Pindos mountain system in Northern Greece and in Southern Albania and feeds mostly on orthopterans. Its small area makes it vulnerable to several anthropogenic effects such as habitat destruction, overgrazing and climate change. Therefore, a thorough data collection on the taxon is important to provide input data for future species conservation efforts. Eubacterial metagenom analysis was performed from faecal samples of three individuals from two different populations from the mountains Trebeshinë (TRE) and Lunxhërisë (LU1 and LU2). Eubacterial 16S rDNA was amplified by PCR then the resulting amplimers were sequenced using an Illumina Nextseq appliance. Alpha diversity of metagenomes was characterized by Shannon and Simpson diversity indices, beta diversity was compared using the diversity profiles by PaSt software. All three samples were dominated by Bacteroidetes, Proteobacteria and Firmicutes clades, but their proportions were highly different. *Bacteroides* spp. and members of the Gammaproteobacteria division appear to be important resident members of the microbiota. Sample TRE was dominated by Proteobacteria, while Bacteroidetes were most prevalent in the other two samples. Sample TRE also showed a lower genetic diversity at all taxonomic levels except the genus level. Diversity within clade Bacteroidetes was similar and the same few species were present in all three samples, while the diversity of Firmicutes differed markedly. Diversity of Proteobacteria was higher in sample LU2 than in the other two samples. Besides these dominant phyla, sequences of eubacterial taxons associated with the orthopteran prey, the soil and the vegetation were also detected including cyanobacterial, plant pathogen and insect endosymbiont sequences. Sequences of snake (*Anaplasma*, *Aeromonas*, *Pseudomonas*, *Salmonella*, *Francisella*, *Coxiella*) and mammalian (*Salmonella*, *Francisella*, *Coxiella*, *Pasteurella*, *Histophilus*) pathogens were also present in all three metagenoms. These preliminary data provide a basis for future investigations testing the dynamics of the microbiota, the effect of the habitat and the potential implications for the conservation of the species.

EFFECT OF ANTIBIOTIC CONSUMPTION PATTERNS ON INCIDENCE OF CARBAPENEM RESISTANT NOSOCOMIAL BACTERIA

GÁBOR KARDOS[1], HAJNALKA TÓTH[1], ORSOLYA GORÁCZ[1,2], ADINA FÉSŰS[1,2], FATEMEH EBRAHIMI[1], BENCE BALÁZS[1], JULIANNA MÓZES[1]

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

The aim of the present work was to examine the relationship between consumption of different antibiotics and carbapenem resistance in *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *E. coli*, and *Klebsiella* spp. between 2004 and 2015 using time-series analysis. Monthly consumption of different antibiotic families or individual drugs was characterized by the number of defined daily

doses (DDDs) consumed per 100 bed-days. Resistance to carbapenems was measured using the incidence density of infections caused by carbapenem resistant bacteria per 100 bed-days. These time-series were analysed by exponential smooting and ARIMA models. Association between consumption and resistance series were analysed by dynamic regression and by vector autoregressive (VAR) models. Time-series analysis was carried out in R statistical environment or using the software Eviews 3.1. In general, antibiotic consumption showed an increasing trend with a strong seasonal component. The most popular drugs were the beta-lactamase inhibitor combinations and fluoroquinolones; consumption of glycopeptides, carbapenems and polymyxins showed the highest increases during the study period. Carbapenem consumption increased from 0.9 DDD/100 beddays (2004-2005) to >2.9 DDD/100 bed-days (2014-2015), which was mostly caused by increased use of meropenem. Forecast estimates predicted further increases in meropenem and ertapenem, but not in imipenem consumption. Yearly averages of the incidence density of infections by carbapenem resistant *A. baumannii*, imipenem resistant *P. aeruginosa* and meropenem resistant *P. aeruginosa* increased from 0.14 to 7.9, from 2.4 to 8.8 and from 1.6 to 5.7, respectively. In case of *Klebsiella* spp., incidense density of carbapenem resistance has always reached 0.1 since 2011, in case of *E. coli* it is still rare. Forecasts predicted a very rapid increase in case of *A. baumanni*, less steep increase in *P. aeruginosa* and slow increase in *Klebsiella* spp. Significant association between carbapenem consumption and carbapenem resistance was demonstrated in case of *A. baumanni* and *P. aeruginosa*, but not in case of *E. coli* or *Klebsiella* spp. both by dynamic regression and by VAR models. In case of *P. aeruginosa* the effect of consumption appeared with smaller lags (0 to 3 months) than in case of *A. baumanni* (3 to 6 months). Meropenem consumption showed stronger association than imipenem or ertapenem consumption. Optimization of carbapenem usage seems to be a key factor in combating carbapenem resistance in *A. baumanni* and *P. aeruginosa*.

G. Kardos was supported by a János Bolyai research scholarship of the Hungarian Academy of Sciences.

STREPTOCOCCUS AGALACTIAE ISOLATES FROM PREGNANCY SCREENING AT THE UNIVERSITY OF DEBRECEN

SZILVIA KARDOS[1], JUDIT SZABÓ[2], TAMÁS MAJOR[3], ZOÁRD KRASZNAI[3], KRISZTINA LAUB[1], ADRIENN TÓTHPÁL[1], ORSOLYA DOBAY[1]

[1]Institute of Medical Microbiology, Semmelweis University, Budapest; [2]Department of Medical Microbiology; [3]Department of Obstetrics and Gynecology, University of Debrecen, Debrecen, Hungary

Streptococcus agalactiae (GBS, group B *Streptococcus*) is one of the most important causes of neonatal sepsis and meningitis, and the source of infection is vaginal colonisation of the mother. This is why it is very important to screen pregnant women and in case of GBS positivity, intrapartum antibiotic prophylaxis can be applied to prevent transmission to the newborn. In this study, we have examined GBS isolates, deriving from pregnancy screening at the University of Debrecen. 95 GBS isolates were collected from pregnancy screening, over a 5-year period (01.2011-02.2016) at the Department of Obstetrics and Gynecology, University of Debrecen. The mean age of the women was 31.2 years (ranging between 24 and 42). Sampling was performed most frequently on week 36 or 37. In two cases in this period, the patient was GBS positive in the previous pregnancy as well. In 49 cases, this was the first child birth. After the thorough identification of the isolates, we determined their antibiotic sensitivity by Etest, and in the case of macrolide resistant strains, we also detected the *erm*, *mef* and *linB* resistance genes. We identified the serotypes of the strains with antisera and PCR, and we detected the presence of major surface proteins (AlpC, Rib, Alp2, Alp3 and Epsilon). The ST-17 hypervirulent clone was detected by hvgA PCR, and the other variant of the same gene (*bibA*) was

also examined. All isolates were fully sensitive to penicillin (max. MIC=0.06 mg/L), levofloxacin and moxifloxacin. On the other hand, macrolide and tetracycline resistance proved to be a major problem. Forty-one isolates (43.2%) were resistant to erythromycin; 36 of these had MIC≥256 mg/L, and all of these carried the *ermB* gene. No *mef* or *linB* genes were detected. 88.4% of the isolates (n=84) were resistant to tetracycline.The serotype distribution of the isolates was the following:V (35.8%), III (28.4%), Ia (14.7%), II (9.5%), IV (6.3%), Ib (5.3%). The surface proteins were detected with the following frequency: rib (34.7%), alp2/3 (33.7%), eps (21.1%) and alpC (10.5%). The serotypes showed strong correlation with the presence of surface proteins: Rib was associated mostly with type III, Alp2/3 with type V, Epsilon with types Ia and IV. Serotypes Ia, Ib, II and III were mostly sensitive to macrolides, while serotypes IV and V showed resistance. The hypervirulent clone ST-17 was detected in 23 cases (24.2%), 22 isolates were serotype III, but one isolate was serotype V. All remaining strains were *bibA* +. In both cases, where the patient carried GBS during the previous pregnancy as well, the isolates proved to be identical. Although serotype III has been previously associated with GBS isolates deriving from pregnancy screening, serotype V was most frequently found in the current study. As this serotype showed macrolide resistance, which would be the choice of drug in intrapartum antibiotic prophylaxis in the case of penicillin allergy, this is a worrying tendency. One quarter of the isolates belonged to the hypervirulent clone ST-17.
These results further support that pregnancy screening should include detection of ST-17 and macrolide resistance in a combined method.
This project was financially supported by the Hungarian Scientific Research Fund (OTKA), grant no. K108631.

MONITORING THE EFFECT OF COMMON BIOCHAR AND PLANT GROWTH PROMOTING RHIZOBACTERIA SOIL AMENDMENTS

ANDRÁS KARI[1], ZSUZSANNA NAGYMÁTÉ[1], BOGLÁRKA RIZÓ[2], CSABA ROMSICS[1], ILDIKÓ PUSPÁN[2], RITA KOVÁCS[2], JÓZSEF KUTASI[2], NIKOLETT PÉK[3], ÉVA KÁRPÁTI[3], MÁRK RÉKÁSI[4], NIKOLETT UZINGER[4], KÁROLY MÁRIALIGETI[1]

[1]Department of Microbiology, Eötvös Loránd University; [2]BioFil Ltd.; [3]Saniplant Ltd.; [4]Institute for Soil Sciences and Agricultural Chemistry, Center for Agricultural Research. Hungarian Academy of Sciences, Budapest, Hungary

Biochar are used to improve soil fertility and mitigate climate change by carbon sequestration. It is produced by the thermal “degradation” of organic materials in the absence of oxygen (pyrolisis). As a soil amendment can improve nutrient retention, increase pH in acid soils, and effect soil biological community structure and abundance. Plant growth promoting rhizobacteria (PGPR) are used for fertilization in agricultural soils. PGPR microorganisms have beneficial effects on plant productivity and crop yield by favoring nutrient uptake (phosphorus, nitrogen, and essential minerals), phytohormon production and decreasing inhibitory effects of various pathogens. The potential of using biochar together with PGPR microorganisms as a soil fertilization technique, offers a new, exciting opportunity for sustainable agriculture. The amendment of alkaline and acidic soils for enhancing plant growth and crop yield was accomplished by 10 different bacterium strains alongside biochar. The aim of the study was to detect the effect of biochar on soil biota and inoculated bacterium strains. Further, the total bacterial community structures of the soils were characterized during the treatment. For detecting and estimating the relative abundance of the applied bacterium strains in treated soils from inoculation till harvesting Terminal Restriction Fragment Length Polymorphism (T-RFLP) was applied. Two different restriction endonucleases had been in silico selected for specific detection of the inoculated stains. Phospholipid fatty acid analysis (PLFAs) was

also used for monitoring the microbial community dynamics. The applied bacterial inoculations contained two strains with the potential of nitrogen-fixation and auxin producing capability. On alkaline and acidic soils the treatment of biochar and PGPR inoculant combination affected 1-5 % increase in relative abundance of the two strains in the 14th and 22th week compared to untreated control. Another strain with siderophore producing possibility, similarly to the phosphate solubilizing bacteria, showed 1-3 % relative abundance increase not only beside the biochar + PGPR inoculant combination, but also by the sole biochar treatment. Only the PGPR inoculation form affected relative abundance increase (1-2 %) of the zinc solubilizer and plant systemic resistance inducing strains. Biochar treatment, both on alkaline and acidic soil, showed a great influence, by T-RFLP analysis, on the autochthonous microbial community structure compared to the untreated fields, and those soils, which were treated only with bacterial inoculants. PLFA analysis so far has not shown correlation between the treatments and microbial community dynamics.

Supported by Norway and EEA grants (HU09-0029-A1-2013).

SCREENING OF dsRNA ELEMENTS IN *MORTIERELLA* SPECIES

TÜNDE KARTALI, BOGLÁRKA SZABÓ, ILDIKÓ NYILASI, CSABA VÁGVÖLGYI, TAMÁS PAPP

Department of Microbiology, Faculty of Sciences, University of Szeged, Szeged, Hungary

Mycoviruses can be found in all major phyla of fungi as they were described in Ascomycota, Basidiomycota species as well as in the different groups of the former Chytridiomycota and Zygomycota phylum. They are transmitted only intracellular routes, as they can only spread with hyphal anastomosis and with the sexual or asexual spores. The majority of mycoviruses have linear double-stranded RNA (dsRNA) genome, although mycoviruses with single-stranded RNA and single-stranded DNA genomes were also described. Mycoviruses can cause smaller or greater changes in the phenotype of their fungal host or they can reduce or enhance the fungal virulence causing hypovirulence or hypervirulence effects. However, most of the mycoviruses are cryptic as their presence in the host is generally symptomless. The aim of this research was the screening of the zygomycete *Mortierella* species for the presence of dsRNA elements and the comparison of the virus harboring feature with their phylogenetic relationships. During our research we have screened 124 *Mortierella* and closely related strains belonging to 71 species. DsRNA elements were isolated with CF-11 cellulose chromatography from a total nucleic acid extract. DsRNA elements could be detected in 15 isolates (13 *Mortierella* and 2 *Dissophora* strains). Most of the strains contained 1 or 2 discrete bands between 2800 and 8000 kb size. We also confirmed the nature of the isolated dsRNA by its resistance to DNase I and S1 nuclease digestions.

We compared this results with our previous phylogenetic data and we observed that dsRNA harboring strains can be found in several *Mortierella* clads without any characteristic pattern. The dsRNA presence also showed a high level of intraspecies variability.

Supported by the National Research, Development and Innovation Fund (TÉT_12_SK-1-2013-0007). The research of TP was supported by the grant of the Hungarian Scientific Research Fund (OTKA NN 106394). This work was funded by the grants Lendület LP2016-8/2016.

IDENTIFICATION OF NOVEL SURFACTIN ISOMERS BY ION TRAP MASS SPECTROMETRY

ANITA KECSKEMÉTI, ATTILA BARTAL, BETTINA BÓKA, CSABA VÁGVÖLGYI, ANDRÁS SZEKERES

Department of Microbiology, University of Szeged, Szeged, Hungary

Surfactins are surfactant compounds of biological origin. They are cyclic lipopeptides consisted of a β-hydroxy fatty acid of various chain length and a peptide ring of seven amino acids linked together by a lactone bridge, forming a cyclic structure in the peptide chain. These compounds are produced mainly by the *Bacillus* species, and possess numerous biological effects such as antiviral, antitumor, anti-inflamatory and antimicrobial activities. The structure of surfactin with the firstly described heptapeptide sequence composed of these amino acids: Glu-Leu-Leu-Val-Asp-Leu-Leu. A mixture of surfactins were extracted from the strain *Bacillus subtilis* (SZMC 6179J) and was examined by HPLC-ESI-IT-MS technique. To increase the separation of the fractions with higher masses a gradient elution was applied reached to non-polar solvent system, which leaded to the elution and characterization of their structures. Both the length of linked fatty acids and the peptide sequences were also investigated during the MS2 spectra analyses of the sodiated precursor ions. These examinations allowed discovering a previously unknown surfactin group containing altered amino acid sequences. The relative amounts of the newly identified isoforms were significant ratios of the produced whole surfactins. The high yields of this new isoform offer an opportunity to separate a pure fraction of these compounds in the future and study its biological activities for further effects.

Connected to the project GINOP-2.3.3-15-2016-00006 and supported by GINOP-2.3.2-15 C113410. A. Sz. is a grantee of ÚNKP-16-4 Scholarship of the Ministry of Human Capacities of Hungarian Goverment

HIGH DIVERSITY OF REPLICATION-ASSOCIATED PROTEIN ENCODING CIRCULAR DNA VIRUSES IN GUANO SAMPLES OF EUROPEAN BATS

GÁBOR KEMENESI[1, 2], BRIGITTA ZANA[1, 2], KORNÉLIA KURUCZ[1], FANNI FÖLDES[1, 2], PÉTER URBÁN[2, 3], ANTON VLASCHENKO[4], KSENIIA KRAVCHENKO[4], IVANA BUDINSKI[5], FARKAS SZODORAY-PARÁDI[6], ABIGÉL SZODORAY-PARÁDI[6], SZILÁRD BÜCS[6], CSABA JÉRE[6], ISTVÁN CSŐSZ[6], PÉTER ESTÓK[7], TAMÁS GÖRFÖL[8, 9], SÁNDOR BOLDOGH[10], VIKTÓRIA NÉMETH[1, 2], KRISZTIÁN BÁNYAI[9], FERENC JAKAB[1, 2]

[1]Virological Research Group, János Szentágothai Research Center; [2] Institute of Biology, Faculty of Sciences; [3]Microbial Biotechnology Research Group, János Szentágothai Research Center, University of Pécs, Pécs, Hungary;[4]Bat Rehabilitation Center of Feldman Ecopark, Lesnoye, Ukraine; [5]Department of Genetic Research, Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia; [6]Romanian Bat Protection Association, Satu Mare, Romania; [7]Department of Zoology, Eszterházy Károly College, Eger; [8]Department of Zoology, Hungarian Natural History Museum, Budapest; [9]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest; [10]Aggtelek National Park Directorate, Jósvafő, Hungary

Bats are increasingly recognized as hosts and sources of viruses with important evolutionary relevance or zoonotic capability even from Europe. Viruses detected in faeces samples are useful to reveal viral diversity of the local ecosystem, with particular regard to dietary viruses, which in the case of European bat fauna, mainly incorporates arthropod-related viruses but also reflects to other viral sequences which are present in the food chain (e.g. plant viruses). In recent years several novel highly divergent circular single-stranded DNA (ssDNA) viruses were described in bats from Brazil, China, USA, and Tonga, Oceania. Faecal samples were collected from multiple localities in Georgia, Hungary, Romania, Serbia and Ukraine by trained chiropterologists during bat ringing and bat rehabilitation activities. Samples were subjected to random primed reverse transcription PCR and semiconductor sequencing by using the Ion Torrent PGM platform. Altogether nine complete

genomic sequences were obtained, representing two viral families of Genomoviridae and Circoviridae and a recently described Gemini-like virus. Phylogenetic analyses revealed distantly related members of the family Circoviridae and novel viral species of the family.
Nucleotide composition analysis was performed for members of the Circoviridae family in order to assess the possible host organism for these newly described viruses. The results of this study provides the first dataset on ssDNA viruses, circulating among European bat species.

FLUORINATED PHOSPHORUS YLIDES AS INHIBITORS OF BACTERIAL EFFLUX PUMPS

ANNAMÁRIA KINCSES[1], ÁGNES MÍRA SZABÓ[1], RYOSUKE SAIJO[2], GENKI WATANABE[2], MASAMI KAWASE[2], JOSEPH MOLNÁR[1], GABRIELLA SPENGLER[1]

[1]Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, Szeged, Hungary; [2]Faculty of Pharmaceutical Sciences, Matsuyama University, Matsuyama, Ehime, Japan

Multidrug resistance (MDR) to antibiotics has become a serious problem in antibacterial chemotherapy. One of the most important mechanisms of MDR is the over-expression of efflux pumps, whereby cells pump out toxic substances to the exterior of the cells. Consequently, it is a major challenge for drug development to discover new efflux pump inhibitors (EPIs). Quorum-sensing (QS) is cell-to-cell communication system in bacteria. This system plays an essential role in bacterial adaptation, survival in the environment and it produces and detects chemical signals called autoinducers (AIs) monitoring the cell density, in addition the inhibition of QS could be a target for antibacterial drug design. Ten phosphorus ylides were compared based on their MDR-reverting activity in *AcrAB-TolC* expressing *E. coli* K-12 AG100 strain and its *AcrAB-TolC* deleted strain (*E. coli* AG100 A). The minimum inhibitory concentrations (MICs) of P-ylides were determined by two-fold broth microdilution method in 96-well plate. The efflux pump inhibition of compounds on the accumulation of the general efflux pump substrate ethidium bromide (EB) was assessed by real-time fluorimetry. The quorum sensing (QS) inhibition of compounds was investigated by agar diffusion method. The relative gene expression level of efflux pump and QS genes was determined by real-time reverse transcriptase quantitative polymerase chain (RT-qPCR) reaction. The compounds (1-10) did not have any antibacterial effect on the MDR *E. coli* strain and the pump deleted *E. coli* strain. The activity of the compounds was compared based on the relative final fluorescence index (RFI) of the real-time accumulation curves. According to the results the most effective derivative was compound 4 and its effect was more pronounced on the *E. coli* AG100 strain compared to the pump deficient AG100 A strain. The compounds are not QS inhibitors but compound 4 could increase the expression of QS gene *sdiA* and the transporter gene *acrB*. Furthermore the most active derivatives 2 and 5 could influence the expression of efflux pump and QS genes after 4 h of exposure. Based on the results it can be assumed that trifluoroacetyled P-ylides could be valuable EPI compounds to reverse efflux related MDR in bacteria. Compound 4 might be an attractive lead EPI for further drug development.

THE EFFECT OF LIGHTING AND BIOREACTOR SETUP ON *CHLORELLA VULGARIS* FERMENTATION

BERNADETT KISS, ÁRON NÉMETH

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

The application possibility of algae are extremely wide from pharmaceuticals through feed and food until agricultural and environmental application for sustainability. For the later, Abiusi et. al. (2016) selected *Chlorella vulgaris MACC555* to utilize ammonia and phosphate content of leachate obtained by dewatering of wastewater sludge. Our main goal was to find an effective bioreactor setup as well as an optimal illumination appropriate for scale up of the above microalgae cultivation for algae biomass production. We compared the cultivations with RGB LED, light bulb, LED-bulb in different scales starting from microplate (1,5 ml) to an own developed plastic air-lift reactor (850 L). The effect of lighting was described in a kinetic model, too.

PHYLOSTRATIGRAPHIC ANALYSIS FOR REVEALING THE EVOLUTIONARY ORIGINS OF GENE FAMILIES PLAYING ROLE IN FUNGAL MULTICELLULARITY

ENIKŐ KISS, ARUN PRASANNA, KRISZTINA KRIZSÁN, LÁSZLÓ NAGY

Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Emergence of fungal multicellularity is one of the major evolutionary transitions of life. Studying the genomic background of fungal complexity can provide directions in unraveling the key molecular mechanisms behind the simple multicellular, filamentous lifestyle. It is well known that signal transduction pathways play a key role in stress response, sensing, cell-cell communication, and the extension of the regulatory toolkit of morphogenesis have crucial role in the development of multicellular structures in various species, but so far there have not been detailed studies of their relevance towards fungal multicellularity. In order to investigate the genomic background of fungal multicellularity we surveyed the literature for known morphogenesis-associated genes. A database consisting 996 genes related to signal transduction, transcriptional regulation, polarized, hyphal growth and adhesion was constructed, covering 25 different model fungi (e.g. *Neurospora crassa*). To understand the evolutionary dynamics of these genes, we combined complete genomes of these 25 species with genomes of another 50 unicellular (including animal and opishtokont outgroups), simple and complex multicellular species. We performed similarity-based clustering (Mcl algorithm) of the genes in the 75 genomes, and found that the 996 genes grouped into 580 clusters of homologues. We identified 7 PFAM domains potentially related to hyphal morphogenesis, which specified additional clusters. These were added to the database. Using the identified orthogroup clusters, we performed phylostratigraphic analysis to uncover the evolutionary origins of gene families related to morphogenesis. Further, using gene trees of 561 clusters, we reconstructed gene duplications and losses along the evolutionary history of fungi, which gave high-resolution view on the dynamics of these putatively multicelularity-related gene families over time. The detailed analysis of multicellularity related gene families revealed that part of the genetic toolkit behind the fungal multicellularity was already present in the most recent common ancestor of eukaryotes. This suggests that in addition to de novo gene family birth and gene duplications, the regulation of the existing genomic toolkit is responsible for the evolution of multicellularity. This is consistent with the Cis-regulatory hypothesis, which is one of the most important theories of Evo-devo and according to which the cis-regulatory elements have the key role in morphological evolution.

INTER- AND INTRASPECIFIC FUNCTIONAL DIVERSITY OF FUNGAL ROOT ENDOPHYTES OF SEMIARID SANDY GRASSLANDS

DÁNIEL KNAPP[1], PÉTER JÁNOS BEREK-NAGY[1], ILDIKÓ IMREFI[1], GÁBOR HERCZEG[2], GÁBOR M. KOVÁCS[1]

[1]Department of Plant Anatomy; [2]Behavioural Ecology Group, Department of Systematic Zoology and Ecology, Institute of Biology, Eötvös Loránd University, Budapest, Hungary

Dark septate endophytes (DSE) represent a worldwide dispersed form-group of root colonizing endophytic fungi, however our knowledge on their role in ecosystem functioning is far limited. Since DSE fungi are relatively frequent in extreme and nutrient-limited environments such as arid and semiarid grasslands, there major importance is hypothesized in these areas. In a previous study on compositional diversity of DSE on Hungarian semiarid grasslands, members of the DSE community were revealed. Our aims were (i) to gain information on substrate degrading capacity of identified DSE fungi of semiarid sandy grasslands using enzyme assays and to test whether functional diversity is coupled with taxonomical diversity and (ii) to test intraspecific functional diversity of DSE fungi using conspecific isolates of three common and dominant DSE species. To assess enzymatic capabilities of fifteen identified DSE lineage, one representative isolate of each species were examined by api-ZYM tests and BioLog FF MicroPlates. To test intraspecific functional diversity of DSE fungi, 8-8 isolates of three common species, *Darksidea alpha*, *Flavomyces fulophazii* and *Periconia macrospinosa* were investigated. Besides genetic information (nrDNA ITS sequences and ISSR pattern) of the conspecific isolates, their symbiotic nature, saprotrophic capabilities and metabolic profiles were characterized by their effect on growth of maize, their growth on medium and using the same two enzyme assays, respectively. Striking differences were found in enzymatic capabilities of DSE species, however all of the substrates tested were utilized by the 15 DSE species. We also found high intraspecific heterogeneity between conspecific isolates. The high intra- and interspecific functional diversity of these root associated fungi can be important for the host performance, since the heterogeneity in function and wide spectra of degrading enzymes of DSE fungi might efficiently help nutrient mobilization and/or uptake of plants from nutrient poor soils.
This study was supported by the Hungarian Scientific Research Fund (OTKA K109102).

DEVELOPMENT OF AN ENVIRONMENTALLY FRIENDLY PROCESS FOR THE PRODUCTION OF GOLD NANOPARTICLES

SZABOLCS KOHÁRI[1], GEORGE SZAKÁCS[1], PÉTER SÁTORHELYI[1], ISTVÁN LAGZI[2], NÓRA ADÁNYI[3], DIANA WEISER[1], BALÁZS ERDÉLYI[1]

[1]Fermentia Microbiological Ltd.; [2]Department of Physics, Faculty of Natural Sciences, University of Technology and Economics; [3]Food Science Research Institute, NARIC, Budapest, Hungary

In recent decades, the special optical, magnetic, electronic and catalytic characteristics of nanoparticles (NPs) have driven scientists to discover new methods to create novel types of NPs with specific properties (size, shape, composition). Conventionally gold NPs (AuNPs) are produced by physical or chemical methods, which usually have some disadvantages (high energy and capital requirements, toxic byproduct formation, rigorous reaction conditions and dangerous chemicals). An environmentally friendly, nontoxic alternative can be the application of biological systems (plant extracts, bacteria and fungi) for the synthesis. In our study we chose to work with filamentous fungi strains, because they can be easily fermented in laboratory conditions as well as on bigger industrial scales, and some fungi strains were reported as effective biocatalysators. 30 different thermophilic

filamentous fungi, fermented on two different fermentation broths (Nano 2, Nano 11) have been screened for their ability to produce AuNPs. Inoculation was performed by fungal spores from Petri plate cultures. After shaken flask cultivation, the fermentation broth was centrifuged and the supernatants were used for the biosynthesis. The biological AuNP formation experiments were carried out at 45°C. For the synthesis reaction tetrachloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$) was mixed with the supernatants in acetate buffer. In the majority of the experiments the distinctive color of AuNP solutions appeared after a half to two hours, the rest needed a 24 hour incubation period. The acquired colors ranged from red through purple to blue depending on the diameter of the AuNPs produced. Twelve of the 60 samples have been chosen for further characterization. The UV-VIS spectra were recorded and the kinetic of the AuNP formation has been measured over a 24 hour period. Further experiments are planned for additional characterization by transmission electron microscopy (TEM). The stability of the solutions have been also measured and it was found that it lasts for a few days, in some instances a few weeks. The reducing agents responsible for the synthesis were also examined in the obtained supernatants. Molecular sieves with different protein size ranges were used to determine the size of these molecules.

Even the fractions below 3 kDa gave positive results, indicating that the molecular mass of the reducing agent is less than 3 kDa. Consequently, the biological reducing agents are not only enzymes with definitely higher molecular mass, but other biological substances.

Supported by the project No. HU09-0118-A2-2016 of EEA/Norwegian Financial Mechanism 2009-2014.

UPSTREAM, DOWNSTREAM AND LABORATORY EQUIPMENT FROM HOLIMEX LTD.

BÉLA KORDA, PÉTER ONDER

Holimex Ltd., Budapest, Hungary

Holimex ltd. is an engineering and trading company founded in 1990, as a part of the Dutch VEKAMAF group. Our profession is to represent machine manufacturers for the chemical, pharmaceutical and related industries. At the beginning Holimex dealt with companies of the Netherland then during the last twenty six years our portfolio was widened with other Western European producers of high quality machines. Experiencing that the traditional chemical synthetic industry lost its importance, Holimex started recently to broaden its portfolio with new partners, who are adept in biotechnology. During our presentation we are introducing our most important partners on the field of biotechnology. The Dutch Applikon BV specialized exclusively to produce bioreactors for bacterial as well as cell culture fermentation, focusing to control, intensive data handling in order to get information as much as possible to shorten the scale-up process. According to this philosophy, the company offers from some millilitres to 5 m3 large fermenters. The vessels are equipped with up-to-dated sensors, data acquisition and corresponding controllers. The Slovenian BIA Separations has developed special monolithic chromatographic columns, with outstanding performance for the separation of large biomolecules, such as proteins, DNS. Promising virus separation and purification process can be carried out by such a preparative size column. The German Cellab, as a pioneer in the field of membrane reactors for cell cultures, was founded in 2014.

Among others we would like to mention Telstar, the producer of biosafety cabinets, cleanrooms and lyophilizers, or SciLog, with single use sensors and filters in its portfolio.

INVESTIGATION OF LACTIC ACID BACTERIA ISOLATED FROM THE EXOTIC ANIMALS OF THE BUDAPEST ZOO AND BOTANICAL GARDEN, HUNGARY

JUDIT KOSZTIK[1], ILDIKÓ BATA-VIDÁCS[1], ERZSÉBET BAKA[1], ÁKOS TÓTH[1], SZABINA LUZICS[1], ENDRE SÓS[2], JÓZSEF KUKOLYA[1]

[1]Department of Environmental and Applied Microbiology, Agro-Enviromental Research Institute, National Agricultural Research and Innovation Center; [2]Veterinary and Conservation Department, Budapest Zoo and Botanical Garden, Budapest, Hungary

The exotic animals that live in zoo have very special gut microflora. It contains bacteria originating from their environment, and animals can also pass down specific bacteria to their descendants when they give birth to them. Since the microbiome of exotic animals was not investigated thoroughly before, the aim of our research is the taxonomic study of the dominant lactic acid bacteria of selected exotic animals. We chose the lactic acid bacteria as the focus of our study, because they are very useful in the production of dairy products and they can be used as probiotics. During the study, we isolated the lactic acid bacteria on MRS plates from the exotic animals of the Budapest Zoo and Botanical Garden (*Gazella dama mhorr*, *Geochelone gigantea*, *Vombatus ursinus tasmaniensis*, *Casuarius casuarius*, *Dromaius novaehollandiae*, *Hystrix indica*, *Heterocephalus glaber*, *Geronticus eremita*, *Macropus fuliginosus*, *Ceratotherium simum*, *Phascolarctos cinereus*, Gorilla, *Ailurus fulgens*, *Medauroidea extradentata*, *Lemur catta*). We used classical and molecular microbiological methods for the taxonomic identification of the lactic acid bacteria strains. First, pure cultures of isolated bacteria were prepared, and then the DNS was isolated. Repetitive PCR method followed by agarose-gel electrophoresis was applied, and the strains were grouped based on their patterns. Representatives of each group were analysed by 16S rDNA sequencing. The novel bacteria were further analysed with the help of housekeeping genes (*recA* and *gyrB*).
The studied animals had very diverse gut microflora. For kangaroo, naked mole rat, and emu the dominant bacteria were solely enterococci, while for gorilla or ring-tailed lemur lactobacilli and lactococci dominated. Overall 171 strains have been isolated belonging to 18 species of the genus *Lactobacillus*, 22 strains belonging to 4 species of the genus *Lactococcus*, 251 strains belonging to the 9 species of the genus *Enterococcus*, 49 strains belonging to 4 species of the genus *Pediococcus*, and 15 strains belonging to 3 species of the genus *Leuconostoc*.

THE *IN VITRO* AND *IN VIVO* EFFICACY OF FLUCONAZOLE IN COMBINATION WITH FARNESOL AGAINST *CANDIDA ALBICANS* BIOFILMS

RENÁTÓ KOVÁCS, ALIZ BOZÓ, FRUZSINA NAGY, ZOLTÁN TÓTH, LÁSZLÓ MAJOROS

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

Farnesol is a quorum-sensing molecule that blocks biofilm formation in *Candida albicans*. Previous in vitro data suggest that farnesol in combination with certain antifungals may have an adjuvant anti-biofilm agent. However, the in vivo efficacy of farnesol is very questionable. Therefore, the in vitro and in vivo activity of fluconazole combined with farnesol was evaluated against biofilms formed by fluconazole susceptible and resistant *C. albicans* clinical isolates using fractional inhibitory concentration index (FICI) determination, time-kill experiments and a murine vulvovaginitis model.

The FICI model expressed as ΣFIC = FICA+FICB = MICAcomb / MICAalone +MICBcomb / MICBalone, where MICAalone and MICBalone are the MIC values of agents A and B used alone and MICAcomb and MICBcomb are the MICs of agents A and B at the isoeffective combinations, respectively. FICI was defined as the lowest ΣFIC. The interaction between fluconazole and farnesol was interpreted as synergistic when FICI was ≤0.5, as indifferent interaction when FICI was between >0.5 and 4 and as antagonism when FICI was >4. The drug concentrations tested in the time-kill experiments were 0.5 mg/L, 8 mg/L, 64 mg/L and 512 mg/L fluconazole with and without 75 μM farnesol against the fluconazole-sensitive strains and against the reference strain as well as 64 mg/L, 128 mg/L, 256 mg/L and 512 mg/L fluconazole with and without 75 μM farnesol against the two resistant strains. The median biofilm MICs of fluconazole-sensitive *C. albicans* isolates ranged between 4->512 mg/L and 150-300 μM for fluconazole and farnesol, respectively. These values were 512->512 mg/L and >300 μM for fluconazole-resistant clinical isolates. Farnesol decreased the median MICs of fluconazole by 2-64-fold for biofilms. Based on FICI, synergistic interaction was observed only in case of the sessile SC5314 reference strain (FICIs: 0.16-0.27). In time-kill studies, only the 512 mg/L fluconazole and 512 mg/L fluconazole + 75 μM farnesol reduced biofilm mass significantly at each time point in case of all isolates. The combination reduced the metabolic activity of biofilms in case of all isolates in a concentration- and time-dependent manner. Our findings revealed that farnesol alone was not protective in a murine vulvovaginitis model. Farnesol was not beneficial in combination with fluconazole in case of fluconazole-susceptible isolates in vivo, but partially increased fluconazole activity against one, but not the other fluconazole-resistant isolate.

EXAMINATION OF LONG-TERM COEXISTENCE OF SOIL INOCULANT STRAINS IN SOIL SPECIFIC BIOFIL PRODUCTS

RITA KOVÁCS, CSILLA IMRE, ILDIKÓ PUSPÁN, ÁDÁM IMRI, MIHÁLY JÁMBOR, JÓZSEF KUTASI

Biofil Ltd., Budapest, Hungary

The process of soil inoculation is well-known for its nutrient supply, plant health promotion and crop yield enhancement purposes. The use of PGPR (Plant Growth Promoting Rhizobacteria) in soil amelioration is becoming more and more widespread all over the world, including Hungary, thus, the quality control of soil inoculant products has received increased attention. In multispecies bacterial cocultures there are different interactions between bacterial populations through primary and secondary metabolites. These interactions can be beneficial, adverse and neutral for the populations. The growth of bacterial populations is determined by the quality and quantity of the available macro and micro elements and the environmental conditions, that can limit opportunities for niche segregation. The developed long-term co-existence is beneficial for the bacterium strain populations which form an association in the products. The community is more stable and more resistant against the environmental effects. The stable system capable to compensate the deviation from equilibrium. Moreover the ecological potential of a microbial community outperforms the sum of the individual species of the community. The aim of our work was to monitor the long-term coexistence of bacterial strains in BioFil® Acid, Alkaline and Normal soil inoculant products, adapted to extreme soil conditions. These bacteria have beneficial effects even on low fertility deteriorated soils, given that a sufficiently high cell count and proper ratio of bacterial strains is present in the inoculant during the spread process. Therefore it is important that the seven bacterial strains are able to maintain long-term coexistence. The bacterial coculture was stored at 4°C in a non-sterile 200 ml plastic bottle. The cell count was determined by plate counting and phase contrast microscopy. The measurements were repeated every week for six months. After the initial cell loss, the total cell count (CFU, colony

forming unit) did not change significantly. The cell count of individual strains showed either an increase or decrease, however. We observed that the cell number of the individual components in BioFil inoculants varied dinamically but the total cell count has changed slightly. The cells are simultaneously proliferating and dying in the closed system due to secreted metabolites and nutrients released by cellulolysis. Nevertheless, owing to niche segregation, all bacterial strains were present in a proper cell count in the culture throughout the six-month examination period.
Supported by Norway and EEA grants (HU09-0029-A1-2013).

DEVELOPMENT OF AN EFFECTIVE BIOPESTICIDE FROM *PHOTORHABDUS LUMINESCENS*

VANDA KNITLHOFFER[1], GÁBOR KISS[2], ÁRON NÉMETH[1]

[1]Department of Applied Biotechnology and Food Science, Budapest University of Technology, Budapest; [2]Nova Scienta Ltd., Soltvadkert, Hungary

Photorhabdus luminescens is an entomopathogenic bacterium from the family of *Enterobacteriaceae*. The prokaryote has a symbiotic relationship with nematodes from the family *Heterohabditidae*. Once the invaded infective juvenile nematode enters the haemolymph of the insect larva, the bacteria is released from the nematodes guts and starts to proliferate rapidly. It then also starts to secrete toxins and enzymes to kill the host, which action also provides a food source both for the bacteria and the nematodes as well. After the host is decomposed the nematodes infect new larvae. This mechanism makes *Photorabdus luminescens* an ideal canditate as a biopesticidal agent. Biopesticides are generally less toxic than the conventionally used pesticides. They affect only the target pest, require lower doses, decompose faster and are not harmful to the enviroment. When used in combination with conventional pesticides we can get the same crop yields while using less synthetic materials. We studied and investigated the *P. luminescens* cultivation for biopesticide production.

INVESTIGATIONS ON THE BACTERIAL DIVERSITY OF NATURAL THERMAL LAKE HÉVÍZ USING METAGENOMIC APPROACH

GERGELY KRETT[1, 2], ATTILA SZABÓ[1], TAMÁS FELFÖLDI[1], KÁROLY MÁRIALIGETI[1], ANDREA K. BORSODI[1]

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

Lake Hévíz is a natural thermal water lake located in Central Europe, Hungary. It is a famous recreational and medical destination since the late 18th century. The water of the lake – which is oligotrophic and rich in sulfur compounds - originates from two crater springs with different temperatures evolving a 33-35°C water temperature in summer that never falls below 22°C even in winter. Due to the high flow of the two crater springs, the whole water volume is replaced in about 3.5 days providing a river-like character of the lake. The aim of the present study was to reveal the bacterial diversity of the warm, cold and mixed spring water originating from the spring cave, the biofilm and sediment of the lake and to explore the seasonal heterogeneity of planktonic bacterial communities using pyrosequencing. Lake water samples were collected from 14 sampling sites in April, July and October 2010 and 2011 while spring cave water, biofilm and sediment samples were taken in June 2015. Comparing the planktonic bacterial community structures, significant seasonal

differences were observed. Summer and autumn samples of 2010 showed the greatest similarity to each other, while spring sample of that year differed mostly from others on genus level according to Bray-Curtis similarity index based cluster analysis. Proteobacteria related sequences were dominant throughout almost all samples, however, major differences were between the sample types at below phylum level. Members of class Alpha and Betaproteobacteria were revealed mainly from the lake water, the mixed water originated from spring cave and biofilm samples. The warm spring water sample was predominated by the genus *Arcobacter* related sequences belonging to the class Epsilonproteobacteria, while the genus *Aeromonas* within the class Gammaproteobacteria was identified in the largest proportion from the cold spring water. Members of the class Gammaproteobacteria were also abundant in the biofilm samples. Dominance of Cyanobacteria and Chloroflexi related sequences and negligible presence of phylum Proteobacteria were revealed from the sediment sample. Owing to the relative richness of reduced sulfuric compounds of the lake water, several potential sulfide oxidizing taxa (e.g. *Rhodobacter*, *Thiobacillus*, *Arcobacter*, unclassified Hydrogenophylaceae) were identified as dominant community component.

WHAT WENT WRONG – POSSIBLE CAUSES OF MULTI-RESISTANT MICROBES GLOBAL SPREAD

KATALIN KRISTÓF

Clinical Microbiological Diagnostic Laboratory, Department of Laboratory Medicine Institute, Semmelweis University, Budapest, Hungary

Antimicrobial resistance in bacterial pathogens is a worldwide challenge leading high morbidity and mortality in clinical settings. Rapid evolution of resistant bacteria calls for new preventive measures to slow down this process, and a longer-term progress cannot be achieved without a good understanding of the mechanisms through which drug resistance is acquired and spreads in microbial populations. Epigenetic inheritance, population structure and heterogeneity, high mutation rates, gene amplification, efflux pumps, horizontal gene transfer facilitated by mobile genetic elements and biofilm formation of bacteria have all been reported as possible explanations for antimicrobial resistance. It has been driven by a diverse set of factors, including inappropriate antibiotic prescribing and sales, compliant behaviour of patients, use of antibiotics outside of the health care sector. The use of an antibiotic (mostly unnecessarily) in any individual may give rise to resistance, among pathogens or normal flora, to that antibiotic alone, to all members of its class, or to additional unrelated antibacterial, or to the acquisition of new bacteria, from a variety of sources (humans, animals, inanimate environment). Antibiotic treatment can damage the intestinal microbiota and, paradoxically, increase susceptibility to infections. Without using the appropriate infection control, resistant bacteria can easily be disseminated to the other patients and the environment. Contamination of waste effluent from hospitals and intensive food animal production with antimicrobial residues is an also a huge global problem. Antimicrobial residues exert selection pressures that influence the acquisition of antimicrobial resistance and virulence genes in diverse microbial populations. The problem of antibiotic resistance poses challenges across many disciplines. The causes of it are complex. One such task is to understand how antibiotics work, and how resistance to them can emerge. If we know the problem, maybe we can fight against it successfully.

POSSIBILITIES OF ANIMAL HEALTH AUTHORITIES TO FIGHT AGAINST ANTIMICROBIAL RESISTANCE

GÁBOR KULCSÁR, MELINDA TERÉNYI

National Food Chain Safety Office, Budapest, Hungary

Antimicrobial resistance is probably one of the major medical challenges of the 21st century. Sales volumes and patterns of veterinary antimicrobial agents as well as AMR monitoring data in Hungary give grounds for special concern. Arrangements should be made to achieve the reduction of antimicrobial consumption in animals and to promote the responsible use of currently available veterinary medicinal products.
The lecture is about the possibilities of animal health authorities to achieve this goal.

CHARACTERIZATION OF A MUTAROTASE GENE INVOLVED IN D-GALACTOSE BREAKDOWN IN *ASPERGILLUS NIDULANS*

LÁSZLÓ KULCSÁR, MICHEL FLIPPHI, LEVENTE KARAFFA, ERZSÉBET FEKETE

Department of Biochemical Engineering, University of Debrecen, Debrecen, Hungary

Aldose 1-epimerases or mutarotases (EC 5.1.3.3) are catalysing the interconversion of α- and β-anomers of hemiacetals of monomeric sugars such as D-glucose and D-galactose, and presumed to play an assessory role in carbohydrate metabolism, as this interconversion takes place spontaneously, too. Although phosphorylation by galactose kinase is generally considered the first step in the Leloir pathway – a ubiquitous route for D-galactose breakdown – it is preceded by mutarotase-catalysed, accelerated conversion of β-D-galactopyranose into the α-anomer, the de facto substrate of galactose kinase. To advance our understanding of fungal lactose and D-galactose catabolism, we identified two putative aldose-1-epimerase genes (*galmA* and *galmB*) in the model organism *Aspergillus nidulans*, and characterized them after generation of single (*galmA*, *galmB*) and double (*galmA/galmB*) deletants, as well as mutarotase overexpressing mutants. Mutarotase activity was quantified as the conversation rate between α- and β-D-glucopyranose anomers. Intracellular- and extracellular activity was determined in cell free mycelial extract and culture supernatant, respectively. In *galmB* single- and galm double mutants, intracellular mutarotation hardly exceeded the spontaneous conversion rate, while *galmB*-overexpressing strains displayed a significantly higher activity than the wild type. We could not detect any mutarotase activity in the culture supernatant, indicating the intracellular nature of the *galmB* gene product. We found that biomass formation and D-galactose uptake rates in *galmB*-deficient mutants were considerably reduced relative to the wild-type strain when D-galactose was present as the sole carbon source. The difference was most profound during the rapid phase of growth. No such effects were observed studying *galmA*.
Although transcription of both studied genes was substantially increased upon transfer to medium containing L-arabinose or L-arabitol, their expression profiles differed. *galmB* expression was consistently high in D-xylose grown cultures and in the later stages of growth, it was strongly expressed on D-galactose. On the other hand, *galmA* transcript could not be detected on D-galactose, although it was observed on D-glucose, D-xylose and L-arabinose. We conclude that *A. nidulans* GalmB is physiologically relevant for the utilisation of D-galactose.

PREVALENCE OF AMINOGLYCOSIDE MODIFYING GENES IN GRAM NEGATIVE BACTERIA COLLECTED FROM AN INTENSIVE CARE UNIT

ORSOLYA KUNGLER-GORÁCZ, BENCE BALÁZS, JULIANNA MÓZES, EBRAHIMI FATEMEH, ANDREA MEGYERI, KINGA FANNI TÓTH, ADINA FÉSŰS, GÁBOR KARDOS

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

The aim of our work was to investigate the occurrence of aminoglycoside resistance genes and integrons in Gram negative isolates collected at an ICU. Altogether 196 isolates were investigated; 76 *Pseudomonas aeruginosa*, 43 *E. coli*, 33 *Acinetobacter baumannii*, 14 *Klebsiella* spp., 14 *Proteus* spp., 7 *Enterobacter* spp. and 5 *Citrobacter* spp. Aminoglycoside modifying enzyme genes *aac*(6')-Ib, *ant*(3")-Ia, *aph*(3')-VIa, *ant*(2")-Ia, *aac*(3)-IIa and *aph*(3')-Ia as well as aminoglycoside resistance methylase genes *armA*, *rmtA* and *rmtB* were sought for using PCR. Integron detection was performed by PCR-detection of integrase genes. The most common aminoglycoside modifying genes were *aac*(6')-Ib (31.6%; 62/196), *ant*(3")-Ia (23.0%; 45/196) and *aph*(3')-VIa (13.3%; 26/196). The gene *ant*(2")-Ia occurred mostly in *Pseudomonas aeruginosa* isolates, and the *aac*(3)-IIa was detectable only in *E. coli*. The resistance methylase gene *ArmA* was found in 12.2% (24/196) of all isolates, mainly in *A. baumannii* (57.6%; 19/33), but also in *Proteus* spp. (2/14) and *Klebsiella* spp.(3/14); *rmtA* and *rmtB* was not encountered. Type 1 integrons were carried by 36.2% (71/196) of isolates, type 2 integrons occurred only in *A. baumanni* (1/196) and *Proteus* spp. (2/196).
Aminoglycoside modifying enzyme genes showed a species-dependent distribution, the most frequent gene was *aac*(6')-Ib. The appearance of the *armA* gene raises concern, because this gene confer resistance to all of the commonly used aminglycosides.
This work was supported by a János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

FIRST MOLECULAR IDENTIFICATION OF *DIROFILARIA* SPP. (ONCHOCERCIDAE) IN MOSQUITOES FROM SERBIA

KORNÉLIA KURUCZ[1], ANETT KEPNER[1, 2], BOSILJKA KRTINIC[3], BRIGITTA ZANA[1, 2], FANNI FÖLDES[1, 2], KRISZTIÁN BÁNYAI[4], MIKLÓS OLDAL[1, 2], FERENC JAKAB[1, 2], GÁBOR KEMENESI[1, 2]

[1]Virological Research Group, Szentágothai Research Centre, University of Pécs; [2]Institute of Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary; [3]Ciklonizacija, Novi Sad, Serbia; [4]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

Dirofilariosis is a common and widespread veterinary health issue in several European countries with notable zoonotic potential. The causative agents are *Dirofilaria immitis* and *Dirofilaria repens* nematoda species which are transmitted by different mosquito vectors. Similarly to other mosquito-borne infections, the knowledge about mosquito species involved in disease transmission is crucial for the complex understanding of local transmission cycles. Since there is no available data on mosquito species, potentially involved in disease transmission from Serbia, 6,369 female mosquito individuals (combined in 180 pools) representing 11 species were retrospectively tested for *Dirofilaria* nematodes, collected from 13 localities in Vojvodina province, Serbia in 2013. Altogether 8.33% of tested pools showed positivity, composed of five mosquito species, mainly *Culex pipiens* and *Aedes vexans,* the most abundant species at the sampled region. *Dirofilaria immitis* (80% of the infected mosquito pools) and *D. repens* (20%) were both detected from multiple localities (n = 6) comprising both urbanized and rural areas, during the whole period of mosquito breeding season

from May to August with a maximum rate in July, which provides the first data on local transmission characteristics regarding mosquitoes from the Balkans.

CHARACTERIZATION OF STRESS SENSITIVITIES OF DELETION MUTANTS OF FUN GENES ENCODING MENADIONE STRESS RESPONSIVE PROTEINS IN *ASPERGILLUS NIDULANS*

ÉVA LEITER[1], MIHÁLY BÁLINT[1], MÁRTON MISKEI[2], ERZSÉBET OROSZ[1], ZSUZSA SZABÓ[1], TAMÁS EMRI[1], ISTVÁN PÓCSI[1]

[1]Department of Biotechnology and Microbiology; [2]MTA-DE Momentum, Laboratory of Protein Dynamics, University of Debrecen, Debrecen, Hungary

Four menadione stress responsive function-unkown (FUN) genes of *Aspergillus nidulans* (Locus IDs ANID_03987.1, ANID_06058.1, ANID_10219.1 and ANID_10260.1) was deleted and phenotypically characterized. In addition, phylogenetic analyses of the tested *A. nidulans* genes and their orthologs among Aspergilli have confirmed only the presence of a TANGO2 domain with NRDE protein motif in the translated ANID_06058.1 gene, exclusively. The gene deletion mutants were exposed to oxidative, osmotic and metal ion stress generating agents and only the ANID_10219.1 deletion mutant were sensitive to 0.12 mmol l^{-1} menadione sodium bisulfite. The gene deletions influenced the stress sensitivities sporadically, for instance some mutants grew more slowly in the presence of various stress generating agents while the ANID_10260.1 deletion mutant showed a similar phenotype to the control strain to all stressors analyzed. Our results are in accordance with earlier studies indicating that the deletions of stress responsive genes do not certainly result in any stress-sensitivity phenotypes, which can be attributed to compensatory mechanisms owing to other elements of the stress response system.

Deciphering the function of genes e.g. by construction and phenotypic characterization of gene deletion mutants may play important roles in biotechnological application of fungi and reducing fungal diseases. Moreover, fungi having recently "non-functional" genes might adapt to different environmental conditions more efficiently in the future.

The project was supported by OTKA (project number: K100464).

MICROBIOLOGICAL INVESTIGATIONS IN DANDÁR BATH (BUDAPEST)

ANETT LIPPAI, SZANDRA KÁLI, SÁRA SZURÓCZKI, ERIKA TÓTH

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

In Hungary there is only limited information on the natural microbiologial communities of thermal baths, the waters are investigated mainly in hygienic aspects and chemical parameters using standardised methods. During our studies the well and three pool waters (indoor 38°C, indoor 20°C, outdoor 38°C) were investigated in Dandár bath (Budapest) using cultivation dependent techniques. To isolate bacteria 10% R2A and minimal synthetic media with agar-agar and gellan gum were used, instead of distilled water well water was used for each media. From the well water sample polyurethane foam-based (PUF) traps were also applied to enrich bacteria. From the isolated bacterial strains DNA was extracted and after PCR and ARDA, bacterial strains were grouped and identified

based on their partial 16S rRNA gene. Next to cultivation even the total cell counts of water samples were determined by epifluorescent microscopy after staining with DAPI. From well water 1.7 x 10^2 CFU/ml, from the indoor 38°C pool 4.01 x 10^5 CFU/ml, from the indoor 20°C pool 1.71 x 10^5 CFU/ml, from the outdoor 38°C pool 1.31 x 10^5 CFU/ml values were detected. Direct cell counts were 1.43 x 10^4 cell/ml from the well, 4.94 x 10^6 CFU/ml from the indoor 38°C pool, 2.6 x 10^6 cell/ml from the indoor 20°C pool and 7.83 x 10^5 cell/ml from the outdoor 38°C pool. In the well water *Brevibacillus choshinensis* (based on literature data this bacterium is able to produce antimicrobial proteins and toxins) and *Brevibacillus brevis* were dominant but they could be observed also in the two 38°C pools. *B. choshinensis* does not grow at low temperature therefore their lack from the 20°C pool is not surprising. *Hydrogenophaga atypica* was isolated only from the indoor 38°C pool most probably due to the location and the water treatment. Based on cultivation the bacterial community of the indoor 20°C pool differed strongly from the other pool waters, most species could be cultivated from this water sample.
Rhizobium herbae was the dominant bacterium but *Porphyrobacter colymbi* and *Sphyngopyxis bauzanensis* were also observed in high quantity. Interestingly the only overlapping betweeen this pool and the well was the presence of *Micrococcus aloaverae*.

MOLECULAR CHARACTERIZATION OF EXTENDED-SPECTRUM B-LACTAMASE-PRODUCING ENTEROBACTERIACEAE STRAINS ISOLATED AT THE OUTPATIENT AND INPATIENT CLINICS OF THE UNIVERSITY OF SZEGED

ÁGNES LIPTÁK, JÓZSEF SÓKI, ANREA LÁZÁR, EDIT URBÁN

Department of Clinical Microbiology Diagnostics, Faculty of Medicine, University of Szeged, Szeged, Hungary

Nowadays, ESBL- producing aerobic bacteria pose significant treatment challenge globally, including Hungary. From 2000 onwards, besides the SHV and TEM types, CTX-M alleles are prevailing in most countries. Currently, we know more than 120 CTX-M alleles that can be grouped into 5 classes where the CTX-M 15 allele belonging to group I is the most prevalent in Europe. CTX-M alleles are usually upregulated by specific insertion sequence (IS) elements. In this study, we aimed to investigate molecular characteristics of ESBL-positive strains in our University Hospital. 49 Enterobacteriaceae isolates were selected that were proved to be ESBL-producers by routine disc diffusion metholds, and they were isolated in 2013 from wide range of clinical samples (24 outpatients and 25 inpatients). They composed of 29 *Klebsiella pneumoniae*, 19 *Escherichia coli* and 1 *Enterobacter cloaceae*. Antibiotic susceptibility tests and ESBL phenotypic characterization were performed using disc diffusion, Etest and agar dilution methods. SHV, TEM and CTX-M genes, type 1 integrons, ISEcp1 and IS26 elements were detected by end-point PCR. CTX-M allele typing was carried out by PCR-RFLP or sequencing. The genetic relatedness of the test strains was investigated by PCR typing (Enterobacterial Repetive Intergenic Consensus - ERIC PCR) and plasmids were isolated from the test strains. Among the 49 test strains 47 proved to be ESBL producer by Etest, all strains were resistant to ampicillin and cefotaxime , and only one strain was susceptible to ceftazidime and amoxicillin/clavulanic acid. Of the 49 test strains 1, 29 and 47 were positive for *SHV2a*, *TEM* and *CTX-M* genes. One *K. pneumoniae* and the *E. cloaceae* did not harbour any investigated ESBL gene. By means of PCR-RFLP analysis, 46 CTX-M-positive cases proved to carry 41 and 5 CTX-M-1 and CTX-M-14 group genes, respectively. Five CTX-M-1 type genes have also been sequenced and all proved to be CTX-M-15 alleles. In 6 strains, we could detect type 1 integrons. *E. coli* strains

displayed heterogeneous ERIC PCR typing patterns and these patterns did not correlate with the origins of the strains. Contrary, in case of the *K. pneumoniae* strains, the ERIC fragment patterns were similar giving fewer types than *E. coli* strains.
We also investigated the plasmid patterns of these strains, and they ranged from 1.4 to 7.3 kb (small plasmids) and from 50 to 64 kb (large plasmids). 8 and 29 IS26 and ISEcp1 IS elements were found in the upstream regions of the CTX-M genes, respectively. As a summary, a high level of dominance of CTX-M-1 type genes was found among the selected ESBL strains isolated in our University Hospital that were activated by IS elements and might be harboured by large plasmids.

SEROLOGICAL SURVEY OF HANTAVIRUS INFECTION AMONG RODENTS IN HUNGARY

MÓNIKA MADAI[1], VIKTÓRIA NÉMETH[2], MIKLÓS OLDAL[1], GYŐZŐ HORVÁTH[3], RÓBERT HERCZEG[4], RÉKA PINTÉR[4], ANNA KUTAS[1], BIANKA DALLOS[4], FERENC JAKAB[1]

[1]Virological Research Group, János Szentágothai Research Center; [2]Department of Dermatology, Venereology and Oncodermatology; [3]Institute of Biology, Faculty of Sciences; [4]University of Pécs, Pécs, Hungary

Hantaviruses are found worldwide and are known to cause serious human diseases. The hosts of these viruses are rodents, soricomorphs and bats. Three types of hantaviruses are circulating in Hungary, Dobrava, Puumala and Tula which are carried by mice and voles. Dobrava and Puumala viruses cause human disease. In Hungarian forests the dominant species of rodents are yellow necked mouse (*Apodemus flavicollis*) striped field mouse (*Apodemus agrarius*) wood mouse (*Apodemus sylvaticus*) and bank vole (*Myodes glareolus*) which are natural reservoirs. The aim of the study was to survey the prevalence of hantaviruses among rodent populations and examine the potential correlation between population densities and seroprevalence. The rodents were trapped at 13 sampling plots on a 149 ha area in the Mecsek Mountains, Hungary from March to Oktober between 2011 and 2014 using a capture- mark- recapture method. Rodent sera were tested for antibodies against the two most significant hantavirus species in Europe, Dobrava- Belgrade (DOBV) and Puumala (PUUV) viruses by ELISA. Among the 3710 tested sera samples 358 were positive for hantaviruses (DOBV, PUUV). In 2011 the seroprevalence was 15 %, but in 2012 despite of the higher number of rodents it was just 7,8 %, in 2013 and in 2014 it was about 6%. The seroprevalence was the highest for the *Apodemus* species in the first two year, then in 2013 and 2014 for the *Myodes glareolus*. In every year among the hantavirus positive rodents the number of males were higher.
In the sampling area, compared to the previous year of 2012, the seroprevalence decreased to half despite of the higher sample size. This result may indicate that the number of infected individuals and community size are not directly proportional. Since in the subsequent years we registered great decrease in population size, but the infection rate stagnated throughout, it may indicate a time delay between population size changing and infection rate alteration. In four years the number of *Apodemus* species which are abundant in the area decreased and the population density of *Myodes glareolus* became higher. Therefore at the last year of the survey of 2014, the Puumala hantavirus seroprevalence was much higher compared to Dobrava seroprevalence.

EBOLA VIRUS STABILITY IN BIOLOGICAL SPECIMENS DURING SHORT TERM STORAGE UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

NÓRA MAGYAR[1], BERNADETT PÁLYI[1], BÁLINT SZALAI[1], ÁGNES FARKAS[1], THOMAS STRECKER[2], ZOLTÁN KIS[1]

[1]Department of Virology, National Center for Epidemiology, Budapest, Hungary; [2]Institute of Virology, Philipps University Marburg, Marburg, Germany

The Ebola outbreak in West Africa posed significant challenges for outbreak response teams to ensure adequate conditions for collection, transport, processing and storage of different biological specimens for reliable laboratory testing. Although the outbreak appears to be under control by now, the persistence of Ebola virus in survivors remains a public health concern. While the adequate storage conditions of specimens for nucleic acid based diagnosis are well studied, only limited information are available for appropriate conditions that preserve virus infectivity. Such knowledge is critical to obtain reliable data of the potential infectiousness of various body fluids of Ebola patients. The infectivity of the Ebola virus in clinical specimens and the effect of different environmental conditions on it have utmost importance not only for transmission but for further analysis such as virus isolation. Therefore, our aim was to determine viability and detectability of the Ebola virus Makona variant during short-term storage in different clinical specimens such as cell culture media, EDTA-whole blood, plasma, urine, semen and saliva under stimulated environmental conditions. Furthermore, optimal storage conditions are critical for extensive virus characterization studies, including virus isolation. The different specimens were spiked with EBOV Makona-C05 to achieve a final 10E5 FFU/ml virus titer, stored at -80°C, +4°C, +23-25°C (room temperature) and +37°C and collected on the hours of 0, 12, 24, 48, 72, 96 and 120. Susceptible VeroE6 cells (cell culture) and fluorescent focus forming assay were used to determine the infective virus titer. In the cell culture media the virus titer decreased uniformly 3 log10 at 37°C and 1 log10 at 25°C by hour 120. The infective titer at 4°C remained unchanged. The virus was found to be the most stable in plasma, the reduction of the infective titer at 37°C was 1 log10 by hour 120.
The virus retained its infectivity at 4°C. Similar result was observed in case of EDTA-whole blood with a 2-3 log10 reduction of infective titer when stored at 37°C. Loss of virus infectivity in urine samples was observed after storing longer than 48 hours. The RNA level in all of the specimens, however, remained stable. Different pH of the urine samples had only a minor effect on the observed decrease of the virus titer. Determination of virus stability in semen and saliva is in progress at the moment. We found that the virus can survive in blood for long time, thereby increasing the success of isolation. Our results may support the work of the field teams in clinical specimen collection for further laboratory analysis and virus isolation.
The study was supported by the EU funded project EVIDENT (GA-666100).

DECREASED KILLING ACTIVITY OF MICAFUNGIN AGAINST *CANDIDA GUILLIERMONDII*, *C. LUSITANIAE* AND *C. KEFYR* IN THE PRESENCE OF HUMAN SERUM

LÁSZLÓ MAJOROS, RENÁTÓ KOVÁCS, GÁBOR KARDOS, ALIZ BOZÓ, QASEM SALEH, ZOLTÁN TÓTH

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

Mortality rates caused by *C. guilliermondii*, *C. lusitaniae* and *C. kefyr* among seriously ill patients are extremely high (53-83%), suggesting that currently used therapeutic approaches, including echinocandin therapy, are insufficient. Furthermore, infections by *C. lusitaniae* and *C. kefyr* have became increasingly prevalent since the introduction of echinocandins. Therefore, the aim of our study was to compare the killing activity of micafungin in RPMI-1640 and in 50% serum against *C. guilliermondii*, *C. lusitaniae* and *C. kefyr*. Micafungin (Astellas) MICs in RPMI-1640 and in RPMI-1640 plus 50% serum (serum from a human male, type AB, Sigma) were determined using the standard broth macrodilution method. Time kill studies were performed in both media at 0.25, 1, 4, 16 and 32 mg/L. Killing kinetics were analysed in both media (RPMI-1640 with and without serum), The mean times to achieve the fungicidal endpoint (T99.9=3/k) were calculated from the k (killing rate) values for each isolate and concentration in both media. *C. guilliermondii* isolates were susceptible to micafungin in RPMI-1640. MICs of *C. kefyr* and *C. lusitaniae* isolates were not higher than the epidemiological cut off values. In 50% serum, MICs increased 32-256-fold. In RPMI-1640, micafungin killing was rapid against *C. kefyr*; mean times to achieve 99.9% growth reduction were shorter than 4.04 hours for all isolates. Contrastingly, 0.25, 1 and 4 mg/L micafungin did not produce any killing against *C. kefyr* isolates in serum. Higher concentrations (16 and 32 mg/L), however, produced fast (≤3.03 hours) killing in 50% serum as well. Micafungin at 4, 16 and 32 mg/L was fungicidal against two of three *C. lusitaniae* isolates in RPMI-1640. In 50% serum, micafungin produced only transient CFU decreases even at 16-32 mg/L against the *C. lusitaniae* isolates. Though micafungin was fungicidal at 32 mg/L against two of three *C. guilliermondii* isolates in RPMI-1640, in 50% serum, k values were always negative (indicating growth). Adding human serum to susceptibility test media drew attention to loss of killing activity in the presence of serum proteins, which is not predicted by MICs in case of *C. kefyr* and *C. lusitaniae* in RPMI-1640. Even elevated therapeutic micafungin doses (150 mg/day) do not produce serum drug levels sufficient for reliable fungicidal activity against either of the three species, which may explain the observed high probability of therapeutic failure in infections with these species.

COMPLEMENTATION OF THE A-FACTOR NON-PRODUCING *STREPTOMYCES GRISEUS* B2682 (AFN) STRAIN WITH A FUNCTIONAL *afsR* GENE

ÉVA MÁRTON, ZSUZSANNA BIRKÓ, SÁNDOR BIRÓ, MELINDA SZILÁGYI-BÓNIZS

Department of Human Genetics, Faculty of Medicine, 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 that makes streptomycetes one of the most important industrial microbial genus since 60% of the known antibiotics derive from their cultures. Morphological development and secondary metabolism are simultaneously affected by various nutritional environments and controlled by extracellular signaling molecules such as γ-butyrolactones e.g. A-factor in *S. griseus*. The lack of A-factor production is accompanied with the block of differentiation and antibiotic production as in the case of the *S. griseus* B2682 A-factor negative (AFN) strain. Shedding light on the cause of A-factor deficiency in the AFN strain might contribute to the deeper understanding of A-factor regulation that might support the screening efforts for new antibiotics. Our aim was to understand the cause of A-factor deficiency

in AFN. A significant mutation was found in the AFN genome by sequencing in the 2642. position of the *afsR* gene where the G → A transition resulted in a nonsense mutation (Trp → STOP codon) that results a 94 amino acid shorter protein. *AfsR* plays prominent role in the regulation of secondary metabolism in *S. coelicolor* via inducing the transcription of afsS transcriptional regulator. However, only controversial information is available about the role of AfsR in the regulation of secondary metabolism in *S. griseus*. In order to confirm the hypothesis that the afsR mutation is responsible for the phenotype of the *S. griseus* B2682 AFN strain we complemented this strain with a functional *afsR* gene. The *afsR* gene and its promoter sequence were amplified by PCR using the *S. griseus* B2682 genomic DNA as template and was ligated to the pHJL401 low copy number shuttle vector using the XbaI and EcoRI cloning sites of the plasmid. In order to increase the transformation efficiency of *S. griseus* the plasmid was transformed to the *E. coli* ET12567 methylation deficient strain at first. The plasmid was purified from *E. coli* by alkalic lysis and was used for the transformation of *S. griseus* B2682 AFN strain. The transformants were selected by thiostrepton resistence. According to the phenotypic studies the sporulation and antibiotic production were restored in the transformants that confirms the role of *afsR* in the regulation of morphological and physiological differentiation in *S. griseus*. As AfsR might be activated by several – yet unknown - environmental stimuli our study suggests a possible explanation for the effect of environmental conditions to the differentiation in *S. griseus* via enhancing A-factor production. Shedding light on the details of this regulation might help screening efforts for new antibiotics by awakening cryptic antibiotic gene clusters.

REGULATION OF THE LINALOOL-INDUCED OXIDATIVE STRESS PROCESSES IN THE HUMAN PATHOGEN *CANDIDA ALBICANS*

GÁBOR MÁTÉ[1], DOMINIKA KOVÁCS[1], ZOLTÁN GAZDAG[1], MIKLÓS PESTI[1], ÁRPÁD SZÁNTÓ[2]

[1]Department of General and Enviromental Microbiology, Faculty of Sciences; [2]Department of Urology, Medical School, University of Pécs, Pécs, Hungary

In this study, the linalool (Lol)-induced citotoxicity and alterations in the accumulation of reactive oxygen species (ROS) and the regulation of the activities of antioxidant enzymes were investigated in acute toxicity tests in a clinical isolate of the human pathogen, petite-negative *Candida albicans*. Lol treatment and cotreatment with miconazole induced dose- and time-dependent decreases in the growth of *C. albicans*, suggesting synergistic interaction. Growth inhibition was manifested in the reduced germ tube formation of the pathogen, indicating a dose-dependent decrease in virulence. For the potential application of Lol in human infections, its effect on the mitochondrial function was investigated. No ability of Lol to induce petite mutants was detected when a petite-positive *Saccharomyces cerevisiae* strain was used. In comparison with the control, the exposure of 10^7 cells ml^{-1} to 0.125 µl ml^{-1} (0.701 mM) or 0.250 µl ml^{-1} (1.402 mM) Lol for 1 h induced 20% and 30% decrements, respectively, in the colony-forming ability.

At the same time, these treatments caused dose-dependent decreases in the levels of superoxide anion radical ($O_2^{\bullet-}$) and total ROS, while there were 1.5 and 1.8-fold increases in the concentrations of peroxides and lipid peroxides, respectively. The Lol treatments resulted in different adaptive modifications of the antioxidant system. In 0.125 µl ml^{-1}-treated cells, decreased specific activities of superoxide dismutases and catalase were detected, while exposure to 0.250 µl ml^{-1} resulted in the up-regulation of catalase, glutathione reductase and glutathione peroxidases.

COMPARISON OF BACTERIAL COMMUNITIES INHABITING THE SALINE AQUATIC ENVIRONMENTS LOCATED IN THE EAST CARPATHIAN BASIN

MELINDA MEGYES[1], JÚLIA MARGIT ASZALÓS[1], JÁNOS MÓGA[2], KÁROLY MÁRIALIGETI[1], ANDREA BORSODI[1]

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

During the Miocen era enormous salt bodies were formed by the evaporation of Paratethys in the Carpathians which provided the operation of numerous famous salt mines throughout Romania and Ukraine from the 18th century. Following the termination of mining, several deep chambers collapsed as a consequence of surface water infiltration. As time passed, these dropout dolines filled up with water and eventually became salt lakes. The water dissolved the underlying salt bodies, effecting in nearly saturated salt concentrations. The studied lakes and creeks located in Aknaszlatina (Ukraine), Aknasugatag and Parajd (Romania) are characterized by a salinity gradient between 2 ppt and 253 ppt. The oldest lake was formed around 100 years ago, while the youngest one appeared less than two years ago. The development of younger lakes is still an ongoing process. Some of them are exposed to anthropogenic effects as well. Therefore, these environments can be regarded as natural laboratories of lake succession. The aim of this study was to reveal and compare the bacterial communities of water and sediment samples from saline lakes and creeks related to the above mentioned old closed salt mines using cultivation based and molecular fingerprinting methods.
Germ count estimation was performed using sea water agar. More than 200 strains were isolated and identified on the basis of the 16S rRNA gene sequence similarity. The bacterial community structures were compared according to their denaturing gradient gel electrophoresis profiles.

EFFECT OF THE VEGETATION ON THE PLANKTONIC BACTERIAL COMPOSITION IN LAKE KOLON (HUNGARY)

ANIKÓ MENTES[1], ATTILA SZABÓ[1], NÓRA TUGYI[2], LAURA JURECSKA[1], BOGLÁRKA SOMOGYI[2], LAJOS VÖRÖS[2], TAMÁS FELFÖLDI[1]

[1]Department of Microbiology, Faculty of Science, Eötvös Loránd University, Budapest; [2]Balaton Limnological Institute, Ecological Research Centre, Hungarian Academy of Sciences, Tihany, Hungary

Planktonic bacterial communities play an important role in aquatic ecosystems, but the composition of the bacterioplankton within a lake can be different due to different environmental conditions. In this research Lake Kolon was investigated, which is a large freshwater marsh in the Duna-Tisza Interfluve (Hungary). There is a major pelagic part (artificial open-water area, dredging) lacking macrophytes and three little inner ponds associated with reed (*Phragmites australis*), bladderwort (*Utricularia vulgaris*), water-lily (*Nymphea alba*). This lake enables the analysis of the effect of different decomposing macrovegetation on the compositon of bacterial communities. Many publications can be found in the literature, where the impact of the possible factors (e.g. pH, temperature, amount of nutrient, salt concentration) were examined on the microorganisms, but little is known about how decomposing macrophytes affect the composition of bacterioplankton. Additionally, bacterial communities in freshwater lakes have been mainly investigated vertically, but few studies discussed the horizontal patterns. Composition of the bacterial community was

determined based on the analysis of the 16S ribosomal RNA gene using next-generation DNA sequencing. Phylum Proteobacteria, Bacteroidetes and Actinobacteria predominated in all samples, however, the relative abundance of these main phyla was dissimilar. Despite the similarities of the measured chemical and physical parameters, the inner ponds had different bacterial communities, probably due to differences in their macrovegetation. All the water samples had brown colour due to the high concentration of dissolved carbon from the large amount of submerged macrophytes. Since low chloropyll-a concentration was measured in all samples, we concluded that the type of macrovegetation defined the composition of the bacterial communities.

This project was supported by the National Research, Development and Innovation Office, grant no. 116275.

TRANSCRIPTOME ANALYSIS OF THE *PSEUDORABIES* VIRUS WITH LONG- AND SHORT-READ SEQUENCING PLATFORMS

NORBERT MOLDOVÁN[1], DÓRA TOMBÁCZ[1, 2] ZSOLT BALÁZS[1], ZSOLT CSABAI[1], MICHAEL SNYDER[2], ZSOLT BOLDOGKŐI[1]

[1]Department of Medical Biology, Faculty of Medicine, University of Szeged, Szeged, Hungary; [2]Department of Genetics, School of Medicine, Stanford University, Stanford, California, USA

Aujeszky's virus (AyV), also known as Pseudorabies virus (PRV) - an animal pathogen – was used to study the transcriptome and gene expression. Its closest human pathogen relatives are Varicella zoster and Herpes simplex 1 and 2. Epithelial porcine kidney cells (PK-15) were infected with PRV and they were incubated for 1, 2, 4 6, 8 or 12h. Total RNA were purified from the samples. Reverse transcription reactions were carried out with gene specific primers. Single-stranded cDNAs were amplified by qPCR. Double-stranded cDNAs were produced using Oligo(d)T and random hexamer primers for subsequent SMRT (PacBio) and Illumina library preparation and sequencing. qPCR data was used for calculating the relative copy number of transcripts. Illumina sequencing was performed on two lanes, data was analyzed using Bowtie, TopHat, BWA and STAR software. For SMRT sequencing we run 38 SMRTCells and data was analyzed with SMRT Analysis and GMAP software. For visualization we used IGV. As a result of our investigation, we discovered numerous novel non-coding RNAs, transcript isoforms, and transcriptional overlaps. Our analyses also revealed novel transcriptional start site (TSS) and poly(A) signals. We also found an extensive antisense RNA expression throughout the entire viral genome. Single Molecule Real-time (SMRT) sequencing allowed us to categorized viral transcripts according to their transcriptional kinetic profiles and also to analyze the potential interaction between genes through their transcriptional machineries.

DISSOLVED OXYGEN LEVELS POSITIVELY CORRELATE WITH ALTERNATIVE OXIDASE EXPRESSION AND THE ITACONIC ACID YIELD IN *ASPERGILLUS TERREUS* FERMENTATION

ÁKOS PÉTER MOLNÁR[1], MICHEL FLIPPHI[1], ERZSÉBET SÁNDOR[2], ERZSÉBET FEKETE[1], LEVENTE KARAFFA[1]

[1]Department of Biochemical Engineering; [2]Institute of Food Processing, Quality Assurance and Microbiology, University of Debrecen, Debrecen, Hungary

A characteristic of filamentous fungal mitochondria is the existence of an alternative respiratory pathway that features the alternative terminal oxidase (AOX). AOX catalyses the direct reduction of

oxygen to water by channeling electrons from the ubiquinone pool directly to oxygen, bypassing cytochrome complex III and IV to recycle the reduction equivalents necessary to drive metabolism and growth. Consequently, the ATP yield is significantly reduced compared to oxidative phosphorylation and most of the released energy is lost as heat. On the other hand, the alternative path via AOX allows the fungus to consume the available growth substrate very fast without the byproduction of detrimental reactive oxygen species by the cytochrome oxidase. AOX was demonstrated to play an indirect but indispensible role during industrial (over)production of fungal secondary metabolites (e.g., citric acid, cephalosporin C). Unlike Cytochrome C, AOX is resistant to cyanide and sensitive to salicylhydroxamic acid, but it has a low affinity for oxygen. The filamentous fungus *Aspergillus terreus* can produce itaconic acid (systematic name: 2-methylidenebutanedioic acid; IA), a natural building block with great protential for the mass fabrication of biodegradable polymers (plastic and coatings) on an industrial scale The process technologies currently used are similar to those applied for industrial citric acid production – another bulk product of so-called overflow metabolism – by the related species *Aspergillus niger*. Citric acid is an early intermediate of IA synthesis, and for the current study we assumed that the involvement of AOX in IA production would be similar to the one in citric acid production. *A. terreus* is capable of growing in the presence of 10 mM KCN. Like other fungal cell factories such as *A. niger*, *A. oryzae* and *Penicillium chrysogenum*, the genome of *A. terreus* specifies two paralog genes, putatively encoding AOX (*aodA* and *aodB*). In submerged fermentations mimicking industrial conditions, *aodB* displayed constitutive but weak expression at each of the time-points tested.
In contrast, the *aodA* expression level positively correlated with increasing dissolved oxygen (DO) levels in the bioreactor as well as with the final IA yield of the fermentation. We conclude that the high DO levels required for successful (high-yield) IA fermentation on glucose facilitate increased alternative respiration by means of the cyanide-resistant alternative oxidase AodA.

EVALUATION OF INNATE AND ADAPTIVE IMMUNE RESPONSE IN CORTICOSTEROID-RESISTANT AND SENSITIVE ASTHMA BRONCHIALE

TÍMEA MOSOLYGÓ[1], ZOLTÁN RÓZSAVÖLGYI[2], NORUDEEN MOMODU[1], VALÉRIA ENDRÉSZ[1], DEZSŐ VIRÓK[1], YVETTE MÁNDI[1], ATTILA SOMFAY[2], KATALIN BURIÁN[1]

[1]Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged; [2]Department of Pulmonology, University of Szeged, Deszk, Hungary

Corticosteroid-resistant asthma bronchiale accounts for a significant proportion of asthma; however, little is known about the mechanisms that underlie the pathogenesis of the disease. Various specific and unspecific triggers have been identified that can lead to an increase in bronchial hyperresponsiveness, inflammation and obstruction. Epidemiological studies have found an association between *Chlamydophila pneumoniae* infection and severe asthma. It is well described that the different cytokines produced by the cells of innate, adaptive immunity and the non immune cells have important role in the pathogenesis of the asthma bronchiale. Human b-defensins (HBDs) are the members of innate immune system. The level of HBD expression varies from individual to individual, and it has been suggested that this variation is because of the copy number polymorphisms of the genes encoding the HBDs. This study was designed to reveal the association between infection caused by *C. pneumoniae* and corticosteroid-resistant asthma bronchiale. Our further aim was to evaluate the immune response of peripheral blood mononuclear cells (PBMCs) obtained from asthmatic patients after in vitro stimulation with *C. pneumoniae* or phytohemagglutinin. Our next

goal was to reveal whether the genetic predisposition of the inflammatory host response may affect the development of asthma bronchiale. Sera and PBMCs obtained from patients with corticosteroid-resistant or sensitive asthma and from healthy blood donors were examined. Total antibodies against *C. pneumoniae* were determined by ELISA. PBMCs were stimulated in vitro with *C. pneumoniae* or phytohemagglutinin. Cytokines (IL-10, IL-17, IFN-γ and TNF-α were measured in the supernatants of stimulated cells by ELISA. Unstimulated PBMCs served as controls. We investigated the copy number variant of the *DEFB4* genes in asthmatic and healthy blood donors. There were no significant differences in the seropositivity to *C. pneumoniae* between the 3 groups. PBMCs of asthmatic patients produced more IL-10 and TNF-a after the in vitro stimulation with *C. pneumoniae*, than that of the healthy blood donors. On the contrary in the supernatants of PBMCs from asthmatic patients, lower levels of IFN-γ were detected, compared to the control group. There were no significant differences regarding the production of IL-17. In the control group, the copy numbers of *DEFB4* gene exhibited a range of 2–8 per genome, with a median number of four copies. In the patients with asthma, the frequency distribution of the subgroups was different from that of the control group. Among the patients with asthma, overall 45% had a lower (<4) copy number comparing with controls. On the basis of our experiment the Th2 cytokines produced by the PBMC are dominant in asthma bronchiale and the lower *DEFB4* copy number can influence its pathomechanism.

DIVERSITY AND CATABOLIC ACTIVITY PROFILES OF SOIL MICROBIAL COMMUNITIES FROM SALINE SODIC SOILS IN HUNGARY

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

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

Solonchak soils in Kiskunság, Hungary are extremely saline and alkaline habitats which host unique biota. While the flora, fauna and the soil characteristics are well-studied, the microbiota is less known, mostly because of their extreme complexity. In the past few decades the groundwater level depletion led to an increased leaching of salts from topsoils, and hence a decrease in salinity and alkalinity in the upper layers. This resulted in changes in the composition of flora. While the vegetation dynamics can be followed relatively easy, the effect of this process on the soil microbiota is less obvious. Our research aimed to reveal and compare the microbiota of solonchak soils with different degrees of leaching. Samples were taken from a saline-sodic area at Apajpuszta (Hungary) from four different vegetation types, representing different stages of leaching. The sampling sites included a saline bare spot ("vakszik"), a *Puccinellia* sward ("mézpázsitos"), an *Artemisia* steppe ("ürmöspuszta"), and a shortgrass pasture dominated by *Achillea* spp. and *Festuca pseudovina* ("sziki legelő"). Three parallel samples were taken from three depths (0-10 cm, 10-30 cm, 30-60 cm) at each site. Soil physical and chemical parameters (texture, water holding capacity, pH, salt content, humus content etc.) were determined. Microbial biomass was estimated by substrate induced respiration. Catabolic activity profiles of soil microbial communities were analysed by multi-SIR method using the MicroRespTM system, using 23 different carbon sources. Cultivation of bacteria was done on three modified agar media. Strains were isolated and grouped by ARDRA, then representatives from each ARDRA group were identified by Sanger-sequencing of 16S rRNA genes. Community structures were compared by genetic fingerprinting of the bacterial communities using PCR-DGGE

based on the 16S rRNA genes. These methods together allowed a detailed description of the physiological status and structure of the soil microbial communities. Major differences between the four sites in both the soil catabolic profiles and dominant bacterial taxa were revealed.
This research was supported by the Hungarian Scientific Research Fund (OTKA) Grant K108572.

IMPACT OF MOLECULAR VIROLOGY ON HIV/AIDS

KÁROLY NAGY

Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary

Human immunodeficiency virus (HIV) is one of the most challenging pathogens science had ever encountered. HIV has been identified as etiological agent of AIDS in 1984. Thirty two years later the number of new HIV infections is still 2.3 million, and every 20 seconds someone dies in AIDS. All over the world the scientific research exert enormous effort to clarify the structure of this retrovirus, the molecular mechanism of HIV infection and the virus cell interactions. These efforts resulted in the development of such highly effective antiretrovirals, which revolutionized the treatment of AIDS. This therapy alone however is not enough, new strategies are needed for conquering AIDS, which require the cooperation of leading scientists, doctors, together with big pharma and governments. Author has been involved in the strategical fight against AIDS since the beginning, with performing molecular virological research on human retroviruses at various local and foreign institutes and universities. This work included the first isolation of HIV in Hungary, the confirmation of the first domestic AIDS case, determination of HIV subtypes dominant in Hungary, detection of the penetration of African circulating recombinant HIV forms (CRF) into the country, and analyzing the significance of primary infections by drug resistant HIVs of therapy naïve MSM population. He performed significant basic research concerning antiviral activities of substrate-based inhibitors of HIV protease, which opened up a new, crucial role of HIV-1 protease in the early phase of retroviral replication. The inhibitory effects were studied by cDNA analysis with PCR followed by Southern blot hybridization and by infectivity assays allowing quantitation of HIV-1 in a single cycle of replication. The results suggest that it is not the inhibition of initiation and progression of reverse transcription but the stability of full-size unintegrated cDNA which is affected in the presence of protease inhibitors. The cleavage of the NC protein is required for the proper formation of preintegration complex and/or for its transport to the nucleus.
Recently his research is focused on to develop and characterize thiolated pyrimidine derivatives with potent antiretroviral effect. These compounds affect –SH groups on the HIV gp120 envelope and the cell surface viral receptor CD4 concentrated in lipid rafts of cell membrane, selectively modifying viral binding sites. HIV viral pseudotypes have been prepared and employed for the quantitative determination of antiviral effects in vitro on recombinant monolayer cells allowing single cycle of HIV replication. These compounds are likely to be new classes of entry inhibitors using effectively to contain HIV infection and block progression to AIDS.

RESULTS AND CONCLUSIONS OF *ZIKAVIRUS* DIAGNOSTICS DURING THE 2016 SUMMER OLYMPIC GAMES

ORSOLYA NAGY[1], ANNA NAGY[1], ENIKŐ BÁN[1], EMŐKE FERENCZI[1], ISTVÁN JANKOVICS[2], MÁRIA TAKÁCS[3]

[1]National Reference Laboratory for Viral Zoonoses, Department of Viral Diagnostics; [2]Department of Respiratory Viruses; [3]Division of Virology, National Center for Epidemiology, Budapest, Hungary

Zika virus is an arthropod-borne Flavivirus transmitted by *Aedes* mosquitoes. Clinical manifestations occur in approximately 20 percent of patients and include mainly mild symptoms such as fever with maculopapular rash, arthralgia, myalgia or nonpurulent conjunctivitis. After the first isolation of the virus in Uganda in 1947, outbreaks of Zika virus infection were reported from the tropical areas of Africa and Southeast-Asia and the Pacific Islands. The first Zika virus infection in the Western hemisphere was detected in February 2014 on Chile's Easter Island and later other cases were registered in May 2015 in Brazil, since then there is a large ongoing outbreak in Latin-America. During the epidemic in 2014 in French Polynesia significant increase of cases of Guillain-Barré syndrome were reported, and in 2015 another important complication of Zika virus infection, the congenital Zika syndrome (microcephalia and other neurological malformations) was discovered in Brazil. Sexual transmission of the virus is also known, the first case occurred in 2008 in the United States and so far 16 more documented cases are listed in the literature. On 1st February 2016 the World Health Organization declared the Zika virus outbreak to be a Public Health Emergency of International Concern. In accordance with the informative of the Chief Medical Officer of Hungary, the Zika virus infection is a mandatory notifiable disease in Hungary from 1st June 2016, and all pregnant women (symptomatic and asymptomatic) with a history of exposure to Zika virus are to be tested as well as patients who met the epidemiological and clinical criteria of Zika virus infection. Difficulties of Zika virus laboratory diagnosis include the serological cross-reactions with other flaviviruses, possible co-infection with Dengue and Chikungunya viruses, which not only share the endemic areas of Zika virus, but have similar symptoms as well, and that most of the cases occur with asymptomatic clinical manifestation. This presentation would like to give a brief summary of the possible diagnostic methods of Zika virus infection and their applicability for diverse sample materials considering the time between the onset of symptoms and the sample collection.
Our aim was also to present the implemented and validated in-house techniques of our laboratory: indirect immunofluorescens method, rRT-PCT and plaque-reduction neutralization assay and the diagnostic results of samples collected from symptomatic and asymptomatic travelers to the endemic region, focusing on the period of the Summer Olympic Games.

INTRATYPIC VARIATION OF HUMAN PAPILLOMAVIRUS TYPE 11 IN RECURRENT RESPIRATORY PAPILLOMATOSIS

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

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

Recurrent respiratory papillomatosis (RRP) is mainly caused by low risk human papillomavirus (HPV) genotypes, HPV6 and HPV11. The infection with HPV 11 is usually associated with a more aggressive clinical appearance of the disease. Our aim was to investigate the genetic characteristics of HPV11 sequences from RRPs with different clinical course. Our recent studies have shown that relationship is assumed between intratypic variation of HPV 11 and the different outcome of the RRP. In this study, HPV11 sequences from four new and two already known patients were analysed and compared to our previous results and GenBank data. After nucleic acid extraction, HPV genotypes were determined in the samples and virus copy number were also estimated by real-time PCR with SYBR Green. After complete genome amplification, amplimers were purified from gel and

sequenced. The assembly of the complete genome of HPV11s and phylogenetic analysis together with GenBank data were performed by CLC Gene Workbench 5.7 software. The HPV11 genomes from recurrent papillomas were identical with the HPV11 sequences from the patients' previous recurrences. In case of a new patient, new nucleotide polymorphisms were not identified, however, in case of two additional patients, previously unidentified nucleotide polymorphisms were found. HPV11 sequences originated from three different localization of an extended papilloma were identical with each other, and the E1 open reading frame (ORF) of these genomes were identical with the E1 ORF of the reference genome derived from an extremely aggressive juvenile onset RRP. Finally, in a sample of a new patient, unique nucleotide polymorphisms causing amino acid changes in E1, E2 and E4 ORFs and T7330G polymorphism in the long control region were also identified. In addition, the previously described T7546C polymorphism, which decreases the activity of LCR significantly, was found in all newly enrolled sequences.

Two main phylogenetic lineages were distinguished in the phylogenetic analysis of the complete genomes available in the GenBank (A1 and A2); all Hungarian HPV11 sequences belong to the group A2. The existence of a sublineage with higher pathogenic potential is also assumed within group A2. This sublineage characterized by polymorphisms in E1, E2, E4, L1 ORFs and LCR; some of them may be related to the severe clinical outcome of the RRP.

This work was supported by János Bolyai Resarch Scholarship of the Hungarian Academy of Sciences.

ISOLATION AND ENRICHMENT OF DECHLORINATING MICROBIAL CONSORTIA TO PRODUCE EFFECTIVE INOCULA FOR BIOAUGMENTATION OF TRICHLOROETHENE CONTAMINATED AREAS

ZSUZSANNA NAGYMÁTÉ[1], LAURA JURECSKA[1], CSABA ROMSICS[1], FANNI TÓTH[1], VIKTÓRIA BÓDAI[2], PÉTER SÁTORHELYI[2], ÉVA MÉSZÁROS[3], ATTILA SZABÓ[1], TAMÁS FELFÖLDI[1], BALÁZS ERDÉLYI[2], KÁROLY MÁRIALIGETI[1]

[1]Department of Microbiology, Faculty of Science, Eötvös Loránd University; [2]Fermentia Ltd., Budapest, Hungary; [3]Institute of Agricultural Sciences, ETH Zurich, Lindau, Switzerland

Halogenated aliphatic hydrocarbons were used as solvents and degreasing agents in industry, agriculture and even in households all over the world. These contaminants can penetrate to soil and groundwater causing serious damages. Under anaerobic conditions halogenated aliphatic hydrocarbons can be reductively dehalogenated thorough dechlorination process which requires special microbial community. In situ biological remediation could be a feasible technique to degrade short-chain halogenated hydrocarbons (tetrachloroethene - PCE, trichloroethene – TCE) by stimulation (biostimulation) microbes involved in decomposition processes or using area specific dechlorinating inocula (bioaugmentation) for complete degradation. Our aim was to isolate and enrich short-chained halogenated aliphatic hydrocarbons degrading microbial consortia in order to enhance complete dehalogenation processes in pilot field project. A new technique was developed to set up and scale up anaerobic three-phase microcosms in laboratory and in operational conditions. TCE contaminated groundwater was used as inoculum to set up microcosms in 0.5L volume which was scaled up to 100L. Biodegradation of TCE to ethene via cis-dichloroethene and vinyl-chloride was observed in all microcosms in accordance with the detection of the reductive dehalogenase (trichloroethene-reductase and vinyl-chloride reductases) genes playing role in dechlorination process. Next Generation Sequencing aided Terminal Restriction Fragment Length Polymorphism revealed the presence of the genera Dehalococcoides which are the key microorganisms in anaerobic,

reductive dechlorination. Fluorescence In Situ Hybridization technique pointed out that the cell counts of Dehalococcoides, Bacteria and Archaea increased after inoculation of the enrichments. The total bacterial and archaeal community structures of the microcosms were altered over time and became significantly different from the community of the groundwater used as inoculum.
The support of HU09-0046-A2-2013 Norway Grant project is acknowledged.

APPLYING AN IMPEDIMETRIC INTSTRUMENTAL METHOD FOR QUANTIFICATION OF ANTI-MICROBIAL AND PREBIOTICAL EFFECTS

ÁRON NÉMETH, ZSOLT BARTA

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

Impedimetric based qualitative and quantitative microbiological test methods are already standardized (like DIN 10115). The Bac-Trac instrument developed and manufactured by Sy-Lab (Austria) follow both the impedance changes of the very specific cultivation media (M-value) and the impedance changes of two electrodes (E-value). According to the suggested methods by the manufacturer for different microorganism either the online monitored M-value or E-value show typical growth curves. The methods also gives specific threshold values for either M or E-values, to which belongs the so called detection time. Since the higher initial cell density will result quicker up growing M or E values i.e. shorter detection time at the same threshold, a calibration curve can be established between initial cell concentration and detection time for quantitative determination. We are going to present our recent results for applying this method for determining and quantifying anti-microbial and prebiotic compounds resulting longer or shorter detection time, respectively.
This method was tested in detection of anti-microbial effects of differently prepared textiles, to determine antibiotic concentration during antibiotic fermentation, and in detection of prebiotic effect of different agricultural wastes and fibres.

EGG PRODUCTS: SAFER APPEARANCES OF EGG

CSABA NÉMETH[1], ADRIENN TÓTH[2], GÁBOR JÓNÁS[2], BERTOLD SALAMON[2], LÁSZLÓ FRIEDRICH[2]

[1]Capriovus Ltd., Szigetcsép; [2]Department of Refrigeration and Livestock Products Technology, Faculty of Food Science, Szent István University, Budapest, Hungary

On surface of shell eggs several microbes may located which often origin of faecal contamination and which have a high risk on human health. To inactivate these bacteria in industry numerous methods are developed for shell disinfection, from these UV-light became most widespread. Countless research results showed that in the industry usually used treatment's time of UV-light is insufficient to reach 5-6 magnitudes decreasing in microbial load, applied 5 minutes UV-lighting. Beside that in aspects of food safety the use of alternative egg shell disinfection methods cause many problems in field of isolation, storage and destruction of egg shell as hazardous waste. Based on mentioned difficulties, in food industrial companies using eggs as ingredient, the application of "ready to use egg products" is spreading. Advantages of egg products is on the one hand that eggs are already separated from egg shell and on the other hand there is the opportunity to buy egg yolk, egg white or whole

eggs to the technology fitting forms: as liquid products, as boiled products, or in form of powder. Liquid egg products are easier to use than powder, because rehydration processes are not necessary. Pasteurization technologies of liquid egg products is developed to prove microbiological safety of products (as less 5 magnitudes decreasing in microbiological load), but in the same time the native attributes of eggs are preserved, like in case of egg white foaming ability, or durability of foam. The importance of time and temperature of pasteurization is manifested - next to the techno functional properties - in *Salmonella*-free product. The *Salmonella*-free product is required in any case, independent from the magnitude *Salmonella* infection in layer hens. In technological aspects this task is easy to solve by knowledge of heat resistance of *Salmonella* strain which are exanimated in egg products. A food producer will use egg products in form of powder, if it has not enough capacity for refrigerated storage, or the use of egg products can't be correctly calculated or planed in long term, or the powder form is easily fitted in the technology. Advantage of egg powders is the long shelf-life (2-3 years) under adequate circumstances, which is due to the low humidity in product (max. 5 m/m%) after drying process. In Hungary is more and more spreading the use of boiled egg products. Boiled egg white, egg yolk boiled whole egg sticks belong to the boiled egg products, as well as the most popular hard-boiled egg removed and cleaned from shell. Advantages of hard-boiled eggs are that food producers don't have to calculate with the loss from shells and boiling, or don't have to adapt the boiling process to the producing technology. Summarizing, the tendency of egg products on market shows increasing, which is explained by food safety aspects and easier using in different production technologies. The assortment of egg products is dynamic growing and analysing the markets of the U.S. and the European Union shows further market expansion.

POTENTIAL INVOLVEMENT OF LONG NON-CODING RNAS' IN *CANDIDA PARAPSILOSIS* VIRULENCE

TIBOR NÉMETH[1], ERNST THUER[2], TONI GABALDON[2], ATTILA GÁCSER[1]

[1]Department of Microbiology, University of Szeged, Szeged, Hungary; [2]Comparative Genomics Group, Centre for Genomic Regulation, Barcelona, Spain

Candida parapsilosis is an opportunistic human pathogen primarily affecting low birth weight neonates and the elderly getting prolonged antibiotic therapy. Microbial infection is a precisely regulated molecular cross talk between a pathogen and the host cells. In our days NGS RNA sequencing approach is the high-throughput golden standard method to reveal these changes in the global transcriptional network. Besides known ORFs these datasets always include large transcripts that for long were believed as transcriptional noise possessing no biological functions. Recently though the significance of these, so-called long non-coding RNAs (lncRNA) has become obvious as the lack or impaired function of these transcripts can end up in malfunction of basic cellular processes and lead to serial developmental disorders in higher eukaryotes. The involvement of lncRNAs in host-pathogen interaction is extensively studied, still there is not much data available on how these transcripts in the pathogen tune the regulatory network and affect the the virulence of the microbe. Here we analysed NGS RNAseq datasets from in vitro infections involving *C. parapsilosis* to identify CplncRNAs related to virulence. Five regions were chosen for deletion out of which three seemed to be taking part in stress tolerance in our in vitro assays. Interestingly one of the mutants showed strong growth defect at 37 °C, a property that is key for being a human pathogen.
Besides, sensitivity to cell-wall stressors and low pH, decreasement of MIC to echinocandins, and growth defect in the presence of oxidative stressors were recorded.

STERIGMATOCYSTIN FORMATION IN LIQUID *ASPERGILLUS NIDULANS* CULTURES ON LACTOSE AND D-GLUCOSE: A KINETIC STUDY

ZOLTÁN NÉMETH[1], ÁKOS PÉTER MOLNÁR[1], ERZSÉBET FEKETE[1], NANCY P. KELLER[2], LEVENTE KARAFFA[1]

[1]Department of Biochemical Engineering, University of Debrecen, Debrecen, Hungary; [2]Department of Medical Microbiology and Immunology; Department of Bacteriology, University of Wisconsin, Madison, USA

Aflatoxins (AT) are among the most carcinogenic natural substances known to date. They are produced by a large diversity of ascomycetous fungal species, mainly from the genus *Aspergillus*. Sterigmatocystin (ST) is structurally similar to AT but is less potent and it is the penultimate intermediate in the biosynthesis of AT in *A. flavus*; however, in several other fungi including the model fungus *A. nidulans*, it is the end product of the corresponding pathway, lacking the last two genes present in *A. flavus* and other AT producing fungi. In addition to being a model system for biochemical and genetic research, *A. nidulans* is also the industrial producer of the antifungal agent echinocandin B, has been used to study the biosynthesis of the beta-lactam antibiotic penicillin, and is employed as heterologous host for the production of the cholesterol lowering drug lovastatin and the immunosuppressive agent mycophenolic acid. In contrast to standard genetic practices where fungi are typically cultivated on agar-solidified media in small plastic plates, these processes are carried out as aerobic, liquid (submerged) fermentations with relatively high mycelial density. Under such conditions, the formation of ST as a contaminant may turn out to be an immense safety issue. While the AT/ST biosynthetic pathway is well-characterized in *A. nidulans* with all the structural genes and intermediates described, many of the regulatory aspects including those related to the carbon source available for the fungus remain enigmatic. This is particularly true for lactose (1,4-O-β-D-galactopyranosyl-D-glucose), the main carbohydrate in cheese whey, which is traditionally considered a cheap and abundant industrial growth substrate for micro-organisms, fungi in particular. The "lactose effect" can be summarized as the inability to block ST biosynthesis by regulatory means upon growth on lactose. To get a first insight, we analysed ST formation on lactose as a sole carbon source in well-controlled, submerged *A. nidulans* fermentations, by comparing kinetic data to those on D-glucose. We demonstrate that ST formation occurs during the stage of minimal (or ceased) carbon utilization that, in turn, results in slow growth.

On D-glucose, this prerequisite prevails only after all the carbon was taken up from the medium, while on lactose – which is a slowly metabolized carbon source – ST is formed throughout the fermentation. In fungi, slow growth coincides with carbon derepression. Due to its slow assimilation rate, lactose generates carbon catabolite derepressing conditions. However, time-profiles of ST formation in mutants lacking the general carbon catabolite repressor CreA either on lactose or D-glucose were similar to the wild-type reference strains, indicating that it may be the slow growth attainable on lactose rather than the lack of carbon repression that – at least partially – is responsible for triggering ST formation in *A. nidulans*.

The project was grant-aided by the Hungarian Scientific Research Fund (OTKA NN116519).

HOW TO IMPROVE DIRECT OBSERVATION OF A WIDESPREAD INTERFUNGAL PARASITIC RELATIONSHIP WITH GFP TRANSFORMATION?

MÁRK Z. NÉMETH[1], MARKUS GORFER[2], PÁL VÁGI[1, 3], GÁBOR M. KOVÁCS[1, 3], LEVENTE KISS[1]

[1]Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary; [2]Austrian Institute of Technology, Vienna, Austria; [3]Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, Budapest, Hungary

Powdery mildew fungi (Erysiphales) are obligate biotrophic plant pathogens, infecting a wide range of angiosperms worldwide. Pycnidial fungi of the genus *Ampelomyces* regularly parasitize powdery mildew colonies in the field and are also known as commercialized biocontrol agents of economically important powdery mildews. To improve direct observation of this widespread mycoparasitic interaction, *Ampelomyces* transformants carrying a visible marker were required. Transformants expressing green fluorescent protein (GFP) were produced by co-cultivating *Agrobacterium tumefaciens* strain AGL1 carrying a plasmid with the hygromycin resistance marker and GFP gene. Resulting transformants were selected on hygromycin-containing medium and were analysed for fluorescence after being grown in culture. Some transformants were used in mycoparasitic tests, i.e. powdery mildew colonies were inoculated with conidial suspensions of the transformants and analyzed with fluorescence microscopy after their intra-hyphal spread and sporulation. Persistence of *Ampelomyces* mycoparasites in powdery mildew-free environments, namely on healthy leaves and in the soil, was also tested with GFP expressing transformants. The transformation method proved to be effective as several transformants emerged on the selective medium and these exhibited strong green fluorescent signal, demonstrating the expression of GFP. In mycoparasitic tests, extensive intracellular colonization of powdery mildew hyphae was evident when powdery mildew-infected cucumber and barley leaves inoculated with transformants were examined using fluorescence microscopy. This indicates that GFP facilitates the direct observation of these mycoparasites inside the hyphae of their mycohosts and also in powdery mildew-free environments.

Supported by a grant of the National Research, Development and Innovation Office (NKFIH NN 100415) and a grant of the Austrian Science and Research Liaison Office/Austrian-Hungarian Action Foundation (90öu16).

DRUG RESISTANCE IN THE THERAPY OF LEISHMANIOSIS

RITA OLÁHNÉ SZABÓ

Research Group of Peptide Chemistry, Hun garian Academy of Sciences – Eötvös Loránd University, Budapest, Hungary

Leishmaniosis is one of the most problematic parasitic infection worldwide, especially in the neglected tropical and in the Mediterranean areas. According to the WHO's annual report approximately 310 million people are affected; 2 million new cases occurs annually, from which 1.5 million cases are cutaneous and about 500 000 are visceral leishmaniasis that is fatal if not treated. About 12 million people can be currently infected. Reservoirs of the parasite can be either humans or animals like dogs, cats, monkeys and rodents. *Leishmania* parasite is taken up by the cells of the reticulo-endotheilal system, mainly macrophages, dendritic cells and neutrophil granulocytes in which the parasites are able to survive and proliferate intracellularly, escaping from the antiparasitic response of the immune system. Current treatment of leishmaniosis is based on chemotherapy. Only a few chemotherapeutics are available including pentavalent antimonials-meglumine antimoniate and sodium stibogluconate, amphotericin B, miltefosine, pentamidine, paronomycin and sitamaquine. Anti-leishmanial agents applied in the clinical practice have either severe side effects or induce the development of drug resistance. Understanding the mechanism of resistance can help in designing/searching new drugs. The combination therapy of two or more drugs administered simultaneously could result also in an increased efficacy via synergistic effect, it can allow shorter

duration of treatment, which can reduce the development of side effects decrease occurrence of drug resistance. As a novel approach conjugation of toxic drugs to amino acids, peptides or proteins is proposed to reduce side adverse effects and also help to circumvent drug resistance by arresting the macromolecular conjugates within the cells. Combination of chemo- and immunotherapy could be a promising perspective in effective cure of the disease. In this lecture our present knowledge and understanding on the development of drug resistance in the therapy of leishmaniosis will be outlined.

PRODUCTION AND GLYCOSYLTRANSFERASE ACTIVITY OF ALPHA-GLUCOSIDASE FROM BIFIDOBACTERIA

PETRA ORBÁN, GABRIELLA STYEVKÓ, ERIKA BUJNA, JUDIT M. REZESSY-SZABÓ, NGUYEN DUC QUANG

Department of Brewing and Distilling, Szent István University, Budapest, Hungary

Bifidobacteria are known as probiotic organisms and they play an important role in human/animal health. Generally, the health promoting effects only observed when applied probiotics are able to colonise and growth in the large colon of host. Now doubt that physiological properties of organism are definitely affected by its enzyme system. While the role of bifidobacteria is well known and documented, whereas the information of its enzyme system are still deficient. In this study, production and glycosyltransferase activity of alpha-glucosidase from bifidobacteria are focused. Nine bifidobacteria strains and three carbon sources contained different glycosidic bonds were applied for screening growth and alpha-glucosidase activity. Four strains *B. lactis* Bb-12, *B. longum* A4.8, *B. breve* B9.15 and *B. adolescentis* grown well on the modified TPY medium (use of maltose instead of glucose). Additionally, these strains synthetized α-glucosidase intracellularly. The best result was observed in the case of *B. lactis* Bb-12 strain (the most commonly used probiotic bifidobacteria strains in the food industry), thus it was selected for further investigation. Optimal maltose concentration and amount of inoculation ratio for production of enzyme were determined to be 1 g/L and 1%, respectively. In this case, the highest intracellular activity (38 U/L) was obtained after 21 h of fermentation. Crude alpha-glucosidase enzyme was prepared base on results of fermentation. The biomass was centrifuged and collected and then three-cycle cell disruption was performed. Bioconversions using crude enzyme preparation were carried out with eight different substrates and monitored by TLC and HPLC methods. Based on the results, alpha-glucosidase from *B. lactis* Bb-12 exhibited both hydrolytic and glycosyltransfer activities. High amount of oligosaccharides were detected in the cases of maltose, sucrose and maltotriose substrates. The results are preliminary, but very promised and may open new opportunities for the production of oligosaccharides with special functions (prebiotics), and for the development of integrated synbiotics where prebiotics can be synthetized by enzyme(s) from probiotic organism.

Supported by the National Talented Program through Project No: NTP-HHTDK-16-0082.

THE BIODEGRADATION OF 5A-DIHYDROTESTOSTERONE BY *RHODOCOCCUS* STRAINS

IVETT OROSZ[1], ERZSÉBET BAKA[2], ADRIENN BALÁZS[1], ANDRÁS TÁNCSICS[3], BALÁZS KRISZT[1], CSILLA KRIFATON[1]

[1]Department of Environmental Safety and Environmental Toxicology, Faculty of Agricultural and Environmental Sciences, Szent István University, Gödöllő; [2]Department of Environmental and Applied Microbiology, National

Agricultural Research and Innovation Centre, Research Institute of Agro-Environmental Sciences, Budapest; [3]Regional University Center of Excellence in Environmental Industry, Szent István University, Gödöllő, Hungary

Primary sources of endocrine-disrupting chemicals (EDCs) are sewage effluents from domestic and industrial facilities. Androgens in human urinary cause the most of androgenic substances in municipal sewage with predominantly domestic input. Out of androgen hormones, testosterone, dihydrotestosterone (DHT), androsterone and the androstenedion are usually detected in the highest concentrations in the environment. To eliminate hormonal effect, understanding steroid metabolism and identifying steroid-dependent gene regulation in steroid-degrading bacteria are key steps. DHT that is a natural androgenic hormone and plays an important role in normal growth and function of the prostate, and responsible for the development of secondary sex characteristics was chosen for our research. The urinary extractions of DHT from a grown man is 14,1 µg/day. Increased levels of this hormone in the environment have adverse effects for living organisms. Reproductive and behavioral disorders were detected in fish, rats and quails during lab experiments. High levels of DHT may promote cell growth that could lead to prostate enlargement, and immunodeficiency. The aim of this work was to screen members of the *Rhodococcus* genus for their DHT degrading potential. Biodegradation experiments were performed in Erlenmeyer flaks in three parallels for 72 hours. Matrix of the biodegradation experiments was LB medium supplemented with DHT in the final concentration of 100 ng/ml. Biodegradation was analyzed by analytical method (gas chromatography–mass spectrometry), while biodetoxification was analyzed by biomonitoring systems. BLYAS (Bioluminescence Yeast Androgen System) is a low cost, rapid bioassay using *Saccharomyces cerevisiae* as a test organism, which measures androgenic potential. BLYAS produces bioluminescence in response to chemicals that bind to the human androgen receptor and then to human androgen response elements (ARE) on a reporter plasmid. In addition, a constitutive control strain (*S. cerevisiae* BLYR) was used to assess whether samples contained cytotoxic substances, which is detected by a decrease in bioluminescence. Based on the S. cerevisiae BLYR bioassay, there were no cytotoxic products in the biodegradation experiments. 17 out of 27 strains of *Rhodococcus* genus could eliminate the hormonal effect with 95-100% in 72 hours. Moreover, 6 strains decreased 90-95% the androgen effect in the biodegradation experiments. However, two strains (*R. fascians*, *R. kroppenstedtii*) decreased only 40% of the androgen effect in 72 hours.

This project was sponsored by the Research Centre of Excellence 9878-3/2016/FEKUT. Special thanks for the BLYES/BLYR strains to the researchers of the University of Tennessee (Knoxville, Tennessee).

DNA BARCODING COUPLED WITH HIGH RESOLUTION MELTING ANALYSIS ENABLES RAPID AND ACCURATE DISTINCTION OF *ASPERGILLUS* SPECIES

MELINDA PAHOLCSEK[1], GÁBOR FIDLER[1], SÁNDOR KOCSUBÉ[2], ÉVA LEITER[3], SÁNDOR BIRÓ[1]

[1]Department of Human Genetics, University of Debrecen, Debrecen; [2]Department of Microbiology, Faculty of Science & Informatics, University of Szeged, Szeged; [3]Department of Biotechnology and Microbiology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

We describe a high-resolution melting (HRM) analysis method that is rapid, reproducible and able to identify reference strains and further 40 clinical isolates of *Aspergillus fumigatus* (14), *A. lentulus* (3), *A. terreus* (7), *A. flavus* (8), *A. niger* (2) *A. welwitschiae* (4) and *A. tubingensis* (2). Asp1 and Asp2 primer sets were designed to amplify partial sequences of the *Aspergillus benA* (beta-tubulin) genes in a closed-, single-tube system. Human placenta DNA, further *Aspergillus* (3), *Candida* (9), *Fusarium*

(6) and *Scedosporium* (2) nucleic acids from type strains and clinical isolates were also included in this study to evaluate cross reactivity with other relevant pathogens causing invasive fungal infections. The barcoding capacity of this method proved to be 100% providing distinctive binomial scores; 14, 34, 36, 35, 25, 15, 26 when tested among species while the within-species distinction capacity of the assay proved to be 0% on the basis of the aligned thermodynamic profiles of the Asp1, Asp2 melting clusters allowing accurate species delimitation of all tested clinical isolates. The identification limit of this HRM assay was also estimated on Aspergillus reference gDNA panels where it proved to be 10-102 genomic equivalents (GE) except the *A. fumigatus* panel where it was 103 only. Furthermore, misidentification was not detected with human genomic DNA or with *Candida*, *Fusarium* and *Scedosporium* strains. Our DNA barcoding assay introduced here provides results within a few hours and it may possess further diagnostic utility when analyzing standard cultures supporting adequate therapeutic decisions.

DETECTION OF A MAMMALIAN-LIKE ASTROVIRUS IN BIRD, EUROPEAN ROLLER (*CORACIAS GARRULUS*)

PÉTER PANKOVICS[1], ÁKOS BOROS[1], TAMÁS KISS[2], ERIC DELWART[3, 4], GÁBOR REUTER[5]

[1]National Reference Laboratory of Gastroenteric Viruses, Regional Laboratory of Virology, Regional Institute of State Public Health Service Government Office of Baranya County, Pécs; [2]Hungarian Ornithological and Nature Conservation Society, Budapest, Hungary; [3]Blood Systems Research Institute; [4]University of California, San Francisco, USA; [5]Department of Medical Microbiology and Immunology, University of Pécs, Pécs, Hungary

Astroviruses are small, non-enveloped viruses with positive sense, single-stranded RNA genomes. The family Astroviridae contains two genera, *Mamastrovirus* and *Avastrovirus*, which – based upon our current knowledge – infect mammals and birds, respectively. However, recent seroprevalence study indicated that people with contact to turkeys can develop serological responses to the turkey astrovirus and minks might have been infected with the avastrovirus. These data suggest that the ‘‘host species/astrovirus genus’’ association should be permeable; however, mamastrovirus infection has not been reported from avian species, yet. The main objectives of this study were the first description, identification and complete genome characterization of a novel mamastrovirus from faecal samples collected from European rollers in Hungary by viral metagenomic analysis and classical RT-PCR methods. Faecal samples (N=19) were collected from clinically healthy European rollers (*Coracias garrulus*) from two breading territories of the Great Hungarian Plain, Hungary, in 2011. Two random faecal samples were chosen to viral metagenomic analysis representing the two breading territories. Based on the GenBank nucleotide and protein database the astrovirus-like assembled contigs were confirmed by RT-PCR method. Specific primers were designed to obtain the complete viral genome and screen further faecal samples. Here, a novel astrovirus was identified by viral metagenomics and RT-PCR methods in 2 (11%) out of 19 faecal samples collected from a wild, carnivorous bird species, European rollers (*Coracias garrulus*) from two breeding territories in Hungary. The complete genome sequence of astrovirus Er/SZAL6/HUN/2011 (KP663426) was 7025 nt-long and had some unique genomic features including an unusually long spacer between the subgenomic RNA promoter and the ORF2 initiation codon. Using the BLASTp Er/SZAL6/HUN/2011 had the highest amino acid identities 35%, 61% and 34% to MAstV 32 (JF713710, host: porcine), to MAstV 23 (JF729316, host: rabbit) and to unclassified porcine astrovirus (JX684071) in ORF1a, ORF1b and ORF2, respectively. The same proteins of Er/SZAL6/HUN/2011 had 25%, 66% and 33% amino acid identities to the corresponding proteins of murine astrovirus (JX544743) as the closest strain. Astroviruses are the third common cause of

gastroenteritis in humans, but astroviruses are supposed to infect wide range of host species (domestic and wild animals). The number of newly discovered astroviruses is rapidly increasing, but mamastrovirus has not been detected from avian species yet. Here we reported the first identification of a mamastrovirus in carnivore bird European roller. The sequence- and phylogenetic analysis indicated that Er/SZAL6/HUN/2011 represents the first member of a novel mamastrovirus species. Data suggest that both mammals and birds could have been exposed to mamastroviruses and avastroviruses providing opportunities for cross-species infection and viral adaptation with cross-class astroviruses especially in carnivorous animals. Astroviruses have high genetic and antigenic diversity and probably it has wider host species spectrum than it was thought before. Supposedly, many novel astroviruses may exist in the nature in birds and/or in other vertebrate hosts.

NON-HOMOLOGOUS END JOINING IS THE DOMINANT REPAIR PATHWAY IN THE DIMORPHIC FISSION YEAST *SCHIZOSACCHAROMYCES JAPONICUS*

LÁSZLÓ ATTILA PAPP, LAJOS ÁCS-SZABÓ, IDA MIKLÓS

Department of Genetics and Applied Microbiology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

Due to certain environmental factors or failures of replication, double stranded breaks (DSBs) can occur in the DNA, resulting in cell cycle arrest. Thus, DNA repair is a crucial process which is essential for all living organisms. Generally, there are two main repair mechanisms which are responsible for this correction. Homologous recombination (HR) repairs DNA strands with homology on the one hand, and non-homologous end joining (NHEJ) on the other, which does not require homologous DNA ends to restore the integrity of the genome. Unlike HR, NHEJ could be called "eukaryote SOS repair" because the main function of NHEJ is to maintain the genomic integrity regardless of sequence homology. Because of this, NHEJ is mutagenic and can cause serious problems, such as chromosomal translocations or activation of oncogenes. Still, NHEJ is the primary repair pathway in most fungal organisms and in humans. In the kingdom of fungi most of the species utilize NHEJ to repair DSBs. Thus, in model organisms, such as *A. nidulans*, *P. pastoris*, *K. lactis* NHEJ makes genetic manipulations difficult. Namely, the targeted gene remains intact and/or the DNA constructions can integrate into undesirable sites. To eliminate NHEJ and its negative effects, genes involved in NHEJ, such as ligase IV, Ku70/80 and Xlf1were deleted. *Schizosaccharomyces japonicus* is a dimorphic fission yeast, which is a relatively new model organism in genetics. However, there have been reports about successful gene deletion in *Sch. japonicus*, these publications did not mention which repair pathway was the dominant process. In our work, we created linear "ends out" deletion constructions to investigate the dominant repair mechanism of *S.japonicus*. We found that NHEJ was the main repair mechanism in the *S. japonicus* which caused several interesting mutant phenotypes. Factors which could promote HR were also identified and ligase IV (lig4) gene was successfully deleted the from the *Sch. japonicus* chromosome.

PREFERENCE OF GLUCOSE, LACTOSE AND PROTEIN UTILIZATION AS CARBON SOURCES FOR GROWTH OF DIFFERENT *LACTOBACILLUS* AND *LACTOCOCCUS* STRAINS

MELINDA PÁZMÁNDI, ZOLTÁN KOVÁCS, ANNA MARÁZ

Faculty of Food Science, Szent István University, Budapest, Hungary

During cheese production whey is formed as a by-product containing mainly lactose and whey proteins. As the consequence of its composition, the biological and chemical oxygen demands (BOI and KOI, respectively) of whey is quite high, thus it requires pre-treatment prior to discharging into wastewater treating facilities. At the same time whey components could be used as raw materials and precursors for further biotechnological processes. The aim of this study was to develop a whey-based drink as a value-added product by utilizing the lactose content of deproteinized whey via lactic acid fermentation. In the course of this study we examined the growth kinetics and fermentation characteristics of 10 lactic acid bacteria (LAB) strains belonging to the genera of *Lactobacillus* and *Lactococcus*. Tryptic soy (TS) broth as a growth medium contained either glucose (G) or lactose (L) as a carbon source. Fermentation experiments were conducted by using deproteinized whey supplemented with various proteins. Based on our previous research results it was understood that ammonium-sulphate as an inorganic nitrogen source did not significantly support the growth and fermentation activity of the strains, therefore we focused on the examination of the organic nitrogen sources (mainly whey proteins). During experiments we monitored the changes in the pH and cell concentrations and we determined metabolism of carbohydrates by HPLC analysis. According to our results glucose supported the growth of each examined strain better than lactose. In glucose containing culture media the specific growth rates were higher than in lactose (for Lactobacilli μ_{maxTSG}: 0.003-0.005 and μ_{maxTSL}:0.0003-0.0008; for Lactococci μ_{maxTSG}: 0.0012-0.003 and μ_{maxTSL}: 0.00056-0.002). The examined lactic acid bacteria did not respond positively to any amino acid supplementation, hence it can be assumed that the strains do not possess any amino acid auxotrophy. Based on our results supplementation of the deproteinized whey medium by whey protein fraction in much smaller concentrations than the original one stimulated growth of the LAB strains. Although adding whey proteins in 1 g/L concentration supported the growth significantly but at the same time all the strains preferred utilization of these proteins as a carbon source in comparison to lactose, thus the lactose content remained almost constant. Decrease of the whey protein concentrations to 0.25-0.5 g/L resulted in a slight decrease of growth rate but at the same time increase in the lactose consumption. Based on our results *Lb. helveticus* PB9, *Lb. rhamnosus* PB10, *Lb. sakei* DSM 20017, *L. lactis* A1 and A3 as well as *L. cremoris* B1 strains were selected for optimization of the fermentation process and examination of their metabolite and aroma production.

This study was financed by the PROST! EU 7. Research Programme PCIG11-GA- 2012-322219.

SEASONAL CHANGES IN THE MICROBIAL COMMUNITY STRUCTURE OF THE WASTEWATER TREATMENT SYSTEM IN A COKE PLANT

ZSUZSANNA POHNER, ZSUZSANNA NAGYMÁTÉ, KÁROLY MÁRIALIGETI

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

The changes in the microbial community structure of the wastewater treatment system in a coke plant were being monitored from autumn until the end of spring. Different groups of bacteria were detected by polymerase chain reaction (PCR): for total bacterial community 16S rDNA was targeted, for phenol-degrading *Comamonas* spp. catechol-2,3-dioxygenase (*C23O*) gene, for ammonia-oxidising bacteria (AOB) ammonia-monooxygenase (*amoA*) gene, for nitrite-oxidising bacteria (NOB) *Nitrobacter* spp. and *Nitrospira* spp. specific fragments of the 16S rDNA and for denitrifying bacteria nitrite-reductase genes (*NirS* and *NirK*) were amplified. The structure of these communities was examined by terminal restriction fragment analysis (T-RFLP). The unicellular Eukaryote organisms indicating water quality were examined by light microscopy. In the bacterial community structure in the autumn there was a strong difference among the different steps of technology (basins), while in the winter they became similar, especially in the case of the denitrifying and nitrite-oxidizing bacteria. However, the T-RFLP patterns of these two groups showed remarkable changes in late winter compared to earlier samples. In the spring the structure of the total bacterial and phenol-degrading *Comamonas* spp. community slightly changed. The AOB organisms were not detectable towards the end of autumn and in winter probably due to the drop in temperature and appeared again only in the beginning of spring. Light microscopic examination showed that in the autumn the bacterial community was not effective enough in eliminating polluting materials from the wastewater as unicellular Eukaryotes sensitive for pollution were not detected. In the winter these organisms appeared but in late winter were not detectable again until the beginning of spring.

TREATMENT OF MULTIRESISTANT *ENTEROBACTERIACAE* INFECTIONS

LÁSZLÓ RÓKUSZ

Medical Centre, Hungarian Defence Forces, Budapest, Hungary

The increasing resistance of Gram-negative bacteria (ESBL+ *K. pneumoniae*, ESBL+ *E. coli*, FQ-R *P. aeruginosa*, NDM-1/New-Delhi metallo-ß-lactamase-1/+ *Enterobacteriaceae*) is a global problem in health care world-wide, resulting in increasing morbidity, mortality and financial burden. The level of inappropriate antibiotic use is estimated to be 25-50% by the infection control surveys and clinical studies. Development of novel antibiotics has extremely slowed down (IDSA 10x20 Initiative). The major risk factors of infections caused by multidrug-resistant (MDR) *Enterobacteriaceae* are the following: urinary catheter, previous antibiotic treatment, diabetes mellitus, malignant diseases, long-term hospitalisation, travelling to high risk countries. Treatment options of MDR *Enterobacteriaceae* infections: ESBL+ and plasmid AmpC+: carbapenems; ß-lactame/ß-lactamase inhibitor (except for AmpC); cephalosporins (for ESBL: effective in case of low MICs, for AmpC: cefepime might be effective); floroquinolons, aminoglycosides (MIC!), fosfomycin (in cystitis); KPC+, MBL+, OXA+: carbapenems, cephalosporins, colistin, fosfomycin, tigecyclin, combinations. Suggested treatment of severe ESBL+ *Enterobacteriaceae* infections: Tigyecyclin monotherapy, or carbapenem (MER, IMP) + 1-2 other AB (ceftazidim or cefepim; colistin; tigecyclin; aminoglycosides, fosfomycin). Novel antibiotics: Plazomicin; avibactam in combination with ceftazidime or ceftaroline; ceftolozan/tazobactam. Individual targeted treatment is required in case of ESBL+ *Enterobacteriacae* infections, we must be aware of resistance data and resistance mechanisms.

Development of novel antibiotics, performance of clinical studies and continuous medical education of infectologists and microbiologists is very important.

BIODEGRADATION OF A TRICHLOROETHENE CONTAMINATED SITE: PILOT-TEST

CSABA ROMSICS[1], ZSUZSANNA NAGYMÁTÉ[1], LAURA JURECSKA[1], FANNI TÓTH[1], VIKTÓRIA BÓDAI[2], PÉTER SÁTORHELYI[2], ÉVA MÉSZÁROS[3], ATTILA SZABÓ[1], TAMÁS FELFÖLDI[1], BALÁZS ERDÉLYI[2], KÁROLY MÁRIALIGETI[1]

[1]Department of Microbiology, Faculty of Science, Eötvös Loránd University; [2]Fermentia Ltd., Budapest, Hungary; [3]Institute of Agricultural Sciences, ETH Zurich, Lindau, Switzerland

Chlorinated aliphatic hydrocarbons (CAHs) are frequent contaminants in soil and groundwater. Under anaerobic conditions, microorganisms can effectively biodegrade CAHs, such as tetrachloroethene (PCE) or trichloroethene (TCE) through reductive dechlorination processes. Microorganisms as electron acceptors use chloroethenes. Several groups of bacteria are able to degrade PCE or TCE to cis-dichloroethene (c-DCE). Only a bacterial group, *Dehalococcoides* is capable of dechlorinating PCE totally to harmful ethene. Aim of the study was to enhance biodegradation of TCE in contaminated groundwater using Pilot-test. Our team developed a contaminated site-specific anaerobic starter culture, a large anaerobic fermentation process and a bioremediation technology previously. The Pilot-test was performed in order to control the effectiveness of the produced culture and the developed technology. Two areas were tested during our project with different contamination. 5% of sour whey was added for one week in order to keep anaerobic conditions. After that anaerobic starter culture was added every 30 days through 3 months, while anaerobic-solution (sour whey) was also used. During the monitoring period 5% of sour whey was injected to maintain anaerobic conditions and to ensure stimulation of dechlorinating microbial population by providing essential nutrients and electron donors. Complex chemical and molecular biological approaches were applied to monitor the Pilot-test. Dynamics of biodegradation (daughter-products of TCE biodegradation, such as cis-dichloroethene, vinyl-chloride, ethane, and methane concentration) was monitored by gas chromatography. Catabolic gene tests (trichloroethene-reductase and vinyl-chloride reductases) were applied to assess the existing dechlorination potential, and to determine the presence and activity of halorespiring bacteria. Changes of microbial community structure was explored by Terminal Restriction Fragment Length Polymorphism and Next Generation Sequencing methods. Cell count values of the total microbial community was determined by DAPI staining, while active members of the community were determined by Fluorescence In Situ Hybridization methods. Community fingerprint analyses revealed that the microbial community had been significantly altered by the bioaugmentation and biostimulation treatments. *Dehalococcoides* spp. were determined in the injection wells with several methods. During the Pilot-test the total CAHs contamination has decreased by 50% in one, and by 90% in the other tested area.

The support of HU09-0046-A2-2013 Norway Grant project is acknowledged.

BIOLOGICAL ACTIVITY OF SILVER NANOPARTICLES PREPARED BY COFFEE AND GREEN TEA EXTRACTS

ANDREA RÓNAVÁRI[1], NÓRA IGAZ[2], DÁVID KOVÁCS[3], ZOLTÁN KÓNYA[1], ILONA PFEIFFER[3], MÓNIKA KIRICSI[2]

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

Due to the obvious disadvantages of the classical chemical reduction methods, the green synthesis of metallic nanoparticles has attracted tremendous attention in recent years. Numerous plant mediated nanoparticle synthesis methods have been developed lately, which are non-toxic, have low cost, are eco-friendly and fairly simple. Among metal nanomaterials, production of silver nanoparticles has always been an important area of research due to their broad range applications in household products, in cosmetics and in the biomedical field. In this study, we report a simple and eco-friendly method for the synthesis of silver nanoparticles using an aqueous solution of coffee and green tea extract as a bioreductant and stabilizing agent. The as-prepared silver 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 delineate major differences in toxicity to human cells, as well as in antibacterial, antifungal and antiviral activities between the silver nanoparticles synthesized using coffee and green tea. The results indicate that both of the coffee and green tea mediated green synthesis was successful, the as-synthesized silver 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. The particles fall into the 'nano' size range which was verified by DLS and TEM measurements. Furthermore, screening of their biological activities confirmed that both types of silver nanoparticles exhibited good antimicrobial activity against all examined microbial species and were toxic to human cells, however the level of toxicity depended largely on the plant extract used for nanoparticle synthesis.

QUALICONT EXTERNAL QUALITY ASSESSMENT: 20 YEARS IN THE SERVICE OF LABORATORY DIAGNOSTICS

ERIKA SÁRKÁNY

QualiCont Nonprofit Ltd., Szeged, Hungary

In Hungary law ordains for health care providers to operate quality management system, and for in vitro medical diagnostic laboratories participation in external quality assessment in different levels, depending on the type and level of competence. In 1996 five scientific societies, among them the Hungarian Society for Microbiology, founded QualiCont, which ensures External Quality Assessment (EQA) of in vitro diagnostic tests by organizing proficiency testing schemes in wide ranges in order to achieve patient safety and health protection in high level.
During its 20 years of operation QualiCont has gone through significant development, which can be described by a series of quantitative and qualitative changes. Since 2014 QualiCont has been a proficiency test provider accredited by the Hungarian Accreditation Board under the NAT-8-0002/2014 registration number; serving 59 accredited proficiency testing schemes, including 10 microbiological ones. In the lecture examples of long-term data analysis of the specific EQA schemes will be presented, which may confirm the practical use of EQA; the test methods, the objective assessment tool of measuring systems, the monitoring of the performance of laboratories, the identification of random and systematic errors and the market surveillance role. It is proven that EQA provides such objective data for the participants, which certify their competence for the patient who takes up their services, the accreditation bodies and other authorities. In health care, the ultimate aim of quality assurance is the continuous ensuring and improvement of patient safety, in which EQA has

an outstanding role: chance and tool in maintaining and developing the quality and reliability of laboratory work, as well as in continuous training of laboratory personnel.

DISTRIBUTION OF *BFT* GENE AND ITS SUBTYPES AMONG *BACTEROIDES FRAGILIS* ISOLATES FROM HUNGARIAN CLINICAL SAMPLES

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

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

Despite *Bacteroides* species are members of the normal human gut flora, these species are the most commonly isolated from clinical specimens as anaerobic aetiological agents. Some B. *fragilis* strains producing a metalloprotease enterotoxin (enterotoxin producing *B. fragilis*, ETBF) that is related to diarrhea in humans and animals. Three different isoforms were identified until this time: *bft-1*, *bft-2*, *bft-3*. We carried out a multicenter study with 200 *B. fragilis* isolates of 5 different Hungarian clinical microbiological laboratories isolated from clinically relevant extraintestinal samples to investigate the distribution of *bft* gene subtypes. These strains were collected from January 2014 to March 2016, and identified by MALDI-TOF MS (Bruker Biotyper, Germany). The RT-PCR reactions for amplification of *bft* genes were performed, the purified DNA amplicons were sequenced with ABI BigDye® Terminator Version 3.1 kit in Series Genome Analyzer 3500 machine (Life Technologies). Durning PCR-RFLP the DNA amplicons were digested by MboI enzyme (Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. Digested products were separated by 1.5% agarose gel electrophoresis. For controls *B. fragilis* R19811 (bft-1), *B. fragilis* GAI 96462 (bft-3) were used. Of 200 *B. fragilis* strains the prevalence of ETBF was 13%. The data were analyzed for differences by Fischer's Exact test using SigmaPlot 12. The most common subtype was the bft-1 (77.0%), whereas 23.0% of the *bft*-positive strains carried bft-2 and there was no strain carrying bft-3. Among the laboratories, strains from the SYNLAB Ltd. harboured most frequently the *bft* gene: 35.0%. Most of *bft-1* subtype carried out among the strains from University of Debrecen 27.0%. Most of the *bft-2* positive strains were isolated in SYNLAB Ltd. (12.0%) and University of Szeged (12.0%). A Hungarian study, published in 2006, 275 *B. fragilis* intestinal and extraintestinal strains were investigated, 8.7% harboured *bft* gene. The *bft* alleles were determined by RT-PCR: the most common allele was the *bft-1* (70.8%), 29.2% of the *bft*-positive isolates carried *bft-2* and there was no isolate carrying *bft-3*. Comparing these data with our results, during the last 10 years, the *bft* prevalence slightly increased, but not significant (p=0.178).

MOLECULAR DETECTION AND ANALYSIS OF A CHROMOSOMAL ELEMENT OF THE *nimB* METRONIDAZOLE RESISTANCE GENE OF *BACTEROIDES* SPP.

JÓZSEF SÓKI, ERZSÉBET NAGY, EDIT URBÁN

Department of Clinical Microbiology, University of Szeged, Szeged, Hungary

We aimed to determine the genetic context of the chromosomal *nimB* gene carried by an international cluster of *B. fragilis* strains and with that to find some experimental clue for the scare spread of metronidazole resistance. Next-generation sequencing was applied for genome sequence determination of *nimB*-positive *Bacteroides* strains and we used genome handling and analysis programs. For exact localization of the ends and transferability of the MTnBf8 transposon we applied outward-oriented PCR and conjugation experiments. The genomic sequence of *B. fragilis* BF8 has been determined which consisted of one scaffold with 5 contigs and 5238821 nt in length. It contained 4373 predicted ORFs and 96 RNA genes. The *nimB* gene was in one copy and two NBU-type integrase genes could be localized downstream (13.6 and 2.4 kb) while a Ser-tRNA sequence could be found upstream (2.4 kb). This implicated that the nimB gene might be on a mobilizable transposon. By means of PCR detection of the covalently-closed circular intermediate, the final sequence spam of the transposon was 16626 nt and the transposon was termed MTnBf8. The ends were downstream the second NBU-type integrase (attL) and in the Ser-tRNA (attR) which harbored 22 nt complete homologies. Besides the NBU-type integrases, the *nimB* and IS1186 sequences the transposon harbored 12 other ORFs. Out of 11 different *nimB*-positive *B. fragilis* strains 8 harbored MTnBf8 by means of the detection of the excised circular form. The conjugal transfer of the *nimB* gene and the metronidazole-resistant phenotype from two strains (*B. fragilis* BF8 and KSB-R) was unsuccessful to 3 different *B. fragilis* hosts. We were able to determine the carrying genetic element of the chromosomal nimB element (MTnBf8), but could not detect its conjugal transfer.
This latter finding may explain the low prevalence or the disappearance of the metronidazole resistance of *Bacteroides* experienced nowadays.

GENOME SEQUENCING PROJECT OF A SHIGA TOXIN PRODUCING *SHIGELLA SONNEI* CLINICAL STRAIN

DOMONKOS SVÁB[1], BALÁZS BÁLINT[2], GERGELY MARÓTI[3], ISTVÁN TÓTH[1]

[1]Enteric bacteriology and alimentary zoonoses, Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest; [2]Seqomics Ltd., Mórahalom; [3]Symbiosis and functional genomics, Institute for Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Shigella strains are one of the main agents of bacillary dysentery, and in recent years *S. sonnei* has emerged as the leading agent of shigellosis in industrialized and rapidly developing countries. More recently, several *Shigella* strains producing Shiga toxin (Stx) have been reported from sporadic cases, and converting phages carrying *stx1* genes have been characterised from *S. sonnei* and *S. flexneri* strains. Here we report the first whole assembled chromosome of a Stx-producing, multidrug resistant (MDR) *S. sonnei* clinical strain isolated in Hungary. The chromosome size of strain 75/02 is approximately 4.9 Mb that corresponds to 4,912 coding DNA sequences (CDS), 97 tRNA genes and 21 rRNA genes. The genome of 75/02 also contains five plasmids, including the 214 kb long pInv harbouring the key virulence ipa and type III secretion system genes, as well as those encoding *Shigella* enterotoxin 2 (Shet2). The remaining plasmids are 59,559, 5,114, 3,619 and 2,690 nt long. The genome contains altogether 19 prophage or prophage-like regions including the earlier-described inducible and transferable Stx1 encoding phage 75/02 of 60.875 nt. The total size of the prophages carried by strain 75/02 is 483 kb. Besides Stx and the factors encoded on pInv, *S. sonnei* 75/02 carries further typical virulence genes associated with *Shigella*, including those encoding the increased serum

survival (iss), *senB* enterotoxin, the shiA protein, which is involved in suppressing host inflammatory response, and also the sigA serine protease autotransporter.
All these results suggest the potential emergence of an increasingly virulent MDR *S. sonnei* strain as well as the dissemination of *stx1* genes mediated by *Shigella* phage 75/02.

INTERFERON-GAMMA RELEASE ACTIVITY AND T-HELPER CYTOKINE mRNA PROFILE

LEVENTE SZAKÁCS, EVELIN BUKTA, BRIGITTA LÁSZLÓ, JÓZSEF KÓNYA

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

Tuberculosis (TB) is most commonly caused by bacterium strains of the *Mycobacterium tuberculosis* complex. T-lymphocytes have a major role in both the pathogenesis and diagnosis of TB. Our objectivs was to evaluate changes in IFN-γ, IL-2, Foxp3, IL-13, IL-17A and IL-22 mRNA expression upon stimulation by TB-specific peptide antigens. In intreferon-gamma release assays (IGRA) blood samples are stimulated with a *M. tuberculosis* specific oligopeptide epitope mix for 16 to 24 hours ex vivo. Changes in cytokine mRNA expression were determined after stimulation, relative quantity was determined using ddCt method. Samples from three groups of patients were analized; patients with clinical criteria of latent tuberculosis infection(n=8) and patients receiving anti-TNF-alpha biological therapy, including rheumatological (n=9) and psoriatic patients(n=15). In latent TB group we observed TB-specific mRNA expression of all six cytokines. In IGRA positive patients with biological therapy, the most common cytokine that demonstrated TB-specific mRNA expression was IL-2 (75%), while TB-specific mRNA expression of IL-13 tended to associate with stronger IGRA reactivity (3,56±2,15 vs 1,37±0,57 IU/mL) as well as that of IL-22 (3,14±2,04 vs 2,08±1,93 IU/mL) and Foxp3 (3,16±1,79 vs 2,31±2,23 IU/mL). Increased TB-specific expression of IL-17A seemed to have the lowest frequency (37,5%) and tended to be independent of the strength of IGRA reactivity (2,89±1,83 vs 2,64±2,06 IU/mL). Although only 54.2% of these samples demonstrated increased IFN-g mRNA expression upon TB-specific stimulation, it also tended to be associated with the strength of IGRA reactivity (3,67±2,13 vs 1,64±1,25 IU/mL).

GENITAL AND ORAL CARRIAGE OF HUMAN PAPILLOMAVIRUSES IN WOMEN WITH HIGH GRADE SQUAMOUS INTRAEPITHELIAL LESION AND THEIR MALE PARTNERS

KRISZTINA SZARKA, ZSÓFIA NAGY, GÁBOR KARDOS

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

Human papillomaviruses (HPVs) are the main risk factor for cancer of the uterine cervix, and may also play a role in rectal, penile as well as head and neck cancer. A major way of HPV transmission is sexual contact, oropharygeal HPVs may derive from oral sexual contact with genitally infected partners. The aim of the present work was to explore genital as well as oral HPV status in women with high grade squamous intraepithelial lesion (HSIL) and in their male partners. Thirty-three women (median age 27; range 19-59) with cytologically confirmed HSIL and their male partners (median age 31, range 23-65) were enrolled; informed consent was collected from all participants. Exfoliated cells from the oral and genital mucosa were collected by cytobrush and placed immediately

in dry ice. All samples from the couples were collected within a month to assess simultaneous carriage of HPVs. HPV detection was performed by MY/GP consensus nested PCR, HPVs were genotyped by sequencing and virus copy numbers were determined using real-time PCR. Prevalence data were compared by chi-square test or sign test, as appropriate. Log copy numbers were compared using Kruskal-Wallis test with Bonferroni correction. ANOVA and Yule's coefficient was used to test for association of HPV status with that of the partner. As expected, the majority of women with HSIL proved to be HPV positive in the genital mucosa (28/33). Their oral mucosa, however, showed significantly lower HPV carriage (7/33, $p<0.001$); all orally positive women carried HPV in the genital mucosa. Similarly, males carried HPV in their genital mucosa more frequently than in the oral mucosa (17/33 vs. 7/33, $p=0.035$), but oral carriers were not always positive in their genital sample. Females carry HPV more frequently than males in their genital ($p=0.004$) but not in their oral samples. The most frequently encountered genotype was HPV16 in both sexes, but high risk genotypes HPV18, 31, 33, 51, 56, 66, 82 and low risk HPV11, 55, 61, 72, 81, 83 and 84 were also found. The genotype was the same in the genital and oral mucosa of 7/7 and 5/7 females and males, respectively. Average copy numbers in positive females were significantly higher in the genital than in the oral mucosa ($1.3x10^5$ vs $1.5x10^3$/ug DNS, $p=0.003$), while in males the copy numbers were comparable ($8.2x10^3$ vs $1.8x10^2$/ug DNS). Females had higher copy numbers than males in the genital ($p<0.001$), but not in the oral mucosa. There were no differences in copy numbers of females with HPV positive or negative partners. In couples, females are more frequently carriers than males in the genital ($p<0.002$), but not in the oral mucosa. In the genital mucosa 15, while in the oral mucosa three pairs shared the same HPV genotype, but neither genital nor oral carriage was found to be associated statistically with the status of the partner. Though transmission between oral and genital mucosa as well as transmission between sexual partners is plausible, proving this association statistically needs higher sample sizes with enrolment of more couples.

This work was supported by a János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

MRSA EPIDEMIOLOGY: WHAT HAPPENED IN THE SOUTHERN PART OF HUNGARY SINCE 2011?

MÁRIA SZÉCSÉNYI, ANDREA LÁZÁR, KÁROLY PÉTER SÁRVÁRI, MARIANNA ÁBRÓK, EDIT URBÁN

Department of Clinical Microbiology, University of Szeged, Szeged, Hungary

As the most common causing agent of nosocomial infections, methicillin resistant *Staphylococcus aureus* (MRSA) was the subject of our investigations in order to compare hospital-acquired MRSA (HA-MRSA) with community-associated ones (CA-MRSA) occurred in different clinical units of Albert Szent-Györgyi Health Centre in Szeged, Hungary in the last five years. Our aim was to find out whether is there any changes in MRSA situation during the last five-year-long period. Data of clinical samples including either methicillin sensitive *Staphylococcus aureus* (MSSA) or MRSA were collected according to the following aspects: 1) in- or out-patient, 2) type of specimen (related to respiratory tracts, blood, surgical swabs or others), 3) clinical unit (surgery, intensive care unit, internal medicine, paediatrics or others), and 4) antibiotic susceptibility profiles. One specimen per person was considered avoiding the unnecessary repetition. The statistical significance of the results was determined using regression analysis. Differences were considered significant at a probability of $P<0.05$. For the distribution of MRSAs, Chi-square values with the Yates' correction were calculated using a 2x2 contingency table with $P<0.05$ considered as significant. Significantly more MRSAs were recorded among the in-patients compared to the out-patients in the last five years (cca. 25% and 10% of total *S. aureus* cases, respectively). Nevertheless, no changes were detected in the number of

MRSAs in both groups since 2011. Considering the various types of specimen, number of MRSAs found in respiratory tract-related samples was significantly higher in hospitalized patients, which was rather resistant to FQs, than in non-hospitalized ones. However, MRSAs detected in surgical swabs and other kind of samples such as urine, stool or specimen from genitalia of in-patients, were more sensitive to FQs in 2015 compared to 2011. Likewise, concerning the total number of MRSAs among hospitalized patients, the ratio of FQ sensitive MRSAs increased between 2011 and 2015 however it cannot be linked particularly to any of the clinical units examined. Notably, ratio of FQ sensitive MRSAs detected in out-patients were always higher than in hospitalized ones in each year. Analysing the diverse clinical units, majority of the MRSA cases were described in the surgical unit with low FQ sensitivity but a slight decrease was observed since 2013. Similarly, numbers of patients having MRSA declined significantly in the intensive care unit during the five years. If we distinguish CA-MRSA as a FQ sensitive one from the HA-MRSA type which is rather a FQ resistant, we can conclude that, in Albert Szent-Györgyi Health Centre, the number of CA-MRSA type increased significantly in the last five years. Presumably, it can be associated with the FQ consumption if we consider the high number of CA-MRSAs recorded in paediatrics.

EFFECTS OF THE FERMENTATION PERFORMANCES ON THE OXYGEN SUPPLY

JENŐ SZILÁGYI

Fermentation, Intrexon Laboratories Hungary, Budapest, Hungary

After a short introduction of my research and development work in the fermentation instrumentation and control the O_2 supply is discussed with the help of an industrial fermentation. Considering the equation of OUR = kLa •(C* - C) the OUR is determined mainly by the fermentation technology while the kLa and the oxygen concentration by the fermentation performances. Beside the environmental parameters (temperature, aeration, pH, agitation, top pressure) the foaming and the working volume can be very important not only in the O_2 supply but in the productivity, too. Very important to emphasise that we are not able to measure the concentration of dissolved oxygen (C) but we can follow the partial pressure of the oxygen (pO_2) during the fermentation. Misunderstandings in the pO_2 measurement are shown in the recent fermentation practices then the calibration of the pO_2 probes and its response during the fermentation are discussed. Relationship between the pO_2 and redox potential (ORP) is also shown. At last some technological parameters (trends of broth weight, foam level and antifoam feeding; aeration demand calculation; relationship between the carbon source feeding and catabolite repression) and technical parameters (fermenter geometry; agitation and aeration systems; flow pattern) of an industrial amino acid fermentation are presented.

IN VIVO TRANSFER OF *SALMONELLA* GENOMIC ISLAND 1 (SGI1) TO COMMENSAL *ESCHERICHIA COLI* IN THE CHICKEN INTESTINE

AMA SZMOLKA[1], ANNA HEGYI[2], FERENC OLASZ[2], BÉLA NAGY[1], JÁNOS KISS[2]

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

Salmonella Genomic Island 1 (SGI1) conferring pentaresistance, was found primarily in *Salmonella* Typhimurium, but has recently been identified in numerous pathogenic *Salmonella* serovars. It has also been identified in several *Proteus mirabilis* and *Acinetobacter* strains, which may refer to the accelerating horizontal spread of the island. However, transfer of SGI1 has not been reported so far to occur in bacteria, such as commensal or pathogenic *E. coli*, constituting integral part of the intestinal flora. Regarding the significance of commensal *E. coli* as part of the normal gut flora and as indicator bacteria for antimicrobial resistance status in animals and man, it was important to see whether commensal *E. coli* could receive SGI1 from potential donor *S.* Typhimurium strains under natural in vivo conditions in the intestine of chickens. To address this issue we have generated the appropriate SGI1-donor strains from the wild type *Salmonella* Typhimurium strain ST919 (details not given here). The majority of resistance determinants of SGI1 helper plasmid R55 was removed by multiple gene KO methods. The streamlined plasmid retained only the *aadB* gene for KmR/GmR, and was conjugated into the asd- and conditionally asd-complemented donor strains (i.e. asd- strains harbouring a plasmid where asd gene is expressed in arabinose-dependent manner from araBAD promoter or a plasmid with thermosensitive replication system where the asd gene is expressed from its original promoter). The resulting *Salmonella* donor strains were successfully tested in conjugation experiments with the laboratory *E. coli*-K12 TG1 NalR and RifR strains as recipients. The reduced growth rate of asd- SGI1-donors suggested that they may disappear faster in vivo from the intestinal flora than the wild type ST919. In vivo conjugation of SGI1 was monitored using a chicken-adapted natural *E. coli* isolate PC2 as recipient, which proved that SGI1 transfer can occur into this strain in chicks' intestine. The wild type and two conditionally asd-complemented ST919 donors were used in two infection experiments, where PC2 recipient was added in 10^7 CFU/chick p.o. and overinfection by either donor strain was carried out 2 hours later in 5 x 10^8 CFU/chick dose. *E. coli* transconjugants and the change of donor titers in chicks was monitored for 13 days of age. We were able to isolate PC2::SGI1 transconjugants from faecal and ceacal samples of the wild type and the arabinose-dependent donor groups most efficiently, when newly hatched (0 day old) chicks were infected. As a result, we have developed an assay to test in vivo horizontal spread of SGI1 in the chicken intestinal microflora. The further fine tuning of the experimental setup is ongoing.

Supported by OTKA-K-105635 (J. Kiss), and J. Bolyai Stipend of Hungarian Academy of Sciences (A. Szmolka).

REVEALING OF THE CULTIVABLE BACTERIAL COMMUNITIES OF FERTŐ

SÁRA SZURÓCZKI, ESZTER SÁRI, KRISTÓF KORPONAI, TAMÁS FELFÖLDI, KÁROLY MÁRIALIGETI, ERIKA TÓTH

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

Fertő, the largest soda lake in Central Europe, is a shallow lake with slightly alkaline pH. In this study, the open water of the Hungarian-Austrian border, the Kis Herlakni inner lake and the water of a reed covered area were investigated. Sampling was carried out in November 2015. The aim of the present study was to reveal the cultivable bacterial communities of the waters of the three sampling points. To cultivate and isolate bacteria R2A and an own developed minimal synthetic medium (prepared with the water of Fertő) with agar-agar and gellan gum were used. 275 bacterial strains

were identified by sequencing their 16S rRNA gene after grouping them by ARDRA. The most probable number (MPN) technique and plating on solid media were applied for the determination the abundance of cultivable heterotrophic, nitrifying, fermentative and sulphate-reducing bacteria. The carbon source utilization profile of the whole bacterioplankton was determined with Biolog EcoPlate. In the case of the open water and the reed covered area we got the highest values of cultivable heterotrophic bacteria (10^4 and 10^5 CFU/ml, respectively) with the usage of the minimal synthetic media prepared with agar. In the case of Kis-Herlakni inner lake the highest CFU value was in the R2A prepared with agar (10^5 CFU/ml). Number of sulphate-reducing bacteria reached the 10^3 magnitude, while the nitrifying and fermentative bacteria were presented in lower numbers (10^0-10^2 cell/ml). Polymers (e.g. glycogen and Tween 40) and carbohydrates (e.g. N-acetyl-D-glucosamin and mannitol) were the most frequently utilized carbon sources at the three sampling sites. Based on cultivation, at all sampling points a diverse bacterial community structure was revealed, though the dominance of Proteobacteria was characteristic for each samples. From the Proteobacteria phylum Alpha-, Beta- and Gammaproteobacteria classes were presented. Representatives of Actinobacteria, Firmicutes and Bacteroidetes were also identified. Strains of Verrucomicrobia phylum appeared only in the reed covered area. In the open water and the Kis-Herlakni inner lake the most frequently isolated genus was *Rheinheimera*. In the reed covered area *Pseudomonas* genus dominated. Most of the identified genera were commonly found in other natural environments: e.g. *Blastomonas*, *Pseudomonas*, *Sphingomonas*, *Flavobacterium*, *Micrococcus* and *Bacillus*. Some new taxa belonging to Proteobacteria, Bacteroidetes and Verrucomicrobia phyla were also isolated, mainly from our novel, own developed oligotrophic medium from the reed covered area.

OPTIMIZATION OF MEDIUM COMPOSITION FOR ENHANCING GROWTH OF *BIFIDOBACTERIUM BIFIDUM* B7.1 AND *LACTOBACILLUS CASEI* 01

LINH TA PHUONG, KRISZTIÁN SÖRÖS, ERIKA BUJNA, SZILÁRD KUN

Department of Brewing and Distilling, Faculty of Food Science, Szent István University, Budapest, Hungary

Nowadays there has been a great interest in producing health enhancing functional foods containing encapsulated probiotic bacteria. The main benefit of this technique is the maintenance of the viability of probiotics through the way from food production to digestion. This can ensure the presence of cells in the large intestine in necessary number of at least around 10^8-10^9 CFU/ml. This number is suggested in many studies for probiotics to take effect. The goal of the study was to optimize the composition of culture medium for the propagation of *Bifidobacterium bifidum* B7.1 and *Lactobacillus casei* 01. The importance of this study is the opportunity to prepare starter cultures with adequate probiotic cell concentration to use for our upcoming investigations regarding encapsulation. Growth of the bacteria was followed by optical density (OD) measurement at 600 nm with spectrophotometer. In case of *B. bifidum* B7.1 three liquid media were compared: TPY (Trypticase Phytone Yeast), RCM (Reinforced Clostridial Medium) and MRS (de Man Rogosa Sharpe), which were supplemented with different nitrogen source (casein-, proteose peptone, soy peptone, yeast extract) and different carbohydrate source (lactose, galactose, prebiotic (isomalto-oligosaccharide). Each of these bifidogenic and growth factors were added in the concentration of 5g/L to each medium. In the experiment with *B. bifidum* B7.1, the highest OD was obtained on MRS broth containing both yeast extract and prebiotics, while the basic RCM showed the lowest OD value. Thus, it can be concluded that the addition of prebiotics gives better result than that of mono- and disaccharides. Presence of extra nitrogen mostly resulted better growth of bacteria. However, in the

case of supplementing MRS with prebiotics and galactose, extra nitrogen sources weren't necessary. In case of investigation of *L. casei* 01 the composition of MRS medium was modified by applying experimental design of Response Surface Methodology, where the following parameters were changed: glucose, yeast extract and meat extract.
The variations of concentration were based on the central composite design. *L. casei* 01 showed best growth activity in MRS broth containing 20 g/L glucose and 5,5 g/L yeast extract with fixed amount of meat extract. When the concentration of yeast extract was not changed, 20 g/L glucose and 6 g/L meat extract containing medium showed the highest OD value. However, the composition of 7.93 g/L glucose with 4 g/L yeast extract, whereas the composition of 7.93 g/L glucose with 8 g/L meat extract made the minimum yield of bacteria. Based on the created response surface plots for the effect of different concentrations of analyzed parameters, better results can be achieved with increasing the amount of both glucose and yeast extract, furthermore with increasing the amount of glucose but decreasing that of meat extract. It can be concluded that glucose has significantly stronger effect on the growth of *L. casei* 01 than yeast extract and meat extract.

MULTIPLE MUTUALIST EFFECTS OF ROOT SYMBIONTS ON SOYBEAN (*GLYCINE MAX* L. MERR.) CULTIVARS UNDER DROUGHT STRESS

TÜNDE TAKÁCS, ANNA FÜZY, RAMÓNA KOVÁCS, KÁLMÁN RAJKAI, IMRE CSERESNYÉS

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

Nitrogen fixing bacterial and arbuscular mycorrhizal fungal (AMF) symbionts are essential in soybean (*Glycine max* L. Merr.) host nutrition, and alleviation of plant stress caused by adverse soil conditions. Soils of areas where soybean is non-native usually lack Bradyrhizobium strains. Especially in organic farming, the role of microbial inoculation of soybean is essential to provide adequate nitrogen supply and promote maximum yield. Multiple mutualist effects were investigated in a pot experiment on two Hungarian registered soybean cultivars, Aliz and Emese, under drought stress. Combined microbial treatments were applied with commercially available products: (1) AMF inoculation with AEGIS Sym Microgranule or SYMBIVIT (10 g pot^{-1}); and (2) bradyrhizobial inoculation with Iregi soybean inoculant powder (1mL $seed^{-1}$; 10^4 CFU mL^{-1}). Control plants were not inoculated. The symbiotic efficiency was tested in organically managed chernozem soil that contained indigenous AMF community. Plants were cultivated for two months in a growth chamber (at 26/18 °C and 16/8 h day/night temperature and photoperiod). The half of plants was adequately watered, while the others were subjected to drought stress. Biomass production (shoot and root dry weight), relative water content (RWC) of leaves, leaf area (LA), chlorophyll fluorescence induction parameters (Fv/Fm) and root electrical capacitance (EC) as an indicator of root system activity were measured. Root colonization of AMF and nodulation parameters were tested to estimate the functionality of microsymbionts. Plant biomass production increased with microbial treatments and decreased by drought stress. Plants inoculated with microbes reached the same weight under drought stress as did non-inoculated plants under optimal water supply. Drought decreased the RWC of leaves in both soybean cultivars, by about 10% in control plants and by 30–60% at treated ones (mainly in Emese). Root colonization of indigenous AMF was high in control plants. However, AMF inoculation could not increase the colonization level, but even decreased the infection in most cases. Microbial treatments significantly improved the root EC and photosynthetic efficiency. Measured EC showed strong linear correlation with root and shoot dry mass and leaf area. Higher N concentration

was found in the leaves of nodulated plants. A dilution effect could cause the reduced P concentration in the leaves of treated plants that produced the highest biomass. Benefits can be not only the result of multiple mutualist effects, but the other inorganic ingredients of the biofertilizer. For the evaluation of the microbial inoculants efficiency, including commercial products, an appropriate methodology (particularly in situ measurements) and comprehensive investigations are needed.

This research was supported by the Hungarian National Research, Development and Innovation Office (K-115714) and the Bolyai János Research Scholarship (BO/00948/15/4).

DNA-SIP REVEALS KEY TOLUENE DEGRADERS UNDER MICROAEROBIC CONDITIONS IN BTEX-CONTAMINATED AQUIFER SEDIMENT

ANDRÁS TÁNCSICS[1], ANNA RÓZA SZALAY[2], MILÁN FARKAS[3], TIBOR BENEDEK[1], SÁNDOR SZOBOSZLAY[3], TILLMANN LUEDERS[2]

[1]Regional University Center of Excellence, Szent István University, Gödöllő, Hungary; [2]Institute of Groundwater Ecology, Helmholtz Zentrum München, Neuherberg, Germany; [3]Department of Environmental Safety and Ecotoxicology, Szent István University, Gödöllő, Hungary

Monoaromatic hyrocarbons such as BTEX compounds are major groundwater contaminants. Biodegradation of these compounds under aerobic circumstances is well characterized, but under oxygen limited conditions the key degraders are still not identified. According to our recent studies, a still uncultured member of the genus *Rhodoferax* and an unclassified Rhodocyclaceae bacterium were identified as possible microaerobic BTEX degraders at the Siklós BTEX-contaminated site in Hungary. To verify this assumption incubation of sediment samples with 13C7 toluene was performed. Groundwater microcosms consisted of 50 ml artificial groundwater medium and 5 g groundwater sediment. Replicate microcosms were amended with 1 mMol labeled (13C7) or unlabeled (12C) toluene. Serum bottles were sealed hermetically and incubated for one week at 20^{o}C. Dissolved oxygen concentration was kept between 0 and 0,5 mg/L and measured non-invasively by using a Fibox 3 trace v3 fibre optic oxygen meter. Toluene concentration was determined by GC-MS. At selected time points DNA was isolated from 12C and 13C7 toluene amended microcosms for DNA-SIP analyses. DNA fractions were analysed by 16S rRNA gene-targeted qPCR and preliminiminary characterization of the bacterial communities was performed by terminal-restriction fragment length polymorphism (T-RFLP). Subseqently, 16S rDNA compositions in the heavy, medium, and light DNA fractions were analysed by barcoded pyrosequencing. Diversity of subfamily I.2.C-type catechol 2,3-dioxygenase genes was investigated by sequence-aided T-RFLP. There were small differences between the 16S rDNA compositions of heavy and intermediate DNA fractions. Results showed the predominance of betaproteobacterial 16S rDNA in the heavy fraction. *Quatrionicoccus*, *Zoogloea* and uncultured Rhodocyclaceae affiliated sequences were the most abundant here. Members of the genus *Rhodoferax* became prevalent in the intermediate fraction. Accordingly, these bacteria could be identified as key toluene degraders under microaerobic conditions. On the other hand markedly different 16S rDNA composition was observed in the light DNA fraction. *Azoarcus* and *Geobacter* affiliated sequences were the most abundant here. Results of the *C23O* gene based T-RFLP analysis showed that a *Zoogloea*-related *C23O* gene was the most dominant in the heavy DNA fraction. Besides, *Pseudoxanthomonas* affiliated *C23O*s were only detectable in the light DNA fraction, suggesting that *Pseudoxanthomonas* species do not play role in the degradation of toluene under microaerobic conditions.

Supported by - TÉT_12_DE-1-2013-0007 and Research Centre of Excellence - 11476-3/2016/FEKUT.

SERUM RESISTANCE PROPERTIES OF COMMENSAL, ENVIRONMENTAL AND CLINICAL *KLEBSIELLA PNEUMONIAE* ISOLATES

ZOLTÁN TIGYI[1], MARIANNA HORVÁTH[2], LÁSZLÓ PÓTÓ[2], GYÖRGY SCHNEIDER[1]

[1]Department of Medical Microbiology and Immunology; [2]Institute of Bioanalysis, Medical School, University of Pécs, Pécs, Hungary

In the past few years large amount of information appeared about the interactions between the environmental microbial ecosystem and the human microbiota. These data suggest that microbial adaptation has much more wider repertoire than the adaptation of the human body to the microbes but both are needed to maintain this ecosystem. *K. pneumoniae* inhabits in the environment and human large bowl as well but it is also known as an opportunistic pathogen which causes different infection types in patients who have predisposing factors. In the past few years at this forum we already presented data on the distribution of different virulence factors e.g. iron uptake systems, adhesins and biofilm production among commensal, environmental and clinical *Klebsiella pneumoniae* isolates. In our current study 113 wastewater (WW) isolates were collected from 47 geographically different sewage treatment plants and 117 commensal (CK) strains were isolated from the stool samples of healthy individuals. We compared the data to those available from our preceding studies which performed on 88 of urinary tract infection (UTI) and same number of blood stream infection isolates (BSI). The clonality of all the strains was checked to exclude the multiple representations in the strain collection with different DNA based methods. Serum bactericidal assay was applied according to Hughes et al for determining the susceptibility of the *K. pneumoniae* isolates to non-immune human serum (NHS). Human blood was obtained from healthy volunteers. Sera of 100 donors were pooled and stored in aliquots at -80°C until use. Bacterial cell suspension was adjusted to 1,1 x 10^5 cells/ml in Phosphate-buffered saline (PBS) 25 µl. of bacterial suspensions and 75 µl. of NHS were mixed in microtiter plate and incubated at 37°C. PBS was added instead of NHS to the cell suspension on a separate microtiter plate to determine the base viable cell counts. Three parallel sample were applied and two parallel aliquots were taken for determining the viable cell counts from one well of plate after 1 and 3 hours of incubation by plating serial dilutions on Luria agar for colony counts. The isolate was taken for susceptible when the viable cell count was ≥90% of the basis cell count at 0 minute and resistant when the viable cells count was higher than 90% of the basis cell count. After 3 hours of incubation the BSI isolates showed the highest serum susceptibility 96,7% than followed by the UTI 93,2%, CK 87,1% and the lowest value was found at WW isolates 82,3%. The statistical analysis revealed that the CK and WW isolates were significantly less susceptible than the BSI isolates, p=0,018 and p=0,001 respectively. Similarly, the WW isolates were significantly less susceptible than the UTI isolates p=0,022. However, the non-clinical isolates showed lower serum susceptibility compare to the clinical isolates those differences were not significant statistically. The result of 1 hour incubations showed same tendency but further statistical analysis showed some other significant differences. This data and further analysis of the dynamics of serum resistance will be presented in the lecture on the forum. Present data do not explain the fact that BSI isolates showed the highest susceptibility to human serum however the serum resistance was thought to be important virulence factor of the bacterium during a blood stream infection.
Further investigations are needed to find explanation.

A NEW *BRETTANOMYCES*-LIKE YEAST SPECIES FROM OLIVE OIL

ANDREA TÓBIÁS[1], NEŽA ČADEŽ[2], DÉNES DLAUCHY[1], LÁSZLÓ FÜLÖP[3], GÁBOR PÉTER[1]

[1]National Collection of Agricultural and Industrial Microorganisms, Szent István University, Budapest, Hungary; [2]Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; [3]Department of Chemistry and Biochemistry, Szent István University, Gödöllő, Hungary

Olive oil is a traditional product of the Mediterranean region, characterized by high nutritional value and excellent organoleptic characteristics. It is well established that olive oil consumption has a positive effect on the health. It was revealed that newly produced olive oil can harbour a rich microbiota, mainly yeasts, which may exert positive or negative effects on the quality of the olive oil. The investigation of the yeasts present in olive oil, revealed that this habitat is colonized by a specific yeast community. In the recent years several new yeast species were described from olive oil and its by-products. During our study two strains of an undescribed yeast species were recovered from spoiled olive oil samples originating from different geographical locations. Comparison of the DNA sequence coding for the D1/D2 domains of the LSU rRNA gene determined for the two strains, with the corresponding sequences available in the GeBank revealed that the two strains are only distantly related to any recognized yeast species.

Multigene Phylogenetic analysis revealed that the new species is related to the *Brettanomyces/Dekkera* clade. Like the known *Brettanomyces* and *Dekkera* species, the two strains of the new species produce acetic acid. The standard physiological characteristics of the two strains were determined and the phenotypic characteristics allow the separation of the new species from the members of the *Brettanomyces/Dekkera* clade. Ascosporulation was not observed in individual cultures or in mixed cultures of the investigated strains. The undescribed species is proposed as new anamorphic member of the *Brettanomyces/Dekkera* clade.

MINERALOGICAL ANALYSIS AND MICROBIAL DIVERSITY OF ARSENIC-RICH THERMAL SPRING OF KÖRÖM, BORSOD REGION, HUNGARY

ÁKOS TÓTH[1], ERZSÉBET BAKA[1], SZABINA LUZICS[1], ILDIKÓ BATA-VIDÁCS[1], ISTVÁN NAGY[2], JUDIT BENYÓ[3], ERZSÉBET TÓTH[3], TAMÁS G. WEISZBURG[3], VIKTOR G. MIHUCZ[4], JÓZSEF KUKOLYA[1]

[1]Department of Environmental and Applied Microbiology, Agro-Enviromental Research Institute, National Agricultural Research and Innovation Center, Budapest; [2]Seqomics Biotechnology Ltd., Mórahalom; [3]Department of Mineralogy, Faculty of Science, Eötvös Loránd University, Budapest; [4]Hungarian Satellite Centre of Trace Elements Institute to UNESCO, Budapest, Hungary

The thermal water well S-3 in Köröm, Hungary (47°59'48.94" N, 20°59'14.22" E) was drilled in 1953. Köröm is located on the NE border the Great Hungarian Plain, an area of elevated geothermal gradient. The water temperature at the well bottom (1880 m deep) was 108°C. Today water flows out at two sites: directly at well head resp. through a pipe in a distance of about 8 m from the well head. The temperature of the outflowing spring water is 83°C, measured at the well head. Both sites intensive travertine formation can be found. Based on XRD studies fresh travertine consists predominantly of calcite. Aragonite is present in subordinate quantities, and no vaterite could be identified. No mineralogical clues could be found for the characteristic colouring pattern of the travertine, observed parallel to the water temperature decrease (of about 20°C), from brown at the outflows through peach colour to white in a distance of a few meters. Move away from the well head

there are four different niche with decreasing temperature (83-60°C) and different colour. In this study we analysed the full microbiom of the four niche by direct isolation, electron microscopy, clone library and metagenome analysis. According to the EM analysis, all sampling spots have a divers microbial community, and we could isolate bacteria that belong to the *Anoxybacillus*, *Geobacillus*, *Brevibacillus*, *Bacillus*, *Thermus* genera. As the clone library analysis revealed eubacterial diversity was fairly high, with *Thermotrix azorensis*, *Hydrogenobacter* and *Thermus* spp. as dominating bacterial components at most pools. Archaeal sequences were not able to detect from the investigated samples, for all that we applied specific primers for the major archeal groups. However, the majority of sequences did not belong to certain species or even genus. According to the comprehensive study carried out by shot gun metagenome sequencing, the most frequent representatives of the communities are the members of Chloroflexi, Aquificae, Proteobacteria, Firmicutes (sample B2); Aquificae, Chloroflexi, Deinococcus-Thermus, Proteobacteria (sample HB2); Aquificae, Deinococcus-Thermus, Firmicutes, Proteobacteria (sample SB); Aquificae, Proteobacteria, Firmicutes, Euryarcheota (sample V1).
Owing to the high throughput of this method, we could identify the archeal representatives. Interestingly we detected DNA sequence of *Pyrobaculum arsenaticum*. This archeon able to use arsenic as terminal electron acceptor, and during arsenic respiration insoluble realgar is formed. Changing arsenic content of the spring may be in relation to this microbe.

INVESTIGATION OF THE BIODEGRADATION PRODUCTS OF HERBICIDES BY CHRONIC *ALIIVIBRIO FISCHERI* TEST

GERGŐ TÓTH[1], JUDIT HÁHN[2], SÁNDOR SZOBOSZLAY[1], BALÁZS KRISZT[1]

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

The usage of pesticides has been gradually increased worldwide since the middle of 20th century. The utilization of pesticides doubled in Hungary from 2000 up to the present day and herbicides account for about 42% of the applied pesticides. The extensive application of these chemicals has raised serious concerns: they are able to contaminate environmental compartments and they are frequently detected in surface water and groundwater. Therefore the application of microorganisms for biodegradation of contaminants gets increasing attention. The aims of our study were to select herbicide degrading bacteria and to examine the bacterial biodetoxification of the active substances of three herbicides (mesotrione, S-metolachlor and terbuthylazine). The biodegradation experiments were carried out using bacteria which are able to degrade various aromatic and halogenated compounds, according to the literature. The herbicide degrading potential of microbes belonging to *Rhodococcus* (10 type strains of 14 strains) and *Olivibacter* genera and 3 consortia created out of these strains were examined. The biodegradation potential of the bacterial strains was examined by analytical chemistry (HPLC). Additional aim was to analyze the biodetoxification and the cytotoxicity of residues from biodegradation experiments by chronic Aliivibrio fischeri bioluminescence inhibition test. The modified chronic test adapted to a 96-well microplate has been used to measure chronic cytotoxicity of the aforementioned herbicides (at contact times of 0-3.5-10-15-25 hours) in our previous studies. According to the analytical results some of these strains and consortia were able to biodegrade two herbicides (S-metolachlor and terbuthylazine) with varying efficiency. In case of the mesotrione we did not detect degradation at all.
Two *Rhodococcus* type strains could degrade S-metolachlor with good degradation potential (59-72%). The biodegradation end-products of one type strain, which has the most effective potential to

degrade the S-metolachlor (72%), caused considerable cytotoxic effect. One consortium was able to biodegrade terbuthylazine with significant degradation efficiency (88%) and the toxicity of metabolites of this consortium measured by *A. fischeri* chronic test has ceased. However, cytotoxic effect (total bioluminescence inhibition) of terbuthylazine residues produced by one *Rhodococcus* strain with a lower degradation capacity (22%) was observed. According to the results of the toxicity test, there is a possibility of the formation of toxic and harmful metabolites in case of strains and consortia which were able degrade the herbicides, too. While the production of highly toxic metabolites may occur at low biodegradation rate, high biodegradation efficiency also does not necessarily mean the decrease or disappearance of the toxicity. These results highlight the importance of the toxicological assessment of the biodegradation residues.

This study was supported by Research Centre of Excellence – 9878-3/2016/FEKUT.

NFAP2, AN ANTI-YEAST PROTEIN FROM *NEOSARTORYA FISCHERI* NRRL 181

LILIÁNA TÓTH[1], ZOLTÁN KELE[2], LÁSZLÓ G. NAGY[3], MÁTÉ VIRÁGH[1], MIKLÓS TAKÓ[1], CSABA VÁGVÖLGYI[1], LÁSZLÓ GALGÓCZY[4]

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

A novel highly stable, cysteine-rich antifungal protein secreted by the filamentous ascomycete, *Neosartorya fischeri* (anamorph: *Aspergillus fischerianus*) NRRL 181 was isolated and partially characterised in this study. This protein was purified from the supernatant of *N. fischeri* NRRL 181 cultivated in minimal medium. After purification with cation-exchange chromatography, protein gel electrophoresis revealed the presence of a ~5.6 kDa protein. Mass spectrometric molar mass measurement of this protein resulted 5556 Da. A peptide fragment was identified by mass spectrometric analysis of the enzyme digested protein sample. BLAST search for this identified fragment on the submitted *N. fischeri* NRRL 181 genome in the UniProt and NCBI databases resulted in a hypothetical protein. The identified peptide fragment covered 31% of the total sequence. The measured molecular mass corresponded well to the calculated mass of the mature form of this hypothetical protein. We termed it as *Neosartorya fischeri* antifungal protein 2 (NFAP2). NFAP2 exerted high antifungal activity on clinically relevant *Candida* species in a broth microdilution test. BLAST searches in genomic databases yielded further 35 NFAP2 homolog proteins in other Ascomycetes. Phylogenetic analysis revealed that NFAP2 and its homologs represent a new group of cysteine-rich antifungal proteins from Ascomycetes beside the well-described *Penicillium chrysogenum* antifungal protein PAF and *Penicillium brevicompactum* 'bubble protein' BP groups. After further investigations of the antifungal mechanism and structure, NFAP2 could be applicable as an antifungal agent against yeasts in the near future.

L.G. holds a Lise Meitner fellowship from Austrian Science Fund (FWF): M1776-B20. Supported by OTKA ANN 110821 and also connected to the project GINOP-2.3.3-15-2016-00006. Authors also thank for the postdoctoral grant OTKA PD 112234 of M. Takó.

NEARLY WHOLE GENOM SEQUENCING AND MOLECULAR CHARACTERISATION OF AN ARENAVIRUS FROM A *BOA CONSTRICTOR* IN HUNGARY

KATALIN TÓTH-IHÁSZ[1], SZILVIA FARKAS[1], SZILVIA MARTON[1], RENÁTA KUGLER[1], GYÖRGY LENGYEL[2], KRISZTIÁN BÁNYAI[1]

[1]Centre for Agricultural Research, Institute for Veterinary Medical Research, Hungarian Academy of Sciences; [2]Medical Centre, Hungarian Defence Forces, Budapest, Hungary

Inclusion body disease (IBD) is a common and important infectious disease of captive snakes belonging to the family Boidae and Pythonidae. Snakes affected by this disease usually have neurological symptoms and secondary infections but there are clinically inapparent carriers. Nowadays IBD diagnosis is based on histophatological examination of tissue samples and the most characteristic features are the eosinophil or basophil intracytoplasmic inclusion bodies detected in the cytoplasm of the central nervous system and in the epithelial cells of internal organs. Snakes diagnosed with IBD are recommended for euthanasia if there is a risk of transmission to other animals. The detection of arenaviruses in samples of snakes with histological evidence of the disease implicates arenaviruses as candidate aetiological agents of IBD. Tissue samples from a captive breeded red-tail boa (*Boa constrictor*) was examined using reverse transcription polymerase chain reaction (RT-PCR) and arenavirus was detected from all examined organs of the snake. Near complete genome sequences of the boa arenavirus was determined applying a combination of next generation and Sanger sequencing techniques. Molecular characterization helps to understand the genome organisation of the pathogen and with phylogenetic examinations we can understand the evolutionary relationships of arenaviruses.

IBD can cause the loss of entire animal collections in exotic snake keepers and currently no treatment or vaccine exist. The results of this study can be used for the development of more effective diagnostic assays and suggest that design of intervention strategies, such as vaccination or the use of antivirals against the multiple species of IBD-associated arenavirus.

COMPLETE GENOME SEQUENCE OF *TRICHODERMA AGGRESSIVUM* F. *EUROPAEUM*, THE CAUSAL AGENT OF GREEN MOULD DISEASE IN THE CULTIVATION OF CHAMPIGNON (*AGARICUS BISPORUS*)

PÉTER URBÁN[1], YOUZHI MIAO[2], CSABA FEKETE[1], KITTI SCHÖNHARDT[1], ANDREA VALASEK[1], LÓRÁNT HATVANI[3], LÁSZLÓ MANCZINGER[3], CSABA VÁGVÖLGYI[3], IRINA S. DRUZHININA[2], LÁSZLÓ KREDICS[3]

[1]Department of General and Environmental Microbiology, Faculty of Sciences, University of Pécs, Pécs, Hungary; [2]Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, TU Wien, Wien, Austria; [3]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Trichoderma aggressivum f. *europaeum* is a filamentous ascomycete species belonging to the Harzianum clade of the genus *Trichoderma*. This species is one of the causal agents of the green mould disease in the cultivation of champignon (*Agaricus bisporus*). The first large green mould epidemic outbreak was reported from Northern Ireland in 1985, which was quickly followed by subsequent epidemics in many other countries. The characteristic symptoms of *Agaricus* green mould

are large patches in the compost which are rapidly turning green. *T. aggressivum* can efficiently compete for nutrients and space, produces toxic secondary metabolites, extracellular enzymes and volatile organic compounds, which may result in significant crop losses. The natural habitat of this green mould species is yet unknown. Potential routes of infection include the air, contaminated clothes of workers, vehicles and animal vectors. Possible management strategies include pasteurization, the application of disinfectants, adjustment of casing pH, chemical and biological control, as well as the breeding of resistant *Agaricus* varieties. The aim of this study was to determine the complete genome sequence of CBS 433.95, a green mould strain isolated in 1991 from mushroom compost in Northern Ireland. The identity of the strain was rechecked and confirmed as *T. aggressivum* f. *europaeum* by the sequence analysis of the internal transcribed spacer (ITS) region and a fragment of the translation elongation factor 1α encoding gene tef1. Fungal genomic DNA was isolated from the strain with Qiagen DNeasy® Plant Mini Kit. For the library construction, NEBNext® Fast DNA Library Prep Set for Ion Torrent was used following the 200 and 400 bp protocols with minor modifications. The whole genome sequencing of *T. aggressivum* f. *europaeum* strain CBS 433.95 was performed on Ion Torrent Next-Generation Sequencing platform both with 200 and 400 bp chemistries on Ion 316 Chip v2. Sequencing of the genome with 200 and 400 bp chemistries revealed 800 Mbp in 3,571,428 reads and 650 Mbp in 2,023,255 reads, respectively. A total of 2104 contigs were obtained with a total consensus length of 38,635,772 bp, a median contig length (N50) of 57,262 bp and a G+C content of 49,6%. Genome assembly was carried out with SPAdes Genome Assembler 3.6.0 (http://bioinf.spbau.ru/spades). The modular genome annotation pipeline TrichoCODE (http://trichocode.com) including a high quality training set for *Trichoderma* was used for ab initio gene prediction in the genome.

Stand-alone BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) of the predicted genes was performed and the results evaluated in tabular format. The availability of the complete genome sequence of *T. aggressivum* f. *europaeum* opens a series of new possibilities to study the genetic background of *Agaricus* green mould disease development.

Supported by grants NKFI K-116475 (National Research, Development and Innovation Office, Hungary), by the EU within the frames of the Széchenyi 2020 Programme (GINOP-2.2.1-15-2016-00006) and by the bilateral grant 90öu3 from the Austrian-Hungarian Action Fund.

TRIBUTE TO DR. JÁNOS VARGA, AN INTERNATIONALLY RENOWNED HUNGARIAN MYCOLOGIST

CSABA VÁGVÖLGYI, NIKOLETTA BARANYI, SÁNDOR KOCSUBÉ

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

This lecture has been prepared to honor the memory of Dr. János Varga, an internationally known Hungarian mycologist who died on June 16, 2016, at age 54. János Varga graduated as a biologist from University of Szeged (formerly Attila József University) in 1986. He subsequently joined to the Department of Microbiology (Faculty of Science) of the same University. He was teacher and leading scientist of this department for 3 decades. Several of his students earned their Ph.D. degrees under his supervision. In 2015 he has been appointed professor at the University of Szeged. The main target of his research was the molecular systematics and mycotoxin production of *Aspergillus* species. He was a scientist with international reputation and spent several years in different laboratories abroad including the University of Birmingham (UK), Wageningen University and the CBS Fungal Biodiversity Center (Netherlands). He was an active and successful researcher taking part in several national and international research projects. He was a member of several national and international

scientific bodies, e.g., the International Commission on *Penicillium* and *Aspergillus* and working groups of the ISHAM. As part of his academic work he wrote several book chapters and edited several books, e.g. *Aspergillus in the genomic era*, (Eds: J. Varga and R. A Samson) and was author of dozens of highly-cited review articles. As a result of this extraordinary scientific activity he published 316 publications which connected to nearly 6 thousend citations. His scientific activity has left a lasting mark on the face of Hungarian microbiology.

ARISA BASED STUDY OF FUNGAL DIVERSITY IN THE (SEMI)ARID SANDY AREA OF THE EXDRAIN-PROJECT

BALÁZS VAJNA[1], GYÖRGY KRÖEL-DULAY[2], GÁBOR M. KOVÁCS[3]

[1]Department of Microbiology, Faculty of Science, Eötvös Loránd University, Budapest; [2]Institute of Ecology and Botany, Centre for Ecological Research, Hungarian Academy of Sciences, Vácrátót; [3]Department of Plant Anatomy, Faculty of Science, Eötvös Loránd University, Budapest, Hungary

As global climate change is an increasing issue, multiannual extreme drought and rain manipulations (ExDRain-project) have been started recently in a sandy grassland near Fülöpháza to study the effect of local climate change in a multidisciplinary way. As fungi have important roles in ecosystem functioning of these areas it is important to monitor their diversity during the experiment. Therefore our present aim was to reveal the diversity of soil fungal communities before the manipulation started and optimize DNA extraction, amplification of locus used for metabarcoding and the applied ARISA (automated ribosomal intergenic spacer analysis) method. This initial state can be used later as a reference point of the postmanipulation results. The experimental site was sampled extensively exactly before the manipulations resulting in 12 composite samples.

Community DNA was extracted from every composite soil sample using MoBio PowerSoil® DNA Isolation Kit in triplicate and MoBio PowerMax® Soil DNA Isolation Kit. In this way we could test whether DNA extraction from about 0.3 g samples in triplicate can compensate one fold DNA extraction from 5 g samples. Fungal community composition was characterized and analysed with ARISA of the nrDNA ITS-1 region. Detailed analysis showed that both DNA extraction methods resulted in similar alpha-diversities of fungal communities per samples, but the four parallel extractions from the same samples might slightly differ. The fungal community fingerprints were compared to the parameters of the soil samples. For a better understanding of fungal communities a Next Generation Sequencing will be carried out in the near future.

This research was supported by the Hungarian Scientific Research Found (OTKA K 112576 and K 109102).

IN VITRO STUDY OF NEW ANTIBACTERIAL SILVER FUNCTIONALIZED PHOTOREACTIVE TIO_2/POLYMER NANOCOMPOSITE COATING FOR DESTRUCTION OF ORAL *STREPTOCOCCUS MITIS*

ANNAMÁRIA VENKEI[1], KRISZTINA UNGVÁRI[1], ÁGOTA DEÁK[2], LÁSZLÓ JANOVÁK[2], JÁNOS MINÁROVITS[1], IMRE DÉKÁNY[3], EDIT URBÁN[4], KINGA TURZÓ[1]

[1]Department of Oral Biology and Experimental Dental Research, Faculty of Dentistry; [2]Department of Physical Chemistry and Material Sciences, Faculty of Science and Informatics, University of Szeged, Szeged; [3]Supramolecular and Nanostructured Materials Research Group of the Hungarian Academy of Sciences,

Department of Medical Chemistry; [4]Institute of Clinical Microbiology, Faculty of Medicine, University of Szeged, Szeged, Hungary

Bacterial colonization and biofilm formation on implanted devices are etiological agents in the infections of bone around the implant. The aim of this study was to test a silver-copolymer nanocomposite surface treatment of titanium implants to restrict bacterial adhesion and proliferation and to investigate these surfaces with illumination. This microbiology study used commercially pure (CP4) sand blasted, acid etched (A, control surface) titanium sample discs (Denti® System Ltd., Hungary) 1.5 mm thick and 9 mm in diameter and two different surface modifications. Two copolymer based nanohybrid layers were tested: B) 60% TiO_2/ 40% copolymer and C) 60% $AgTiO_2$/ 40% copolymer ([Ag] = 0,001 m/m %). *Streptococcus mitis*, which is a first colonizer in titanium dental implant associated infections was cultured on the coated discs. The discs were then exposed to UV-visible light source-in to different time periods. The antibacterial effect of the photocatalysts in each group was determined by MTT and protein assay after different time (5, 10, 15 minutes) illumination. The number of the attached bacteria on all titanium surfaces was reduced depending of time. According to MTT results we observed the least amount of living bacteria on the surface with silver (C) and protein assay confirmed these results. MTT results demonstrated that55 % of the bacterial cells have been destructed on $AgTiO_2$ composite film after 15 minutes illumination compared with control titanium discs in dark set. Our study demonstrated that the silver nanoparticle-modified titanium surface shows more intense antibacterial effect than the control. This effect is intensified after 15 minutes UV-visible light illumination. The silver nanocomposite-coated titanium has antibacterial and antiadhesive activities to *Streptococcus mitis* cells.

Support of „ TÁMOP-4.2.2.A-11/1/KONV-2012-0035 Investigation of the interactions of environmental and genetic factors in development of immune-mediated and cancer diseases" project is acknowledged.

WHEY UTILIZATION WITH PROPIONIBACTERIA UNDER MICROAEROFIL CONDITIONS

ALADÁR VIDRA, ÁRON NÉMETH

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

Whey is a major co-product of the diary industry with its volume increasing at about the same rate as milk production volume. Whey exerts a considerable chemical oxygen demand (COD) and its cost-effective disposal or utilization has become increasingly important to the modern dairy industry. Whey is foremost a dilute solution and many of the difficulties encountered in whey handling stem from this fact. We investigated the possibilities of whey utilization in a microbiological way. In these investigations aerotolerance and propionic acid production by *Propionibacterium freudenreichii* subsp. *shermanii* and *Propionibacterium acidipropionici* was studied using whey as a carbon source in culture media. Propionibacteria are gram-positive, nonmotile, and anaerobic to aero-tolerant bacteria producing propionic acid, acetic acid, and carbon dioxide as products of the fermentation of sugars and lactic acid. Strains of the genus *Propionibacterium* are used in several industrial processes. Propionibacteria are used by the dairy industry for the production of starters for the Swiss-type cheese manufacturing, but are also used industrially as silage inoculum, as a probiotic agent, and finally for the production of vitamin B_{12} and propionic acid. Propionic acid has many and varied applications as an antifungal agent in foods and feeds. As a preservative, propionic acid extends the shelf life of food products by inhibiting molds and some bacteria. Although preservatives deriving

from *Propionibacterium* fermentations are available, most propionic acid used by the food industry is produced by chemical synthesis. Several processes have been patented for producing propionic acid via fermentation. Batch methods that use a variety of substrates typically produce 1 to 3% propionic acid in 7 to 14 days. Other processes, including fed-batch, cell immobilization, continuous, semi continuous, and multistage processes have been used to improve the yields of propionic acid. Despite these efforts, the maximum reported yield of propionic acid obtained by fermentation is still too low to be economically competitive with chemical synthesis. Our obtained results showed that the *P. acidipropionici* is more tolerant against oxygen. The *P. freudenreichii* utilized whey faster than *P. acidipropionici*. The two strains consumed the lactate content first and the lactose content secondly. The *P. acidipropionici* utilized lactose much slower than *P. freudenreichii*. In lactose-free whey *P. acidipropionici* is more suitable than *P. freudenreichii* because of its better aero tolerance.

A STREAMLINED, QUANTITATIVE PCR BASED MEASUREMENT OF HERPES SIMPLEX VIRUS ANTIVIRAL SUSCEPTIBILITY AND HERPES SIMPLEX VIRUS-SPECIFIC NEUTRALIZING ANTIBODIES

DEZSŐ VIRÓK[1], ILDIKÓ ESZIK[1], TÍMEA MOSOLYGÓ[1], VIRÁG KISS[1], KAMIL ÖNDER[2], VALÉRIA ENDRÉSZ[1], KATALIN BURIÁN[1]

[1]Institute of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Hungary; [2]Department of Dermatology, Paracelsus Medical University, Salzburg, Austria

Herpes simplex viruses (HSV) are common human pathogens that can cause painful but benign manifestations and recurrent complaints, but can cause significant morbidity and mortality on infection of the eye or brain and on the disseminated infection of an immunosuppressed patient or a neonate. HSV growth inhibition measurement by plaque or yield reduction is a key task in the development of novel antiviral compounds but the manual methods are very labour intensive. The sensitive and specific PCR technology might be an effective method for quantitation HSV DNA related to virus replication; however the currently described PCR approaches have a major limitation, namely the requirement of purification of DNA from the infected cells. This limitation makes this approach unfeasible for high-throughput screenings. The monitoring of the HSV specific antibody titre is essential in vaccination trials and in the improvement of HSV-based oncolytic virotherapy. Usually, conventional cytopathic effect-based and plaque reduction neutralization tests are applied to measure the neutralization titre, but these methods are time consuming. To overcome this, we developed a quantitative PCR (qPCR) method for the detection of HSV-2 DNA directly from the infected cells and the qPCR method was further adapted to measure the titre of HSV specific neutralizing antibody in human sera. The conditions of qPCR assay were optimized to measure the antiviral activity of known and novel antiviral substances. Using HSV-2 seronegative and seropositive patients' sera, the validity of the qPCR neutralization test was compared to traditional cytopathic effect-based assay. Our direct qPCR method was able to detect the HSV-2 DNA quantitatively between multiplicity of infection (MOI) 1/64 and 1/4194304, indicating that the dynamic range of the detection was approximately 65500 fold with high correlation between the biological and technical replicates. As a proof of the adaptability of the method, we applied the direct qPCR for antiviral inhibitory concentration 50 (IC50) measurements of known and novel antiviral compounds. The measured IC50 of acyclovir was ~0.28 μg/ml, similar to the previously published IC50 value. The IC50 of novel antiviral candidates was between 1.6 – 3.1 μg/ml. The qPCR-based neutralization titres of HSV positive sera were 1:32-1:64. We got the same neutralization titres using a traditional neutralization assay. The negative sera did not inhibit the HSV-2 replication in either of

the tests. Our direct qPCR method for the HSV-2 growth determination of antiviral IC50 and neutralization titre is less time-consuming, less subjective and a more accurate alternative to the traditional plaque titration and growth reduction assays.

THE RELATIONSHIP BETWEEN FUNGAL COLONIZATION, HOST-TREE PHYSIOLOGY AND ENVIRONMENTAL CONDITIONS ON SUMMER TRUFFLE PLANTATIONS

TORDA VARGA[1], ZSOLT MERÉNYI[2], ZOLTÁN ILLYÉS[2], JUDIT VIKOR[2], GABRIELLA TAMASKÓ[2], ÁKOS KUND ORCZÁN[2], GÁBOR VÖLCZ[2], PÉTER PITLIK[2], ÁDÁM SOLTI[2], ZOLTÁN BRATEK[2]

[1]Institute of Biochemistry, Synthetic and Systems Biology Unit, Biological Research Centre, Szeged; [2]Department of Plant Physiology and Molecular Plant Biology, Faculty of Science, Eötvös Loránd University, Budapest, Hungary

Summer truffle (*Tuber aestivum*) is a valuable ectomycorrhizal fungus since it is a common partner of several tree species of temperate climate. Moreover, its sporocarp is a popular and high-prized crop all over the world. Truffles have been cultivated since the middle of 19th century and even though the cultivation methods were advanced after 1970, unexpected decrease in the production and unfertile orchards occur all over the world. Truffle orchards are complicate biological systems where the fungal partners, the host trees and the environmental conditions affect each other. Thus, our goal was to find ecological driving factors of the truffle ectomycorrhizal colonization and find connections between fungal colonization efficiency and physiology of the host trees. We monitored 11 summer truffle plantations in Hungary at three and six years after orchard plantation. The colonization rate of summer truffle and other (contaminant) fungi were determined by examining root samples. Height and stem diameter of host trees were measured to estimate biomass production. In case of five plantations the maximal quantum efficiency of photochemical system II, referring to the vitality of host trees was determined by PAM 101-102-103 chlorophyll a fluorometer. All together almost 1000 individuals belong to six tree species were examined. The pedological properties (pH, macro-, microelements, lime content, etc.) of each orchards were determined from soil samples. In some cases the pH of individual soil samples around host trees were measured to examine spatial effect of pedological properties. Meteorological data were supplied by the Hungarian Meteorological Service for every half year period before a sampling day. To describe the pedological and meteorological properties of orchards we performed a principle component analyses (PCA) and the first two principle component were used in further analyses. General linear models (GLM) were built to find out driving forces on truffle colonization and on the vitality of host tree. Spatial effects were analyzed by Mantel and Partial Mantel Tests. We found that the biggest variability of colonization rate of fungi and vitality of trees were among tree species and orchards. Thus GLMs were built for each tree species separately. Our results weren't biased by spatial effect, because we found no spatial correlation between variables. Generally, the fungal colonization rate, the biomass as well as the nitrogen and phosphor content of soil affected the vitality of the plants.

Moreover, the vitality of host trees and nitrogen and phosphorus content of the soil affected summer truffle colonization. In all cases, the contaminant fungal colonization had a negative effect on summer truffle colonization rate. However, in some cases the truffle colonization was unchanged or increased in parallel to the increased contaminant fungal colonization.

Supported by MIKOQUAL Jedlik Ányos program and QUTAOMEL National Technology program.

EXAMINING EARLY FRUITING BODY DEVELOPMENT OF *COPRINOPSIS CINEREA* BY HISTOLOGICAL INVESTIGATION AND SINGLE CELL RNA-SEQ: A METHODOLOGICAL STUDY

TORDA VARGA, KRISZTINA KRIZSÁN, ÁRPÁD BÁLIND, PÉTER HORVÁTH, ISTVÁN NAGY, LÁSZLÓ G. NAGY

Institute of Biochemistry, Synthetic and Systems Biology Unit, Biological Research Centre, Szeged, Hungary

The histological and molecular background of fruiting body development in mushroom forming fungi is poorly known. *Coprinopsis cinerea* is the most studied basidiomycete in this respect, with a relatively well-documented development, however, even in this species, patterns of cap and stipe differentiation early in the development are hardly known. During *Coprinopsis* development, cap and stipe rudiments appear in Stage I primordia, however, how the cap initial differentiates from a homogeneous secondary hyphal knot is not known. Thus, our main goal is to explore tissue differentiation of *Coprinopsis cinerea* and compare the temporal and spatial mRNA expression profiles of different cell populations to find out the main molecular mechanisms of early fruiting body development. We performed a histological study of the early developmental processes of *Coprinopsis cinerea* with the aim to identify key cell lineages for RNA-seq. We have chosen laser-capture microdissection (LCM) coupled with single cell RNA sequencing (scRNA-Seq) to examine differentially expressed mRNA. Because coupled LCM and scRNA-seq haven't been done on Basidiomycetes our further goal was to optimize protocols: cryo-sectioning, LCM, scRNA extraction and amplification using the Smart-seq2 method. We grew C. cinerea strain A43mutB43mut on YMG media to obtain hyphal knots. After hyphal knots appeared, we sampled five primordia in every ~6th hours for two days. Samples were fixed, embedded in sucrose solution and frozen at -40°C. Ten micrometer thick sections were cut and microscoped. We identified three new developmental stages, which link the secondary hyphal knot and the stage I primordium stages. During these stages a dense structure appears on the auxiliary region of the secondary hyphal knot, which will serve as a cap initial of the fruiting body. To obtain scRNA expression profiles, we first optimized microdissection, RNA extraction and amplification protocols. The fixation and embedding procedures were optimized for RNA integrity, as opposed to histological quality of the sections. We found that fixation of stage I primordia or earlier stages with Farmer's fixative for 30 minutes is sufficient, while larger primordia or fruiting bodies need longer incubation times. We used 10 μm thick cryo-sections of stage I primordia and PEN membrane glass slides to ensure precise tissue catapult by LCM. We performed LCM on a Zeiss PALM MicroBeam microscope optimized LCM parameters to obtain 7.000-30.000 μm^2 sized areas with only a 20 μm wide burned perimeter. We compared the impact of different RNA protective agents (none, 2-mercaptoethanol, Qiagen RNAlater) and cell lysis techniques (using buffers from two vendors' kit) on the quality of RNA recovered from embedded tissues. We found that protective agents have little impact on the quality of RNA during embedding. To disrupt cells we introduced a heating-cooling-vortexing procedure before Pico Pure RNA isolation.

TAXONOMICAL CHARACTERISATION OF ENDOPHYTIC FUNGI OF *TAXUS BACCATA* AND ANTIMICROBIAL EFFECTS OF THEIR SECONDARY METABOLITES

BETTINA VOLFORD[1], SARUUL ERDENEBILEG[1], ARUNA VIGNESHWARI[1], OTTÓ BENCSIK[1], ANIKÓ NÉMETH[2], LÁSZLÓ KREDICS[1], CSABA VÁGVÖLGYI[1], ANDRÁS SZEKERES[1]

[1]Department of Microbiology, Faculty of Science and Informatics; [2]Botanical Garden, University of Szeged, Szeged, Hungary

Endophytes are a group of highly diverse microorganisms that reside within plant tissues without causing diseases, moreover, they can be beneficial for their host. These endophytic fungi have been recognized as important and novel sources of natural bioactive products with potential application in agriculture, medicine and food industry. Nowadays, a number of research efforts has been focused on the isolation of endophytic fungi from medicinal plants, discovering many undescribed endophytic fungal species as well as undescribed chemical substances, which have potential to be used as medicines in the future. These are also the major reason of the extensive research searching for endophytic fungi from *Taxus* species, which are common e.g., in Western and Southern Europe or in Western Asia. In our study, *Taxus baccata* samples were collected from the Hungary in late spring of 2015. Small pieces of these plants were washed, surface-sterilized and placed in triplicates onto the surface of two types of agar media. Finally, 95 isolates were recovered, purified and deposited into the Szeged Microbiological Collection. The initial identification of isolates was performed on morphological bases, which was confirmed by molecular biological techniques. The species diversity of the isolates was remarkably high representing the genera of *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium* and *Trichoderma*. After liquid culture cultivations of the isolated endophytes, their ferment broth was extracted sequentially by equal volumes of n-hexane, chloroform and ethyl acetate and finally evaporated to dryness. Then the concentrated extracts were tested against six bacterial strains. Among the 95 isolates, 25% of the isolates showed significant inhibitory activity against the bacterial strains. Our results confirm that the natural products of *Taxus* endophytes could be potential candidates for the antimicrobial applications.

Connected to the project GINOP-2.3.3-15-2016-00006 and supported by GINOP-2.3.2-15 C113410.

PRODUCTION OF HOST METABOLITES BY ENDOPHYTES OF *HYPERICUM PERFORATUM*

ARUNA VIGNESHWARI[1], ANIKÓ NÉMETH[2], LÁSZLÓ KREDICS[1], CSABA VÁGVÖLGYI[1], ANDRÁS SZEKERES[1]

[1]Department of Microbiology, Faculty of Science and Informatics; [2]Botanical Garden, University of Szeged, Szeged, Hungary

The natural products are naturally derived compounds playing a very important role in health care and prevention of diseases in a number of cultures on the Earth for thousands of years. Natural products could be presented as metabolites or byproducts from broad range of organisms including microorganisms, plants or animals, which are almost infinite resources for drug discovery to provide new medicinal agents to the human health care and therapy. Furthermore, the number of secondary metabolites produced by fungal endophytes is a remarkable part of the nowadays known natural products. These organisms could live within plant tissues synergistically for all or part of their life cycle and cause no apparent infections and it has been discovered that their secondary metabolites are occasionally the same as those produced by the respective hosts, which have been exclusively isolated from higher plants. In our present study, 8 fungal endophytes were isolated after a surface sterilization procedure on PDA plates from the medicinal plant *Hypericum perforatum* sampled from the Botanical Garden of University of Szeged (Szeged). After the purification, the strains were characterised taxonomically based on molecular techniques with the PCR amplification and sequencing of ITS region of the genomic DNA. For the secondary metabolite production, the isolates were inoculated into liquid media and after the cultivation the organic solvent extracts of both the

ferment broth and mycelia of cultured isolates were prepared. These crude extracts were investigated concerning their hypericin and emodin content, where these compounds were identified by HPLC-UV and HPLC-MS techniques confirmed by comparison with authentic standards. The antimicrobial activities of the extracts were also examined against different bacterial strains in bioassays. Based on our results one fungal strain produced the napthodianthrone derivative hypericin in the rich mycological medium under shake flask fermentation conditions. Emodin was also found both in the extracts of this fungus and the extracts of two other isolates, which were also able to produce of this compound. The mycelial extracts of the isolates showed significant inhibitory activity, while the broth extracts showed lower inhibitory activity against all bacteria. Moreover, the effects of the most active extracts were reached to the level of the applied antibiotic control.
The isolated endophytic fungi, which were able to produce valuable host metabolites have significant scientific and industrial potential in a cost-effective biotechnology.
Connected to the project GINOP-2.3.3-15-2016-00006 and also supported by GINOP-2.3.2-15 C113410.

INVESTIGATING THE ROLE OF THE SYK/CARD9 PATHWAY IN THE IMMUNOLOGICAL RESPONSE AGAINST *CANDIDA PARAPSILOSIS*

ERIK ZAJTA[1], ADÉL TÓTH[1], KATALIN CSONKA[1], JANKA ZSÓFIA CSEPREGI[2], TAMÁS NÉMETH[2], ATTILA MÓCSAI[2], ATTILA GÁCSER[1]

[1]Department of Microbiology, University of Szeged, Szeged; [2]Semmelweis University School of Medicine and MTA-SE 'Lendület' Inflammation Physiology Research Group of the Hungarian Academy of Sciences and Semmelweis University, Department of Physiology, Budapest, Hungary

A major signaling pathway initiated by C-type lectin receptor (e.g., Dectin-1 and -2, Mincle) ligation is mediated through Syk and CARD9. This route has been implicated in infections caused by several fungal pathogens like *Candida albicans*. *Candida parapsilosis* is an important opportunistic human pathogenic yeast. It is common cause of candidaemia and poses a special threat to neonates. Despite its clinical relevance, little is known about the immunological mechanism of *C. parapsilosis* infections. The aim of this new project is to investigate the role of Syk and CARD9 in *C. parapsilosis*-triggered in vitro innate immune cell responses and in in vivo murine infectious models. We generated bone marrow chimeras with wild type (WT), Syk- or CARD9-deficient hematopoietic systems. Peritoneal macrophages (PMs) and bone marrow derived macrophages (BMDMs) were cultured or differenciated. PMs and BMDMs were infected with *C. parapsilosis* or *C. albicans* and supernatants were analysed for cytokine content by ELISA. The animals were infected intravenously with *C. parapsilosis* or *C. albicans* and the number of CFUs was determined from organ homogenates and blood. The lack of Syk or CARD9 led to significant reductions in IL-1β and TNFα secretion in vitro. Both Syk- and CARD9-deficient mice were more susceptible to *C. albicans* than WT mice on day 2 post infection. To a lesser extent, Syk- and CARD9-deficient mice were also more sensitive to *C. parapsilosis* on days 2 and 5 post infection. Preliminary data from other experiments (LDH release, the ability of phagocytosis of *C. parapsilosis* by macrophages may also be presented. Our data suggest that both Syk and CARD9 influence *C. parapsilosis* induced host responses. This work contributes to a better understanding of the immunological background of non-albicans *Candida* infections which are an increasing global concern.

EXPANDING OUR KNOWLEDGE ON TWO CLOSELY RELATED *HYGROPHORUS* SPECIES IN HUNGARY

ERIK ZAJTA[1], TORDA VARGA[2], GÁBOR M. KOVÁCS[3], BÁLINT DIMA[3]

[1]Department of Microbiology, University of Szeged; [2]Biological Research Centre, Szeged; [3]Department of Plant Anatomy, Faculty of Science, Eötvös Loránd University, Budapest, Hungary

Hygrophorus penarius s.l. is an ectomycorrhizal basidiomycete is considered as not rare in Hungary. In 2007, specimens associated with *Fagus sylvatica* and *Quercus* spp. were recognized as separate species, *H. penarius* and *H. penarioides*, respectively. This was based on ecological, morphological and molecular traits, however, on a very small sample size. Our aims were to revise a range of *Hygrophorus penarius* s.l. specimens collected in Hungary by molecular phylogenetic analyses to gain information about the distribution of *H. penarius* and *H. penarioides* and to test if results of molecular analyses remain coherent with the morphological species descriptions. The nrDNA ITS regions were amplified and sequenced from 25 specimens of the Macrofungi Collection of the Hungarian Natural History Museum and three other specimens. Fruiting body and spore sizes were measured. Statistical analyses were performed to test morphological differences between *H. penarius* and *H. penarioides* specimens. Our phylogenetic analyses revealed that 21 specimens were *H. penarioides*, three *H. penarius*, two *H. poetarum* (protected in Hungary), one *H. eburneus* and one belonged to an unidentified *Hygrophorus* species. *Hygrophorus penarioides* and *H. penarius* clustered in separate clades. In addition, we found a characteristic polymorphic region in the ITS2 of *H. penarioides* sequences. We detected some discrepancies between molecular identification and the current species concept of the taxa. Nevertheless, there was no prominent difference in fruit body size nor spore size between our specimens of the two species.
Our results suggest that mycorrhizal host tree, fruitingbody size and spore size cannot unambiguously distinguish between *H. penarius* and *H. penarioides* without molecular phylogenetic analyses. We also conclude that *H. penarioides* is far more common in Hungary than *H. penarius*.

METAGENOMIC ANALYSIS OF BAT GUANO SAMPLES REVEALED THE PRESENCE OF POTENTIAL PATHOGENIC VIRUSES TO *APIS MELLIFERA* IN HUNGARY

BRIGITTA ZANA[1, 2], GÁBOR KEMENESI[1, 2], PÉTER URBÁN[2, 3], KORNÉLIA KURUCZ[1], FANNI FÖLDES[1, 2], SAFIA ZEGHBIB[1, 2], MIKLÓS OLDAL[1, 2], FERENC JAKAB[1, 2]

[1]Virological Research Group, János Szentágothai Research Center; [2]Institute of Biology, Faculty of Sciences; [3]Microbial Biotechnology Research Group, János Szentágothai Research Center, University of Pécs, Pécs, Hungary

The predominance of dietary viruses in bat guano samples had been described recently suggesting a new opportunity to survey the prevalence and to detect new viruses of arthropod of even plant infecting viruses circulating locally in the ecosystem. Here we describe the diversity of viruses belonging to Picorna-like virus group in Hungarian insectivorous bat guano samples. The conducted metagenomic analysis on our samples have revealed the significant predominance of Aphid lethal paralysis virus (ALPV) and Big Sioux River Virus (BSRV) in Hungary for the first time. Phylogenetic analysis were used to clarify the relationship to previously identified honey bee infecting ALPV and BSRV strains, showing that our strains possess close genetic relationship with the strains that have been already described as honey bee pathogenic.

Furthermore, the previously confirmed ability of these viruses to replicate and the presence of mature virions in adult honey bees indicate their virulence and their frequent incidence suggest their significant role in honey bee health. With the identification of two recently identified honey bee pathogenic viruses for the first time in Hungary and on the basis of previous results about the causative agents in elevated honey bee losses in Europe and the USA we can suggest our results may possess major importance to the detriment of Hungarian honey bee colonies.