# ABSTRACTS

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## INVESTIGATION OF THE FUNCTION AND STRUCTURE OF ACE2P - A CONSERVED REGULATOR OF CELL SEPARATION

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During cytokinesis, a three-layered cell wall has to be produced allowing the separation of daughter cells in the fission yeasts *Schizosaccharomyces*. The formation and cleavage of the septum requires strict coordination to prevent cell lysis. These processes are under the control of complex networks of genes and proteins. The transcription factors Sep1p and Ace2p play essential roles in cell separation through regulation of numerous genes such as *agn*1 and *eng*1 that encode enzymes of septum degradation. It has been proven that the mentioned regulators are conserved as their putative orthologs can be found in many fungi. However, they are diverse in the protein and gene sequence length. The zinc-finger transcription factor Ace2p has some *Schizosaccharomyces* specific structural characteristics. The aim was to deepen our knowledge on the structure and function of the Ace2p by further analysis of its sequence characteristics with bioinformatical and experimental methods. As more genome sequences of related species became recently available, we performed BLAST search using the protein sequence of *Sch. pombe*. After generating phylogenetic trees we analysed the sequences deeply for finding new conservative blocks. Then we examined the protein sequences with the IUPred algorithm to find intrinsically disordered regions. As the protein sequences from the N-terminus to the first conserved blocks seem to be ordered but diverse among the species, we created a trunked version of Ace2p to learn its role.
The transformation of this product to our *Sch. pombe ace*2Δ strain is underway.

## HOW COULD MEIOTIC DNA BREAKS DEFINE THE FATE OF GENE ORDER IN RELATED SPECIES?

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Most sexually reproducing organisms have recombination processes during meiosis, which could contribute to the rearrangement of gene order on their chromosomes. This phenomenon could be advantageous for the organism as the new gene order being formed can elevate its fitness. Otherwise it could lead to complete disaster, if it occurs near the centromeres or within repetitive DNA. Henceforth, in proper circumstances recombination occurs just in specific regions, called hotspots. Meiotic hotspots are often motif associated, initiated by transcription factors (Tfs) such as Atf1-Pcr1, Rst2 and Php2-Php3-Php5 complex in *Schizosaccharomyces pombe*. It is supposed that Tfs play a role in shaping and evolving DNA double strand breaks (DSBs), but forming of DSBs are not caused directly by them. In *Sch. pombe*, developmentally programmed DSBs are generated by a topoisomerase-related protein called Rec12 (Spo11 in *Saccharomyces cerevisiae*). A near single-nucleotide resolution DSB map has been made by other laboratories. As this DSB landscape and sequence motifs are available, we are able to examine how meiotic DNA breaks could affect the reshuffling of gene order in the fission yeasts.

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First, we analysed the synteny relationships in the four *Schizosaccharomyces* species by finding conserved collinear blocks that consist of at least five genes. Second, we compared the positions of those blocks and of Rec12 cleavage sites in the genome of *Sch. pombe*. After that we examined the association of DSB sites and local clustering of essential genes. Moreover, we were curious about the function and co-expression rate of genes located near the cleavage sites. At last, we compared the locations of known meiotic hotspot motifs with our map of conserved collinear blocks.

## ROLE OF MALDI-TOF METHOD TO IDENTIFY BACTERIAL SPECIES FROM INFLAMED PERIODONTAL POCKETS

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All forms of periodontitis are multifactorable diseases, but the role of strict anaerobic bacteria is obvious in the procession of the diseases. In the subgingival biofilm, we can group the component bacteria into complexes. We worked with two patient groups: 10 people had aggressive periodontitis and 6 people had chronic periodontitis. The aim of this study was to compare the presence of bacterial species in this two disease forms. We also proposed to compare a rapid biochemical identification system and MALDI TOF mass spectrometry (Bruker Daltonik, Germany) in the identification of anaerobic bacteria. Samples were collected in the Department of Periodontology, in our University. All patients underwent general dental examinations, including pocket depth and bleeding measuring, according to criteria established by WHO. The microbiological sample collection was performed with sterile paper points at isolated and dried pockets. Sample preparation and microbiological culture were carried out according to the routine microbiological practice. After the cultivation procedures all of the isolated bacteria were identified by biochemical and by MALDI-TOF methods. In patients with aggressive periodontitis the average periodontal pocket depth is 6.2 mm (5-8 mm) and the average isolated bacterial species number is 8.2/pocket (4-16/pocket), while in case of patients who had chronic periodontitis the average periodontal pocket depth is 6.8 mm (6-9 mm) and the average isolated bacterial species number is lower: 5.5/pocket (3-10/pocket). Altogether 189 bacterial strains were isolated in the two groups: 111 (58.7%) obligate anaerobic and 59 (31.2%) facultative anaerobic bacteria. Using the biochemical identification method, only 31.1% of the strains were identified at species level. In contrast, using the MALDI-TOF method 172 isolates (91%) were identified at species level, and in case of 19 isolates (10.1%) identification was not reliable. In this study we used MALDI-TOF mass spectrometry to study the presence of various bacterial species from periodontal pocket samples in cases of aggressive and chronic periodontitis patients. We managed to identify at species and at genus level difficulty identifiable obligate anaerobic bacteria using MALDI TOF. Our result also emphasize that this identification method can be useful for the rapid identification of bacteria from clinical samples. We also managed to identify 7 bacterial strains at species level, which are hardly or not identifiable by biochemical methods.

## THERMOPHILIC BIOFILM AND WELL WATER PROKARYOTIC COMMUNITIES FROM BUDA THERMAL KARST SYSTEM REVEALED BY SEM AND MOLECULAR CLONING

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Phylogenetic approaches and chemical-physical analyses have been used to investigate the diversity of thermophilic archaea and bacteria in a geothermal karst system located in Budapest (Hungary). Karstic thermal water of 73.7 °C and 6.2 pH and biofilm extracted from an outflow pipeline have been analyzed with the help of molecular cloning and scanning electron microscopy (SEM). On the scanning electron microscopic observations it can be clearly seen that calcium carbonate minerals serve as surface for colonization for bacteria and the cross-section of the hollow morphology suggests that bacteria may have played a role in the formation of minerals accumulated in their cell surface. The vast majority of the bacterial clones proved the dominance of the chemolithoautotrophic sulfur oxidizer Betaproteobacteria in the water sample, while that of the hydrogen oxidizer Aquificae in the biofilm. The most abundant phylotypes both in water and biofilm archaeal clone libraries were closely related to thermophilic ammonia oxidizer *Nitrosocaldus* and *Nitrososphaera* but phylotypes belonging to methanogens were also detected.

The results show that in addition to the bacterial sulfur and hydrogen oxidation, mainly archaeal ammonia oxidation may play a decisive role in the studied thermal karst system.

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

## DIVERSITY OF EXTREMOPHILIC BACTERIA IN THE HIGH-ALTITUDE LAKES OF OJOS DEL SALADO VOLCANO, DRY ANDES

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Bacterial communities inhabiting the high altitude lakes in a mountain desert area of volcano Ojos del Salado (Dry-Andes) were revealed by molecular biological and cultivation dependent methods. Water and sediment samples from lakes located between 3770 and 6500 meters above sea level were taken in February 2014. Diversity of the bacterial communities was investigated with 16S rRNA based molecular methods. General diversity of the total number of 17 samples was compared by DGGE, a molecular fingerprinting method. Detailed analysis of the bacterial diversity from 4 different altitudes was performed with 16S rRNA gene based clone libraries. A total of 17 different bacterial phyla were revealed in the studied 6 samples, and the phylogenetic distribution within the phyla were different according to the sampling sites.

Molecular clones showed the highest similarities to sequences from cold environments and high altitude or high latitude permafrost soils. Presence of litotrophic (Fe, S oxidizing), psychrophilic and acidophilic organisms were detected with both molecular methods. Seven samples taken from sediments were cultured using two modified R2A media with different NaCl concentrations. Average cell counts were estimated, and isolated strains were identified.

## *CORYNECOCCUS HYSTRIXII* GEN. NOV., SP. NOV., A XYLAN-DEGRADING BACTERIUM ISOLATED FROM THE FACES OF *HYSTRIX CRISTATA* FROM THE BUDAPEST ZOO AND BOTANICAL GARDEN, HUNGARY

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A Gram-positive mesophilic bacterium, designated strain TSL3, was isolated on xylan-containing agar plate from faces of *Hystrix cristata* from the Budapest Zoo and Botanical Garden, Hungary. The novel strain was non-spore forming, non-motile, catalase- and oxidase-positive. Optimal growth of strain TSL3 occurred at 20-37 °C and pH 6.0. Morphology of TSL3 investigated by EM resembles to the members of *Arthrobacter* genus: the shape of the cells changes during the growth cycle, typically forming short, coccoid rods in young cultures (1-2 days) and cocci appears in the old cultures or stationary phase (4-7 days). The cells group together in young cultures almost the same way as coryneform bacteria, forming „Chinese letter" like cell aggregations. Strain TSL3 shows the peptidoglycan structure A4 alpha L-Lys - Gly - L-Glu. It differs from the known peptidoglycan structure A11.40 in displaying L-Glu instead of D-Glu in the interpeptide bridge. The main fatty acids of this novel bacterium are anteiso 15:0 and iso 16:0. Polar lipid profile contains PG, DPG, PI, 3 PL and 2 unknown glicolipid. Menaquinone composition of the novel bacterium is MK-7, -6, -8 (91:4:3). The G+C content of genomic DNA was 65.2 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain TSL3 fell within the Micrococcaceae family near to *Artrobacter-Micrococcus-Citricoccus* genus. Its closest neighbours were unidentified strains from the above mentioned genus in the range of 96.1-96.4%. Based on phenotypic, chemotaxonomic and phylogenetic properties, strain TSL3 is considered to represent a novel genus and species, for which we proposed the name. Based on our polyphasic taxonomic characterization, we propose that strain TSL3 represents a novel genus and species, for which we propose the name *Corynecoccus hystrixii* gen. nov., sp. nov. (type strain TSL3T = NCAIM B.02604T), within the family Micrococcaceae.

## IDENTIFICATION OF A NOVEL DENSOVIRUS IN THE DARKLING BEETLE *ZOPHOBAS MORIO*

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Within the last decades increasing number of people keep lower vertebrates (birds, reptiles, amphibians or fish) as companion animals. Some species – especially exotic ones – require special diet, including insect larvae. *Zophobas morio* F. is a species of darkling beetle, whose larvae are

known by the common name superworm or zophobas, and frequently used as mealworms, mainly in the reptile pet industry. A Hungarian zophobas breeder experienced significant losses in her colony. Larvae became inactive, showed unusual behaviour, failed to pupate and died. Larvae were submitted to the Faculty of Veterinary Science to diagnose the aetiology of the disease. On the basis of clinical signs, virus infections were suspected. Tissue homogenates of diseased pupae were subjected to negative contrast electron microscopy investigations, which revealed relatively high numbers of spherical virions with approximately 20 nm in diameter. Because until now no viruses were described in this species, next generation sequencing technology was implemented to detect and determine virus-specific nucleotide sequences in the tissues of the larvae. Larval homogenates were purified by filtering and gradient ultracentrifugation, followed by pelletisation. Nucleic acid was extracted from the pellets and was subjected to reverse transcription. DNA sequences were determined by the IonTorrent technology. Reads were subjected to BLAST search and sequences of viral origins were identified. The highest nucleotide identity (97%) was found with the *Blattea germanica* densovirus-like virus (BgDVLV) detected in bat faeces samples, which were collected in Hubei province, China in 2007 (GenBank accession number JQ320376). By the assembly of virus-specific overlapping reads a 5160 nucleotide long sequence of the virus DNA was obtained. The sequence contains five putative open reading frames (ORFs): The putative ORF1 consist of 593 amino acids (aa) and shows 98% identity with the predicted structural protein of BgDVLV. It contains a putative conserved domain of the Densovirus VP4 superfamily. The predicted ORF2 contains 256 aa, including a putative conserved domain of parvovirus coat N protein. It shows 99% sequence identity with the BgDVLV ORF2. The hypothetic ORF3 is composed of 533 aa and contains a conserved domain motif of parvovirus NS1 protein. It is 98% identical, with the ORF3 of BgDVLV. The predicted ORF4 overlaps with ORF3 and encodes a 256 aa polypeptide, which is 97% identical with the BgDVLV putative non-structural protein. The predicted ORF5 is coding for a 211 aa protein, which is at the C-terminal part 98% identical with the putative non-structural protein of BgDVLV. The ORF1 and ORF2 aa sequences have shown high (>97%) sequence identities to deduced aa sequences detected in *Gregarina niphandrodes* (Apicomplexa), a septate eugregarine, described from the adult yellow mealworm, *Tenebrio molitor*. Based on the nucleotide sequences conventional and real-time polymerase chain reaction assays were developed.
With the diagnostic methods viral nucleic acid was detected both in diseases and clinically healthy zophopas larvae of the same colony. The results of the study indicate that a densovirus, related to other insect densoviruses can infect *Z. morio* and may be a pathogen in this species.

## STUDY ON THE GLUTATHIONE METABOLISM IN THE FILAMENTOUS FUNGUS *ASPERGILLUS NIDULANS*

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The tripeptide glutathione (gamma-L-glutamyl-L-cysteinyl-glycine, GSH) plays key roles in the maintenance of mitochondrial structure, membrane integrity as well as in the cellular defence mechanisms for xenobiotics and reactive oxygen species. GSH itself is able to react non-enzymatically with reactive oxygen species. Moreover, GSH biosynthesis enzymes including NADPH-dependent GSH-regenerating reductase, glutathione S-transferase and peroxide-eliminating glutathione peroxidase are also contributed to the response to oxidative stress. This study is focused on the glutathione metabolism of the model organism *Aspergillus nidulans* by the phenotypic

characterization of the deletion mutants of the glutathione reductase (*glr*A; AN932) and the gamma-L-glutamyl-L-cysteine ligase (*gcs*; AN3150). Deletion of *glr*A resulted in reduced growth at 37 oC without GSH supplementation, higher sensitivity to oxidative stress inducing agents menadione sodium bisulfite (MSB), diamide and hidrogen-peroxide and concomitant elevation of catalase enzyme activity similar to earlier studies of Sato et al. [1, 2]. Some interesting phenotype also came up in our work, namely this mutant showed sensitivity to osmotic stress inducing agents NaCl, KCl and sorbitol as well as antimycotics, e.g. fluconazole and amphotericin B and cell wall stress generating agent Congo red. Conidiospores of *Δglr*A were sensitive to heat (50 $^{o}$C) and oxidative stress ($H_2O_2$), too. Gene disruption of gcs was lethal in *A. nidulans* in the absence of glutathione similar to *Saccharomyces cerevisiae*. In *Δgcs* strain deprivation of GSH also resulted in the elevation of catalase activity in submerged culture. It has also been proven that GSH is essential to not even the sporulation but the germination of conidiospores in both of the mutants studied.

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Fruzsina Bakti and Anita Király contributed equally to this work.

[1] Pócsi, I. et al. (2004) Adv Microb Physiol 49, 1-76.
[2] Sato, I. et al. (2009) J Biol Chem 284, 8042-8053.
[3] Sato, I. et al. (2011) J Biol Chem 286, 20283-20291.

## INVESTIGATION OF *CANDIDA PARAPSILOSIS* SUSCEPTIBILITY TO CASPOFUNGIN

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The number of *Candida* infections have been groving in the last few years. As an opportunistic pathogen mostly cause infection in immunocompromised people. The patients at intensive care unit have a higher risk to get infected with this kind of fungi. In the first line, treatment of candidiasis is done by azole-type drugs. The too intensive and unneccesary usage of azoles is resulted in resistance to this drug in many *Candida* species, like *C. parapsilosis*. Because of this phenomenon have made a requirement of new drugs with new target sites. During our work we were examined the effect of caspofungin, an echinocandin type drug on 179 *C. parapsilosis* strains from the collection of Department of Microbiology, Faculty of Science and Informatics, University of Szeged. This strains are originated from different health care institutes of different geographical areas. Our examination were done according to the M27-A3 protocol. Three strains of the collection were hypersensitive (strain nr.: 22, 70 and 243) and two of them were resistant (strain nr.: 47 and 74) to caspofungin. We are already investigated the strains of the collection for the susceptability to fluconazole. Here we were compared the MIC valuesfor the two antifungal drugs. Comparing our findings with the fluconazole MIC values we were found that the caspofungin resistant strains had an average MIC value for fluconazole. The opposite of this phenomenon is also right: the less susceptible strains to fluconazole have had an average MIC to caspofungin.

## URINARY TRACT INFECTIONS CAUSED BY *TRICHOSPORON*

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*Trichosporon* species are basidiomycetous yeasts widely distributed in soil and water. They can also colonize the human skin and gastrointestinal tract. *T. asahii, T. inkin* and a few other *Trichosporon* species cause opportunistic infections, such as white piedra, hypersensitivity pneumonitis and invasive infections. However, data about urinary tract infections caused by *Trichosporon* species are scant. The goal of this study was to describe *Trichosporon* urinary tract infections (UTI) diagnosed in the Microbiology Unit of the Mures County Hospital between 2010 and April 2015. In the aforementioned period 3 cases of UTI caused by *Trichosporon* species were detected. Patients' age was 42, 72 and 82 years; regarding gender, there was a woman and two men. All patients were introduced to hospital for life-threatening diseases, intestinal occlusion, inguinal hernia and ileus. They underwent surgical interventions and were treated in the Intensive Care Unit of the Mures County Hospital. Urinary catheters were inserted for all patients. Because of the high risk of infections due to indwelling devices, urine tests and cultures were performed. Urine tests had no modifications for most of the parameters, excepting erythrocytes, proteins and pH. The latter was 5 in each sample. Erythrocytes varied from 30-50 to 300/µl, while proteins showed values of 3 and 10 mg/ml. Urine cultures were negative for bacteria, but were positive for fungi ($>10^5$ CFU/ml for all patients). Two of the fungal isolates were identified as *T. asahii* and one as *T. inkin* by Vitek 2 Compact System, respectively. Identity of one of the *T. asahii* isolates was confirmed by molecular technique based on the sequence alignment of the amplified 23S rDNA sequence to that found in the GeneBank. In case of this isolate the MIC values were low for voriconazole (0.25 µg/ml). Although haemocultures were negative, evolution of the cases ended with the decease of the patients. In conclusion, among severely debilitated patients, rare fungal pathogens can colonize the urinary tract. Antifungal susceptibility testing is needed in order to select the appropriate antifungal drugs.

## THE EFFECT OF MICROWAVE, RADIO FREQUENCY TREATMENT, AND STEAMING ON THE CONTAMINATING MICROFLORA AND COLOUR OF SPICE PAPRIKA POWDER

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The EU-FP7 project SPICED "Securing the spices and herbs commodity chains in Europe against deliberate, accidental, or natural biological and chemical contamination" focuses on contaminating agents based on e.g. frequency of natural occurrence, possible impact on human health, and relevance for food terrorism. One of its tasks is the evaluation of available means and techniques for the decontamination of spices. Steaming of spices is a proven method for microbial decontamination. Microwave and radio frequency treatments for this purpose are in their research phase. In our work we have studied the effect of these decontamination methods on the microflora and the quality (colour) of spice paprika powder. Steam treatment (saturated dry steam, 108-125 °C for 20-120 sec) reduced mesophilic aerobic total bacterial count from $1.8x10^5$ CFU/g to $6.0x10^2$

CFU/g, and moulds from $1.3x10^2$ CFU/g to under the detection limit. Yeasts, coliforms, *Escherichia coli*, and Enterobacteriaceae could not be detected in the samples. Microwave heating was performed by Daewoo Kor-630A laboratory equipment (800 W, treatment moisture content: 20.3%, treatment temperature: up to 95 °C, total treatment time: 100 s (30 g sample, 1.5 mm layer thickness with 20 s heating periods). No relevant reduction of the mesophilic aerobic total bacterial count could be observed following the treatment. The colour of the paprika powder got darker and had a brownish character. Radio frequency treatment was done by Laboratory equipment with 10 kW Brown Boveri generator, 13.5 MHz (treatment moisture content: 20.3%, treatment temperature: up to 95, 105, 115 °C, total treatment time: 50-90 s (100 g sample in 105x35x70 mm wooden box with 30 sec heating and cooling periods). The microbial load of the samples showed no reduction even for the most severe treatment. The colour of all treated samples were significantly darker than the control, they had a burnt character.

Molecular methods were also applied in the detection, enumeration, and identification of the dominant bacteria of control and steam treated paprika powders.

All bacteria are spore forming rods of the family Bacillaceae. *Bacillus methylotrophicus*, *Bacillus pumilus*, *Bacillus vallismortis*, *Bacillus sonorensis*, and *Bacillus amyloliquefaciens* represented 99% of the dominant bacteria, regardless of treatment.

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## OPTIMIZATION OF THE CULTIVATION CONDITIONS OF *BIPOLARIS ORYZAE*

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Ophiobolins are a unique family of the sesterterpene-type secondary metabolites produced by certain filamentous fungi belonging into the genera of *Bipolaris, Cochliobolus, Drechslera* and *Aspergillus*. Until now more than 30 of these compounds have been described and assigned into several subgroups based on their characteristic structure. Ophiobolins have numerous biological activities such as calmodulin antagonism, antimicrobial, cytotoxic and nematocid effects. In this study we determined the optimal cultivation conditions of *B. oryzae* related to the ophiobolin production. Using a previously developed HPLC method, we have monitored five different ophiobolin analogues: 6-epi-ophiobolin A, ophiobolin A, ophiobolin I, 3-anhydro-ophiobolin A and 6-epi-3anhydro-ophiobolni A. For detection and quantification of these secondary metabolites, a modular HPLC system equipped with a Prodigy C18 150 x 4.6 reversed phase column was used. During the isocratic elution the mobile phase composition was water:acetonitrile (30:70). The detection was carried out using UV-VIS detector at 230 nm. During the optimization experiments, three different cultivation conditions were tested in three replicates including inoculation procedure, temperature, and carbon sources, while the ophiobolin contents were analyzed in each days of the 14 days long cultivation period. 600 µl culture supernatant was taken and replaced with 600 µl PDB daily and extracted three times with equal volumes of ethyl acetate. The extracts were evaporated to dryness under nitrogen steam and resolved in 1 ml acetonitrile, which were injected to the HPLC system. To determine the optimal inoculation procedure agar plugs from 5 days old B. oryzae culture as well as conidia suspensions in different concentrations were inoculated into 50 ml media and were cultivated for 14 days. No considerable difference was observed between these inoculation procedures. The ophiobolin production was examined at four different temperatures, 20 °C, 25 °C,

28 °C and 37 °C, from which the 28 °C proved to be the most optimal for the ophiobolin production and at the highest examined temperature there was no observed fungal growth.
Furthermore, ten different carbon sources were tested to determine the most suitable cultivation media to gain the highest ophiobolin amounts. The results of this work can provide optimized fermentation conditions for the further investigation of highly valuable ophiobolin compounds.
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## ANALYTICAL MONITORING OF THE EARLY ELEMENTS OF NICOTINIC ACID DEGRADATION PATHWAY IN FILAMENTOUS FUNGI

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The HPLC technique coupled to various detectors is a powerful tool in the monitoring of selected metabolites in the complex biological systems. In our present study, this analytical tool was applied to the separation and detection of pyridine-related compounds, which was extracted from filamentous fungi and has a great importance in pharmaceutical and agricultural industries as intermediates. One of the prominent members of these compounds is the nicotinic acid (NA), which is a carboxylic acid and the basic component of NADP playing an essential role in living organisms. The microbial metabolisms of NA were described in prokaryotes, which are able to grow on a media containing this substance as the sole carbon and nitrogen source. The sequential prokaryotic degradation involves the introduction of a hydroxyl group at C6-position of the pyridine ring (6-hydroxynicotinic acid, 6-HNA) as the initial step and the formation of 2,3,6-trihydroxypyridine through an oxidative decarboxylation (2,5-dihydroxypyridine, 2,5-DHP) or hydroxylation (2,6-dihydroxynicotinic acid) followed by ring cleavage and formation of maleamic acid.
The biochemical pathways involved in the degradation of this N-heterocyclic aromatic compounds could be used as a source of novel and unusual enzyme activities, thus it is important to monitor the presence and quantity of the intermediers during different eukaryotic fermentations carried out on NA. The HPLC separation of some known metabolites involved in the microbiological degradation of NA was carried out on the reverse phase column using a diode array detector, which allows the detection of both the pyridine ring and the resulted aliphatic compounds.
The effects of a mobile phase composition and pH were investigated and optimized for the best resolution of the available standards including NA, 6-HNA and 2,5-DHP. Using an aqueous buffer solution as a mobile phase, a baseline separation was achieved. Under the optimal conditions the linearity was tested and the limit of detection and quantitation were determined. Using this analytical method we have successfully analyzed several filamentous fungal strains able to growth on media containing NA as carbon source to investigate the eukaryotic degradation of NA. Our results provide a good basis for investigation of unexplored microbial pathways of NA metabolism.
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## IS SITE-SPECIFIC RECOMBINASE XERC OF *MYCOPLASMA SYNOVIAE* INVOLVED IN ITS *VLH*A GENE RECOMBINATION?

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A major poultry pathogen *Mycoplasma synoviae* synthesizes haemagglutinin VlhA which enables the attachment to the host cells and immune evasion. The *vlh*A locus contains the expressed gene and about 70 pseudogenes arranged as tandem repeats upstream of the expressed gene, The VlhA functional and antigenic variants are generated by site-specific gene conversion in which 3-end sequence of the expressed *vlh*A gene is replaced by a pseudogene sequence. We found in the genome of *M. synoviae* a gene for the site-specific recombinase XerC .The gene is located near gene encoding recombinase RecO and gene encoding protein with RecF/N domains. Western blot analysis with specific antibodies showed that *M. synoviae* strains expressed XerC. This recombinase has capability to cause DNA breaking / rejoining and can be involved in *vlh*A gene conversion. Since *M. synoviae* lacks *rec*BC genes it probably uses the RecFOR pathway for the homologous recombination (HR) and/or DNA repair. *M. synoviae* expresses RecA which plays a central role in HR and homologous DNA sequence pairing. In the *vlh*A gene conversion a pseudogene sequence is used as a template and is transcribed to replace an appropriate sequence in the expressed *vlh*A gene. Recombinations frequently occur immediately downstream of the conserved sequence 5-AGCGGATCGGTAACT-3 which is present at 5' end of up to 20 *vlh*A pseudogens. Identification of the XerC recombinase would enable targeted studies about its role in the *vlh*A gene conversion.

## CHARACTERIZATION OF *CANDIDA PARAPSILOSIS* NULL MUTANTS GENERATED IN THE FRAME OF DELETION LIBRARY

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Recently, the increase in the prevalence of fungal diseases has focused attention on understanding the interactions between the pathogens and the host. Despite the growth of sequence information, a large number of fungal genes are uncharacterized and the function of genes are based solely on sequence homology. To characterize gene function in fungi such as the opportunistic pathogen *Candida parapsilosis*, gene knockout methods can be applied. In our previous work we have identified several fungal genes using RNA-Seq data, that were overexpressed during host-pathogen interactions. To investigate their functions the creation of a knock out library is under progress. We have recently adapted a novel gene knock out strategy. Fusion PCR method was applied to generate gene specific deletion constructions in order to disrupt genes from the genome of *C. parapsilosis* CLIB leu-/his- auxotrophic strain. All of the null mutant strains were tested under different conditions such as growth abilities on certain temperatures and media along with survival in the presence of various stressors. Resistance to antifungal drugs was also examined. We found null mutants that show differences in appearance such as increased pseudohyphae formation (CPAR2 200390), regressed growth on different temperatures (CPAR2 303700) and alkali-phobic phenotype (CPAR2 100540). Difference in the virulence of these null mutant strains was also found when using both *in vitro* and *in vivo* infection models. Significant difference was found in the clearence of

all three deletion mutants in both liver and spleen after *in vivo* infection comparing to the wild type. CPAR2 200390 null mutants were found to be killed less efficiently by murine macrophages *in vitro*, and showed decreased biofilm formation. In contrast, null mutants of CPAR2 303700 showed a significant increase in biofilm formation and also increased fungal burden was detected in the kidneys following *in vivo* infection. Furthermore, more murine macrophages were found to phagocytose CPAR2 100540 deletion mutants, however the killing efficiency was lower comparing to the reference strain. In the future using this method we will be able to identify key regulatory factors that may play a role in the virulence of *C. parapsilosis* during host-pathogen interactions.

## DIVERSITY OF dsRNA VIRUSES INFECTING THE *CAPSICUM* GENUS

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Double-stranded RNA (dsRNA) viruses of the Partitiviridae and Endornaviridae families frequently occur in many plant genera. They usually do not cause any symptoms in the host plant and to our present knowledge can only be transmitted by seed and pollen. All viruses depend on the contribution of their host for multiplication and dispersal. This is surely also true for partitiviruses which only encode the viral replicase (RNA-dependent RNA polymerase, RdRp) and a coat protein. Considering the life-long association of partiti- and endornaviruses with their host plant we have to ask the question how specific the interactions with the host are and by what mechanisms the continuous persistence of the viruses is being ensured. Trying to find an answer to these questions we analysed the occurrence of partiti- and endornaviruses in different species of the *Capsicum* genus (*C. annuum, C. baccatum* var. *baccatum* and var. *pendulum*, *C. frutescens, C. chinense, C. pubescens* and *C. eximium*). We found that the distribution pattern of these dsRNA viruses followed the phylogenetic tree established in the same gene bank collection by Nagy et al. [1]. The effect of intensive breeding was evident in *C.annuum* where the most variable pattern was observed. Interestingly, partitiviruses did not occur in any of the *C. baccatum* lines investigated, but endornavirus was always present. A complex pattern of dsRNAs in the size range characteristic for partitiviruses was observed in most other *Capsicum* species, suggesting that different partitiviruses may be present. In *C. annuum* two such viruses, PCV1 and PCV2 were described in the literature [2], but we were unable to detect them in *C. chinense* lines by RT-PCR. Therefore, we started to clone and sequence a putative partitivirus of *C. chinense*. The sequence determined up to now is the sequence of the viral RdRp and is clearly different from that of PCV1, i.e. it represents a new pepper cryptic virus tentatively designated as PCV3 by us. It was shown by RT-PCR that the new virus also occurs in *C. frutestens* and *C. chacoense*, but is missing from all *C. annuum* cultivars investigated in our experiments. To find out whether partiti- and endornaviruses can be transmitted and become persistent in species which normally do not carry them, we carried out interspecific crosses between *C. annum* and *C. baccatum*, as well as between *C. chinense* and *C. annuum*. Although we obtained many seeds, the germination and survival rate of seedlings was very low. Preliminary results on virus transmission will be shown.

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[1] Nagy et al. (2007) Genetics 50, 668-678.
[2] Valverde et al. (2011) Virus Genes 43, 307-316.

## DOSE ESCALATION STUDIES WITH CASPOFUNGIN AGAINST *CANDIDA GLABRATA*

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Dose escalation of echinocandins has been suggested to improve the clinical outcome against *Candida glabrata*. To address this possibility, we performed *in vitro* and *in vivo* experiments with caspofungin against four wild-type *C. glabrata* clinical isolates, a drug susceptible ATCC 90030 reference strain and two echinocandin resistant strains with known FKS mutations. MIC values for the clinical isolates in RPMI-1640 were ≤0.03 mg/L but increased to 0.125-0.25 mg/L in 50% serum. In 50% serum, the replication of *C. glabrata* was weaker than in RPMI-1640. Caspofungin in RPMI-1640 at 1 and 4 mg/L showed fungicidal effect within 7 hours against three out of four clinical isolates, but was only fungistatic at 16 and 32 mg/L (paradoxically decreased killing activity). In 50% serum, caspofungin at ≥1 mg/L was rapidly fungicidal (within 3.31 hours) against three out of four isolates. In a profoundly neutropenic murine model all caspofungin doses (daily 1, 2, 3, 5 and 20 mg/kg) decreased the fungal tissue burdens significantly ($P<0.05$-$0.001$) without statistical differences between doses, but the mean fungal tissue burdens never fell below $10^5$ CFU/gram tissue. The echinocandin resistant strains were highly virulent in animal models and all doses were ineffective. Results confirm the clinical experience that echinocandin dose escalation does not improve efficacy.

## BIOGAS PRODUCTION FROM CHICKEN MANURE

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Biomass based technologies have several benefits: the necessary substrates are available in large quantities, the generated energy carriers are environmentally friendly, easy to carry and store. Biogas is a renewable energy carrier which is similar to natural gas and can be used in practically all applications to replace natural gas. It is produced by anaerobic fermentation of the organic materials. The problem of poultry waste management has been an increasing concern in many parts of the world due to the huge amount of this waste stream. Pollutants from improperly managed poultry waste can cause serious environmental problems in terms of water, air, and health quality. Chicken manure (CM) contains two main forms of nitrogen: uric acid and undigested proteins, which represent 70% and 30% of the total nitrogen in CM, respectively. Anaerobic decomposition of uric acid and undigested proteins in CM results in high amounts of unionized ammonia and ammonium ions. Excess ammonia inhibits the anaerobic microbial community and thus methane production. The possibility of washing CM with water was tested with the aim of decreasing the inhibitory nitrogen content of the substrate. After two days soaking CM in tap water, the liquid and

solid phases were separated and significant nitrogen content was detected in the water phase. Removal of excess nitrogen-containing compounds improved the suitability of CM as sole substrate for anaerobic digestion. The results demonstrated that anaerobic fermentation became sustainable when the reactors were fed with washed CM, which confirmed that ammonium concentration was indeed the limiting parameter in the anaerobic digestion of CM. The simple and inexpensive method of removing nitrogen-rich water soluble components from CM, however, resulted in a large volume of water with high nitrogen and other dissolved organics content as a residual waste. In order to improve the economic value of the process, the separated liquid phase (CM-water) was used as a nutrient solution for algae because algae need a significant amount of nitrogen source for growth. A *Chlorella* sp. strain was cultivated in these experiments. In order to avoid light limitation CM-water was further diluted with water. At the optimal dilution ratio (CM-water : distilled water) the *Chlorella* culture grew vigorously and reached higher optical density than in its default growth medium. The harvested algal biomass could be recycled into the biogas generating process. CM has low C/N ratio which is not favorable for evolving biogas with high methane content.
To solve this problem we developed a co-fermentation scheme using the solid fraction of washed CM and corn stover, to increase this ratio. These experiments yielded promising results.
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## IMPACT OF INTERFERON-GAMMA ON THE GENE EXPRESSION OF HUMAN EPITHELIAL CELLS

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*Chlamydia trachomatis* is an obligate intracellular bacterium responsible for the acute and chronic infections of the eye and urogenital tract. Fibrosis induced by chronic infection could eventually lead to debilitating diseases such as blindness and infertility. Interferon-gamma (IFNG) is a key cytokine involved in the defense of chlamydial infections. The major antichlamydial mechanism induced by IFNG is the degradation of intracellular tryptophan pool by the induction of the host gene indoleamine-2,3-dioxygenase (IDO). However other, less described IFNG induced mechanisms could also play a role in the antichlamydial defense. To explore these potential mechanisms, we measured the IFNG induced global gene expression in HeLa human epithelial cells by DNA-chip technology. HeLa cells were treated with 20 IU/ml IFNG for 6, 12 and 24 hours, the total RNAs were extracted, amplified according to the Affymetrix RNA amplification protocol and hybridized on the Affymetrix Human Genome U133 Plus 2.0 Arrays. 3 replicates were tested in a particular timepoint. Identification of significantly changed genes was performed using the Significance Analysis of Microarrays (SAM) method in the TMEV software environment. Functional analysis of the significantly changed genes was performed using the STRING and DAVID databases. IFNG had a significant impact on the HeLa gene expression with close to 300 up- and downregulated genes at least in one timepoint. Functional analysis of the upregulated genes revealed that Gene Ontology (GO) functional classes, such as defense response, response to virus, inflammatory response, antigen processing and presentation were found to be significantly enriched in this geneset. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the significantly upregulated genes showed that several genes could be mapped to the pathways antigen processing and presentation, cytokine-cytokine receptor interaction, complement cascade, proteasome and toll-like receptor signaling pathway. IFNG had a strong impact on epithelial genes expression, inducing

diverse defense related pathways that could induce adaptive and innate immune responses extracellularly, and also inducing defense genes that could combat the chlamydial growth intracellularly. Further experiments are being designed to address the role of novel inducible genes/ pathways in the antichlamydial defense.

## IMPACT OF *CHLAMYDIA TRACHOMATIS* INFECTION AND INTERFERON-GAMMA TREATMENT ON THE HUMAN POLYMORPHONUCLEAR LEUKOCYTE TRANSCRIPTOME

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*Chlamydia trachomatis* is an obligate intracellular bacterium that causes a variety of medically important diseases including conjunctivitis, trachoma, pelvic inflammation, infertility and lymphogranuloma venereum. The histopathological background of these diseases includes a profound acute inflammation that frequently leads to chronic inflammation and fibrosis. The neutrophil granulocyte is a key cell type involved in the *C. trachomatis* mediated inflammation and histopathology. Interferon-gamma (IFN-gamma) is the major cytokine in the elimination of *Chlamydia* infection. Despite the importance of neutrophil granulocytes, the impact of *C. trachomatis* infection and/or IFN-gamma treatment on the neutrophil gene expression has not been previously studied. RNA sequencing was used to discover the impact of the *Chlamydia trachomatis* serovar D infection and/or IFN-gamma treatment on the transcriptome of human neutrophil granulocytes. Our results show that the *C. trachomatis* infection alters the expression of a variety of host pro-inflammatory genes including matrix metalloproteinases, complement cascade members, Fc receptors and various pro-inflammatory cytokines and chemokines. Interestingly, several adaptive immunity-related genes were also induced, especially in the presence of IFN-gamma, including members of the antigen processing and presentation machinery and the T-cell chemokines MIG, I-TAC, IP-10 and RANTES. Protein level measurements of MHC-I, MIG and IP-10 confirmed the expression data obtained with next generation sequencing.
Finally, we identified altered expression of several members of the long non-coding RNA (lncRNA) family, such as the induction of lincRNA-p21. Our experiments support the role of neutrophil granulocytes in the *C. trachomatis* mediated inflammation and also highlight their role in linking innate and adaptive immunity via T-cell recruitment, activation and antigen presentation.

## THE EFFECT OF A SEWAGE PLANT RECONSTRUCTION ON THE WATER QUALITY OF THE RIVER ZAGYVA AT JÁSZBERÉNY CITY

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The quality and quantity of the water-soluble organic and inorganic substances have a crucial role in the water-qualification. Examination of the water quality (e.g. COD, BOD, ammonium, nitrite, nitrate, etc.) with chemical analysis is important to explore whether water purification technology follows the water quality standards. However, these data are inefficient to examine the combined effects on the ecosystem. For that reason integrate application of standard tests and ecotoxicological tests in WWTPs should be applied. In our study the waste water sewage plant of Jászberény city was analyzed. The communal sewage is treated by activated sludge. The sewage plant belongs to Jászberény city, with 24000 inhabitants. It was built in early 1980s, and in 2010 it could not hold the limit values restricted by the authorization. The preparation and raising fund took 4 years. In 2014 the reconstruction work began. The effect of a sewage plant reconstruction (2013 November – 2015 March) was monitored on the water quality by different ecotoxicology tests and by measuring organic and inorganic parameters. We investigated the upper stream, the waste water effluent, and the outfall sampled from the down stream area, at the discharge point of the waste stream into the body of water that is the oxbow of the Zagyva river. The monitoring period covered operation of the old technology, the whole period of the reconstruction, and the operation of the new technology. The water samples were investigated by SOS-Chromotest for genotoxicity, BLYES/BLYAS test for estrogenicity/androgenicity and Microtox for citotoxicity. Parallely chemical and biological oxygen demand (COD, BOD), total nitrogen, ammonium, orthophosphate, pH were measured. Ecotoxical effect was detected in the effluent waste water of the WWTP, when it operated with the old technology and during the technological recovery. This effect was present in the natural water, too. High ecotoxicity was observed typically in the summer months and after the dysfunction of the WWTP. After the new technology stabilized the effluent did not have harmful effect on the water quality in the receiving oxbow. Interestingly, in several cases, when physico-chemical parameters corresponded to threshold limits, ecotoxicity was measured. For that reason, only the restriction to physico-chemical parameters is not enough to support the good quality of living waters.
Further investigations of this paradox could improve the effectiveness and decrease the cost of the current expenses of a sewage plant in the future.
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## THE MITOCHONDRIAL DNA RFLP ANALYSIS OF *CANDIDA ZEMPLININA* YEAST STRAINS

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*Candida zemplinina* is a wine-associated yeast species, frequently isolated from grapes. One of the most promissing non-*Saccharomyces* yeast which could be potentialy used in winemaking as co-starter with *Saccharomyces cerevisiae* strains. Study of the diversity in *C. zemplinina* strains is important for knowing more about the non-*Saccharomyces* wine yeasts and also for the yeast starter industry. Pfliegler et al. [1] highlights the facts that the comparison of nuclear genome of several *C. zemplinina* isolates from different geographical origins using different fingerprinting methods showed less genetic diversity nevertheless the physiological/oenological diversity of these strains may be higher. It raised the question if the mitochondrial DNA of the examined strains are diverse

or not. For this reason, we examined the possible polymorphism of the mtDNA of *C. zemplinina* isolates studied [1]. We isolated the mitochondrial DNA of 20 strains, seven of which were isolated from botrytized grapes and six were isolated from sweet botrytized musts in Tokaj region (Hungary). Five strains were originated from grapes and musts of different European wine growing regions, one was isolated from soil in South-Africa and one was isolated from a *Drosophila* sp. in the USA. For the mtRFLP analysis we used EcoRV, HinfI, MboI, DraI and HaeIII restriction enzymes. To test the diversity of *C. zemplinina* strains, band patterns of the mtRFLPs were used to create dendograms with the UPGMA method. We observed high level mitochondrial genom polymorphism. The patterns of the 5 restriction enzymes allowed to differentiate 14 groups among the 20 strains tested. The strains grouped in 2 clusters in the case of MboI, DraI and HaeIII band patterns, and they generated 3 clusters with the HinfI and EcoRV. The strain, isolated from *Drosophila* sp. in the USA, was found to be most distanly related to the type strain of the species. The geographical origin and source of the strains was not reflected on the dendograms. 8 strains, isolated from botrytized grapes and sweet botrytized musts in Tokaj were in the same cluster, while the other isolates from Tokaj and different countries were grouped together in another cluster.

[1] Pfliegler, W. P. et al. (2014) Microbiol Res169, 402-410.

## PULMONARY TUBERCULOSIS IN LIBYA: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF PULMONARY TUBERCULOSIS IMMIGRANT AND NATIVE PATIENTS IN CHEST CLINIC IN LIBYA

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Tuberculosis (TB) is still a common disease in both developing and developed. The aim of the study is to investigate the socioeconomic factors (age, gender, occupational, unemployed) among the PTB patients. A retrospective cross-sectional study was conducted in Misurata chest hospital in Libya in the period from January 1st 2005 to December 31st 2007. Medical records of 743 PTB patients were reviewed and grouped according to their demography and socioeconomic. PTB prevalence was determined. The selection of cases was confirmed by positive smear for presence of *M. tuberculosis* and chest X-ray. A total of 20779 patients who registered, of them 743 who had PTB. The prevalence of PTB patients was 3.6%. There were 608 (81.8%) males and 135 (18.2%) were females. 51% were immigrants and 49% were Libyans. Of all patients, 66.4% (493) fall in the age group 20-40 years. Of the total, 6.6% (49/743) of the patients had HIV-co-infection. The infection rate among the males was 6.6% and 6.7% was among the females. The HIV infection rate among the Libyans 22.4% and 77.6% was among the immigrants. There were 7.3% (54/743) had hepatitis co-infection. Of them 24% were Libyans and 75.9% were immigrants. 60.2% of the Libyan patients and 75% immigrant patients were unemployed. The housewife represents 19.5% (71/364) of the Libyan patients and 4.2% (16/379) of the immigrants who had PTB. The occurrence of PTB tended to be higher with younger age which represents 81.5% patients. The PTB disease of Libyans and immigrants may associated with different demography or socioeconomic factors. The prevalence of PTB among the immigrants and the native were nearly similar. This study with limited sample and representing patients in one chest clinic may not represent the whole population of the country, but it could be a model for other studies in different Chest hospitals or clinics in the country.

# ANTAGONISTIC EFFECT OF MICROORGANISMS ISOLATED FROM FOOD PROCESSING ENVIRONMENT ON FOOD-BORNE PATHOGENIC BACTERIA

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Along the food production chain (during production, processing, distribution and preparation) one of the most important purposes is to ensure the safety of the products. As contamination can occur at any steps of the chain effective inhibition of food spoiling and pathogenic bacteria is needed. Several techniques (heating, freezing, drying, salting, fermentation and smoking) can be applied for food preservation, however these methods may contribute to changes in the products' sensory properties. Preservation with chemicals is another possibility for enhancing the safety of the products, nevertheless consumers more frequently reject the application of these compounds in food. Thus, biocontrol can be an alternate technique to inhibit the growth of food-borne pathogens and ensure food safety without affecting the products' nutritional values. The aims of this work were the isolation and characterization of bacteria with antagonistic activity on food-borne pathogens like *Escherichia coli, Listeria monocytogenes, Salmonella* Hartford and *Yersinia enterocolitica*. Altogether 44 bacteria were isolated from three different food processing environments: twenty isolates originated from an abattoir, six strains were isolated from vegetable processing environment, while 18 isolates were cultured from surface samples of an egg processing plant. The isolates were grouped into 37 clusters by their colony morphological characteristics based on shape, color and acid production on WL nutrient agar plates. Inhibition assays were carried out by co-culturing the pathogens and the test strains on CASO agar plates. Approximately $10^6$ cells of the isolates were dropped onto the agar's surface massively inoculated by the pathogenic bacteria to see whether they had any inhibitory effect on the pathogens' growth. The plates were incubated on eight different temperatures (5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 37 °C, 42 °C ) to be able to determine the optimal temperature for inhibition. Out of the tested 37 bacterium isolates fifteen could inhibit at least one of the tested pathogens, and the most effective inhibition was detected between 20 °C and 30 °C. To characterize and identify these potential biocontrol bacteria physiological and biochemical tests were done. All 15 isolates proved to be mesophilic, neutrophilic, and based on the OF tests 10 of them can be characterized by fermentative carbohydrate utilization. Using the KOH test seven isolates were clustered into Gram-negative and eight into the Gram-positive groups of bacteria. For preliminary identification miniaturized identification kits were used: BBL Crystal Gram-Positive ID kit for Gram-positives and API 20 tests for Gram-negative strains. The result showed that the isolates belong to *Pseudomonas, Staphylococcus, Acinetobacter, Corynebacterium, Lactococcus, Enterococcus, Sphingomonas* and *Helcococcus* genera. For more accurate identification molecular biological analyses are in progress. Moreover, determination of inhibitory effect for cell-free supernatants generated from cultures of the isolates has also been started. Our results demonstrate that potential biocontrol bacteria can be found in food production environments and may be applied for inhibition of pathogenic bacteria that can cause severe food-borne diseases.

## SCREENING OF EMERGING VIRAL INFECTIONS UNDER THE CLIMATE CHANGE IN HUNGARY

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Modeling of the climate change in Germany suggests that the probable rise in temperature up to 2100 will be between 1.4 and 5.8 °C. In a number of cases, Mediterranean, subtropical and tropical infectious diseases were introduced or transmitted by vectors in recent years. 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. Indirect immunofluorescence (IIF) with BIOCHIP slides (Euroimmun Med. Lab. AG., Lübeck, Germany), and PCR and RT-PCR methods have been introduced for the screening of blood donors and different risk groups. In this abstract we summarize the antibody results achieved with IIF. 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 persons and IgG antibodies by 21, 17, 16 and 11 persons. 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 persons, while IgG was negative. The intensification of migration, the growth in density of the population, the susceptibility to infectious diseases, the decline of human immunity in consequence of insolation (UV effect), and incorrect nutrition, all tend to make humanity sensitive to infectious illnesses. Prevention, early diagnosis and treatment are important to counter local endemics and epidemics.

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## DETECTION OF *FUSOBACTERIUM NUCLEATUM* IN SALIVARY SAMPLES OF HEAD AND NECK CANCER PATIENTS BY REAL TIME PCR

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Recently, *Fusobacterium nucleatum* was implicated in colon carcinogenesis. Because *F. nucleatum* is a member of the oral flora and our earlier observations indicated that there was an association of *F. nucleatum* with oral carcinomas, we explored the possibility to evaluate *F. nucleatum* as a potential salivary biomarker of head and neck carcinomas (HNCCs). Unstimulated saliva samples were collected from patients with head and neck carcinomas (N=19) and healthy controls (N=21)

and the corresponding biopsy samples were processed for pathological examination. DNA samples isolated from saliva were amplified using *F. nucleatum*-specific primers by real-time PCR and analysed in a StepOne (LifeTechnologies) instrument.
Quantitation was made based on Ct valuses compared to a standard curve. The relative number of *F. nucleatum* DNA copies was higher in the salivary samples of HNCC patients than in controls, although the difference was not statistically significant. Further studies are necessary to clarify the role of *F. nucleatum* in the generation of HNCC.

## DETECTION OF PLASMID-MEDIATED QUINOLONE RESISTANCE DETERMINANTS IN ESBL PRODUCING ENTEROBACTERIACEAE STRAINS

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Increasing number of quinolone resistant strains are detected among clinical isolates. The mechanism of resistance is explained by mutations in the target molecules of fluoroquinolones and by plasmid-mediated quinolone resistance determinants (PMQR). The PMQRs include Qnr proteins (QnrA, QnrB, QnrC, QnrD and QnrS), aminoglycoside acetyltransferase (6')-Ib-cr enzyme and efflux pumps (OqxAB, QepA). The PMQRs lead to low-level quinolone resistance and facilitate the selection to higher level of resistance. The detection of PMQRs is difficult but their presence may lead to therapy failure. Our aim was to determine the occurrence of the qnr genes in Enterobacteriaceae strains. Microbiological data of the patients treated at intensive care units of Semmelweis University were analysed between 2010-2014 and isolates with extended spectrum beta-lactamase enzyme (ESBL) production from blood culture were choosen for further investigation. Identification of isolates was performed by MALDI-TOF. Minimal inhibitory concentrations (MIC) were determined by microdilution method for ciprofloxacin, levofloxacin and moxifloxacin after the EUCAST recommendations. The presence of *qnr*A, *qnr*B, *qnr*C, *qnr*D and *qnr*S genes were detected by multiplex and simplex PCR. In our study a total of 108 strains were included namely, 52 *Escherichia coli*, 55 *Klebsiella pneumoniae* and one *K. oxytoca*. MIC values showed that 42 *E. coli* (38.88%) and 51 *K. pneumoniae* strains (47.22%) were resistant to ciprofloxacin, 41 *E. coli* (37.96%) and 47 *K. pneumoniae* strains (43.51%) were resistant to levofloxacin, and 88 strains including 42 *E. coli* (38.88%) and 48 (44.44%) *K. pneumoniae* were resistant to moxifloxacin. *K. oxytoca* was susceptible to ciprofloxacin and levofloxacin and showed reduced susceptibility to moxifloxacin. The overall coverage of qnr genes was 9.25%, as 10 strains 2 *E. coli*, 7 *K. pneumoniae* and one *K. oxytoca* carried one of the determinant. Two strains (one *E. coli* and one *K. pneumoniae*) were found positive for *qnr*D and eight strains (one *E. coli*, 6 *K. penumoniae* and one *K. oxytoca*) carried *qnr*S. No *qnr*A, *qnr*B and *qnr*C were detected. All *qnr* positive *E. coli* and *K. pneumoniae* strains were resistant to ciprofloxacin (≥64 mg/L), levofloxacin (≥ 4 mg/L) and moxifloxacin (≥ 64 mg/L) with high MIC values according to the EUCAST guideline. The ciprofloxacin, levofloxacin and moxifloxacin MICs of *K. oxytoca* were < 0.25 mg/L, < 0.25 mg/L and 1 mg/L, respectively.
Our data show similar coverage as other European studys. In our investigation we found that qnr determinants are present in fluoroquinolone susceptible and resistant Enterobacteriaceae strains and we should be aware of their presence as they may cause therapy failure.

## CHANGES IN THE DISTRIBUTION OF ASYMPTOMATICALLY CARRIED ESBL-PRODUCING ENTEROBACTERIA AND THEIR ESBL GENES AMONG HEALTHY INDIVIDUALS

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This study aims at making a survey on the faecal carriage rate of ESBL producers among healthy individuals and compare it to our previously results obtained on the same population few years earlier. The stool samples from 1004 healthy individuals (779 from individuals screened for employment purposes and 225 from applicants for long-term care (LTC) facilities were analyzed from November 2013 and May 2014 and were compared with the previous results in 2009-10 on the same populations. Isolates were recovered on eosin methylene blue agar supplemented with 2 mg/l cefotaxime and identified by MALDI Biotyper and were confirmed by species-specific PCRs. ESBL genes, integron content of ESBL producers as well as phylogenetic analysis of *Eschericia coli* isolates were analyzed by PCRs. The rate of fecal carriage of ESBL-producing isolates dropped from 7.2% to 5.3% among applicants for LTC, while the rate was consistent in employment screening group (2.0% vs 2.3%). CTX-M-15 type ESBLs were more frequently detected in screening group in the present study (77.8% vs 22.7%; $p<0.001$). Class 1 integron occurrence was similar in both studies (40.6% vs 55.6%). Carriage of integrons were associated with ant(3")-Ia and aph(3')-Ia genes in both studies. Phylogenetic group A (associated with commensal strains) were more represented among strains from screening group in the former study (63.6% vs 27.8%), while in the present study, group B2 (associated with extraintestinal pathogenic strains, 44.4% vs 9.1%; $p<0.001$) were the most represented among strains from the same population. Furthermore all the *E. coli* strains with phylogenetic group B2 belonged to the clone ST131.
The present study highlights the change of commensal *E. coli* strains to extraintestinal pathogenic strains in the community over time (as it happened in several countries). The high rate of CTX-M-15-producing *E. coli* in the screening group may show the increase and further maintenance of these enzymes in the community settings and suggest easy spread of them. These healthy humans could act as resistance gene reservoirs as well as outbreak sources.

## DOES CORE ENVIRONMENTAL STRESS RESPONSE EXIST IN *ASPERGILLUS NIDULANS*?

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Genome-wide transcript changes caused by different stress conditions including 5 oxidative stress - menadione sodium bisulfite (MSB), $H_2O_2$, t-butylhydroperoxide (tBOOH) and diamide - and salt stress (NaCl) treatments were studied in *Aspergillus nidulans* cultures. We intended to address the question whether the core environmental stress response exist or not in this important model organism. Possible connections between environmental stress response and cross-stress protection

phenomena were also studied. Genes showing unidirectional expression changes under MSB, tBOOH and dimaide stress (the three stress conditions inducing very similar stress responses regarding the number of regulated genes) were defined as core oxidative stress response (COSR) genes. Although the numbers of COSR genes were high (873 genes) the subsequent inclusion of either $H_2O_2$ or NaCl stress treatment data sets into the analysis reduced markedly the number of core stress response genes. Only 13 genes showed unidirectional transcriptional changes in all stress exposure experiments. While the number of co-regulated genes was small, the number of genes regulated exclusively by one certain type of stress was well above 1,000. This together with the low pairwise correlation found between transcriptome profiles suggests that the different stressors had characteristic stress-specific effects on the transcriptome. Cross-stress adaptation experiments demonstrated that pre-treatments of the cultures with low concentrations of either $H_2O_2$ or diamide decreased the growth inhibitory effects of MSB and pre-treatments with a low concentration of MSB decreased the growth inhibitory effect of NaCl, suggesting the possible presence of acclimation or adaptive stress response. In conclusion, our data do not support the existence of the core environmental stress response in *A. nidulans*.
We assume that the observed co-regulations were most likely the consequences of the overlapping physiological effects of the stressors especially in the case of severe stress treatments. Our study also supports the view that molecular processes independent of the environmental stress response can explain the cross-stress protection phenomena in *A. nidulans*.

## INVESTIGATING THE ROLE OF ATFA IN CONTROLLING STRESS RESPONSES IN *ASPERGILLUS NIDULANS*

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AtfA is a b-Zip transcription factor of *Aspergillus nidulans* which plays a key role in regulating stress responses in this filamentous fungus. To gain insight into the regulatory function of AtfA, genome-wide mRNA changes caused by different stresses in the presence or absence of the functional *atf*A gene were investigated. The lack of *atf*A affected mRNA accumulation of more than 1200 genes under unstressed conditions. High number of genes (more than 1500 genes) also showed AtfA-dependent regulation under menadione sodium bisulfite (MSB) stress. In contrast, the transcriptome profiles of the *Δatf*A mutant and the control strains were quite similar under NaCl, t-butylhydroperoxide (tBOOH), diamide and $H_2O_2$ treatments. Stress-dose dependent activation of Atf1 was not detected in the presence of $H_2O_2$. Several GO terms related to stress signaling and regulation ("phosphorelay signal transduction system", "regulation of protein phosphorylation", "calcium ion transmembrane transport" as well as "response to stimulus") were typical of the group of down-regulated genes in the *Δatf*A mutant under unstressed conditions. According to these findings, we hypothesize that the main function of AtfA is coordinating the activity of certain regulatory genes under both stress and unstressed conditions. Due to the networking nature of signaling, the lack of AtfA was likely compensated by functions of other regulatory proteins under $H_2O_2$, tBOOH, diamide, NaCl stress, which resulted in transcriptional profiles similar to those observed in the control strain. In case of MSB or in the absence of any external stress stimuli, the

signaling network was unable to substitute AtfA satisfactorily, which resulted in serious disturbances in cell homeostasis and, subsequently, altered the transcription of a large group of genes. Further research is in progress to identify the AtfA target genes responsible for the efficient stress response under MSB stress.

## EXAMINATION THE BIOFILM FORMATION OF *LISTERIA* SPECIES AND *PEDIOCOCCUS ACIDILACTICI*

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*Listeria monocytogenes* is an ubiquitous food-borne human pathogenic bacterium that has a significant public health risk. Nowadays there is an increasing consumer demand for less treated food products that may allow the growth of *L. monocytogenes*. Hence the food manufacturers interesting in natural antimicrobial substances like bacteriocins produced by lactic acid bacteria. However, the European Food Safety Authority was reported the occurrence of *L. monocytogenes* was increased in fermented food products. The aim of our study was examined the co-growth of *Listeria* species and lactic acid bacteria in biofilm formation in different model and natural media. *L. monocytogenes* L4 (isolated from milk industry), *L. monocytogenes* 1486/1 (from cheese), *L. monocytogenes* 971 (from hamburger), *L. innocua* CCM 4030, *Pediococcus acidilactici* HA 6111-2 (isolated form "alheira") were investigated. Stainless steel coupons were substrates used for biofilm formation, since this material is frequently used for the manufacture of food processing equipment. Different matrices (model media: BH and MRS; milk; and minced meet) selected for examine the biofilm formation, those food matrices able to support the growth of *Listeria* spp. and lactic acid bacteria. It was obtained *L. innocua* CCM 4030 could overgrow *L. monocytogenes* L4 during biofilm formation. This phenomenon could be a beneficial for the food industry, since the *L. innocua* is not human pathogen bacteria. When *L. monocytogenes* was inoculated together with lactic acid bacteria it was clearly demonstrated the *P. acidilactici* HA6111-2 inhibit the *L. monocytogenes* 1486/1 and 971 in model media and minced meat biofilms. The *P. acidilactici* was able to produce a high amount of antilisterial bacteriocin in biofilm. That could be an explanation why *P. acidilactici* HA 6111-2 could inhibit the growth of *L. monocytogenes* strains. It was also demonstrated *P. acidilactici* HA6111-2 was not able to produce bacteriocins in milk when *L. monocytogenes* was also present. Consequently, the *L. monocytogenes* 1486/1 and 971 could overgrow the *P. acidilactici* HA 6111-2. Our data suggest in the food industry we can inhibit the biofilm formation of *L. monocytogenes* by lactic acid bacteria, but it is depend on the food matrices. Further examination needed to extend this conclusion to the other lactic acid bacteria and to define what is the exact reason when *L. innocua* inhibit *L. monocytogenes*.

## DEVELOPMENT OF A QPCR BASED METHOD FOR THE QUANTITATIVE MEASUREMENT OF *CHLAMYDIA TRACHOMATIS* GROWTH

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*Chlamydia trachomatis* urogenital serovars are obligate intracellular bacteria involved in acute and chronic pelvic inflammatory diseases. Testing of antimicrobial compounds and cellular elimination mechanisms is an important task, but the enumeration of chlamydial growth with fluorescent microscopy is labour intensive, low-throughput and subjective. HeLa cells were infected with *Chlamydia trachomatis* serovar D at various MOIs ranging from 32 to 0.008 in the presence and absence of antibiotics moxifloxacin, tetracycline and the novel antimicrobial compound PCC00213. The extracted DNA was used as a template in a qPCR reaction to measure the relative change in *Chlamydia* genome copy numbers. The parameters of the qPCR reaction were optimized including optimization of the primers, the annealing temperature and the testing of various qPCR master mixes. Our qPCR method was able to detect the applied MOI range in a 4 log scale, with high correlation between the biological and technical replicates.
qPCR based growth quantitation revealed that the MICs of moxifloxacin, tetracycline and PCC00213 were 0.031 μg/ml, 0.031 μg/ml and 6.2 μg/ml respectively, identical or close to the already published MIC values. Further testing of other already described antichlamydial antibiotics and novel compounds is being performed. The qPCR based method for determination of chlamydial growth and antibiotic MIC values is less time-consuming, more objective and orders of magnitude more sensitive than the manual or automatic fluorescent microscopy based methods.

## TRACKING THE ENRICHMENT OF FE(III)-REDUCING BACTERIA IN TWO SLIGHTLY DIFFERENT ENRICHMENT MEDIA

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*Rhodoferax* species and other Fe(III)-reducing microbes, like members of the genus *Geobacter* are frequent members of oxygen-limited, BTEX contaminated subsurface environments. It is well known that some *Geobacter* species harbor benzylsuccinate synthase (*bss*A), the key gene of anaerobic degradation of toluene, while some *Rhodoferax* species might have a role in the microaerobic degradation of aromatic compounds and might harbor subfamily I.2.C-type catechol 2,3-dioxygenase (C23O) genes. The main objective of this study was to enrich and identify the abovementioned Fe(III)-reducing microbes and understand they role in the degradation of BTEX compounds. Groundwater sample from the Siklós BTEX contaminated site was incubated anaerobically for two weeks to decrease the number of strict aerobes. After incubation enrichment media were inoculated with groundwater samples under anaerobic circumstances. Since we did not find obvious methods in other studies for the enrichment of *Rhodoferax* species, two slightly different kinds of enrichments were set up. In both cases phosphate buffer based acetate enrichment media were supplemented with trace element solution and vitamins. Fe(III)NTA was added to the media to provide Fe(III) as electron acceptor. The only difference between the two enrichment media applied was the type of the magnesium source: in type I enrichment $MgCl_2$ was used, while in type II enrichment $MgSO_4$ was added to the medium. Thus, in the latter case $SO_4^{2-}$ was also present in the medium as an available electron acceptor. Whit this enrichment setup we aimed to answer two questions: (i) does the presence of $SO_4^{2-}$ as sulfur source enhance the stable enrichment of *Rhodoferax* species, and (ii) does the presence of $SO_4^{2-}$ increase the diversity of enriched

bacteria. T-RFLP and 16S rRNA clone libraries were used to track the enrichment of Fe(III)-reducing bacteria in the different enrichments during 5 consequtive transfers. It was found that the composition of the initial bacterial community considerably changed during the enrichments and after 3 consequtive transfers the enrichment communities became to be stable. Dominance of *Geobacter psychrophilus, Romboutsia lituseburensis* and a *Sunxiuqinia* related bacterium was observable in both enrichment cultures. Furthermore *Clostridium thiosulfatireducens* and *Pelosinus fermentas* related species in case of the $MgCl_2$-amended enrichment, while *Pseudomonas azotoformans* in the $MgSO_4$-amended medium were also dominant. Unfortunately, selective enrichment of *Rhodoferax* species was not successful.

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## IDENTIFICATION AND EXPRESSION ANALYSIS OF A SECOND LACTOSE PERMEASE THAT COMPLETES THE UPTAKE OF THIS SUGAR IN *ASPERGILLUS NIDULANS*

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Lactose (milk sugar) is the main carbohydrate in whey which for most micro-organisms is a gratuitous carbon source that is slowly assimilated. To optimize fermentation processes that employ whey residue, we study lactose catabolism in the genetic model *Aspergillus nidulans*, a soil-borne saprophyte fungus. Recently, we characterised the lactose permease LacpA, responsible for a considerable part of the uptake in *A. nidulans*, and showed that uptake rather than hydrolysis is the limiting step in its lactose catabolism. We have now identified a second physiologically relevant lactose transporter, LacpB. Mutants deleted for both *lacp*A and *lacp*B only appear to take up minute amounts of lactose and mycelia of the double deletant strains are unable to produce new biomass from lactose. Although transcription of both *lacp* genes was strongly induced by lactose in pregrown wild-type mycelia, their inducer profiles differ markedly.

*lacp*B responded also strongly to beta-linked glucopyranose dimers, cellobiose and sophorose, while these inducers of the cellulolytic system did not provoke any *lacp*A response. Nevertheless, *lacp*A/B double mutants grew like wild type on cellobiose which suggests that cellobiose uptake in *A. nidulans* is mediated by mechanisms independent of *lacp*B.

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## UNCONVENTIONAL STRATEGIES FOR THE CULTIVATION OF NEW BACTERIAL STRAINS FROM AQUATIC ENVIRONMENTS

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For most prokaryotes, there are no pure laboratory cultures available, however many yet-not-cultivated bacteria are potentially culturable even with the application of cheap and simple techniques. Experiments for enhancing the cultivation of bacteria were performed using samples taken from markedly different aquatic habitats (a peat bog lake, a saline lake and a bioreactor). In situ cultivation (polyurethane foam blocks impregnated with culture media), special gelling agent (gellan gum) and special culture media (using pH, salt concentration and nutrient content values corresponding to parameters of the sample) were applied, furthermore long-term incubation was performed to support the growth of slow-growing bacteria. Based on the results of cultivation-independent analyses targeting the taxonomic composition of bacterial communities (pyrosequencing and terminal restriction fragment length polymorphism), it was shown that even incubation conditions supposed to be close to those present in nature provide strong selection pressure to bacteria. Nevertheless, pure cultures of several potential new genera and species were obtained, mainly belonging to the bacterial phyla Proteobacteria and Bacteriodetes, which supported the efficiency of the applied non-conventional cultivation methods. It has been demonstrated that with the applied strategy, up to 7.5% cultivation efficiency could be achieved.
It seems that cultivation efficiency is not only significantly affected by medium composition, but also by the type of the applied gelling agent and incubation conditions.
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## INACTIVATION OF *ENTEROCOCCUS FAECIUM* UTILIZING PEROXYACETIC ACID OR ULTRAVIOLET RADIATION

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Disinfection is considered to be the essential process for the inactivation and destruction of waterborne pathogens, in order to protect human health and also the environment. Chemical disinfectants such as chloramines, chlorine dioxide etc. and especially chlorine are commonly used for drinking water disinfection as well as disinfection of various wastewaters such as from procedures for laundering hospital textiles because of their low cost and ease of handling. However disinfection procedures using chlorine substances are not environmentally friendly due to the formation of carcinogenic or mutagenic by-products such as trihalomethanes and haloacetic acids, especially with waters containing organic matter such as laundry wastewater; therefore alternative disinfection procedures such as UV radiation, ozonation, chemical decontaminants such as oxidizing agents etc. need to be investigated. In this study two disinfection processes were used to determine the disinfection effect of water and hospital laundry wastewater artificially contaminated with *Enterococcus faecium*. Different concentrations of peroxyacetic acid and different exposure times with ultraviolet radiation were tested on inoculated water and wastewater. The number of cfu after incubation on kanamycin agar base was determined for each experiment. It was found that 70 mg/L PAA was sufficient to reach a 5-log10 reduction within 35 min treatment for hospital laundry waste water, whilst 5 min treatment time was reached by 110 mg/L PAA. 80 mg/L ensured a 5-log10 reduction after 15 min treatment time. For water inoculated with *Enterococcus faecium* 80 mg/L PAA was sufficient to reach a 5-log10 reduction within 5 min. However minimal recovery was noted

and subsided after 40 min, thus proving that the hospital laundry wastewater already contained inhibitory substances shortening the necessary treatment time.
Ultraviolet radiation for 1½ hours also proved to be efficient for hospital laundry wastewater with inhibitory substances preventing any dark repair after 18 hours. Thus indicating that such method could be used for laundry wastewater or similar water that is stored overnight and then reused, if scale-up and cost-effective studies prove to coincide with these results.

## HAS THE USE OF FLUOROQUINOLONES CONTRIBUTED TO THE WIDESPREAD DISSEMINATION OF THE MAJOR INTERNATIONAL CLONES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND EXTENDED-SPECTRUM B-LACTAMASE-PRODUCING *KLEBSIELLA PNEUMONIAE* IN THE HEALTHCARE SETTING?

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We recently demonstrated that fitness cost associated with resistance to fluoroquinolones is diverse across clones of both methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* in adult hospital wards. We observed that major clone MRSA and ESBL-producing *K. pneumoniae* strains with high-level resistance to fluoroquinolones retain their fitness while the vitality of other *S. aureus* and *K. pneumoniae* isolates become compromised during the development of resistance to these antibiotics [1, 2]. This mechanism could have contributed to the widespread dissemination of both pathogens, a notion subsequently confirmed by Knight et al. [3] investigating the dynamics of MRSA clones in a British hospital. In addition, our findings were propped up by the observations of Holden et al. [4] and Lawes et al. [5] studying the clonal shifts of MRSA in England and Scotland, respectively. Our results demonstrating a link between resistance to fluoroquinolones and clonality strongly suggest that a more judicious use of these antibiotics would significantly decrease the rates for both MRSA and ESBL-producing *K. pneumoniae*; a concept supported by most of the related literature [6].

[1] Horváth, A. et al. (2012) Eur J Clin Microbiol Infect Dis 31, 2029-2036.
[2] Tóth, Á. et al. (2014) Eur J Clin Microbiol Infect Dis 33, 837-843.
[3] Knight, G.M. et al. (2012) J Antimicrob Chemother 67, 2514–2522.
[4] Holden, M.T. et al. (2013) Genome Res 23, 653-64.
[5] Lawes, T. et al. (2015) British Med J Open 26, e006596.
[6] Füzi, M. (2014) Acta Microb Immunol Hung 61, 399-405.

## GENETIC DIVERSITY OF PIGEON CIRCOVIRUSES IN HUNGARY

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The pigeon circovirus (PiCV), a member of the genus *Circovirus* (family Circoviridae), is thought to play a crucial role in the so-called young pigeon disease syndrome, a multifactorial disease. The general signs include lack of appetite, weight loss, diarrhea, respiratory problems, and decreased lymphocyte count. PiCV is known to have immunosuppressive effect. Isolation or *in vitro* replication

of the virus is not solved yet, and no specific prevention methods are available either. The objective of this work was to study the genetic diversity of PiCVs in Hungary. Organ samples from dead domestic (racing, meat and fancy) pigeons, originating from flocks in Jász-Nagykun-Szolnok County, were screened by PCR. Additionally, two feral pigeons, found dead in Budapest, as well as cloacal excrements collected from live birds of a couple of other columbid species were examined. A consensus nested PCR, described for the amplification of a fragment from the gene (rep) of the replication associated protein, was used for the initial screening. The positive samples were further investigated with another PCR, suitable for the acquisition of the entire gene (cap) of the capsid protein. The nucleotide (nt) sequence of the PCR products was defined and, based on these, specific PCR primers to amplify the missing genome parts were designed. The newly obtained sequences were assembled, annotated and compared by phylogenetic calculations to previously published PiCV sequences. All live-bird samples gave negative results, while seven dead pigeons were found to be positive. Six full circular genomes were assembled; these are the first complete PiCV sequences from Hungary. In one rep-positive sample, both the cap-targeting, and the invers PCRs gave negative results. The full genomes showed 94−99% nt identity when compared to each other and to sequences retrieved from the GenBank. Phylogeny inference by Maximum Likelihood (ML) method, based on the cap sequences, confirmed the existence of five genetic groups as described previously. Interestingly, a sixth lineage, formed by two new cap sequences, also appeared. The topology of the full-rep-based ML tree was markedly different showing a divergent grouping.

Our results not only increase significantly the number (around 60 worldwide) of complete PiCV genomes but also provide novel data about the pronounced genetic variability of these viruses, i.e. every genome seems to be unique though the majority of the usually slight nt sequence differences do not always result in amino acid changes. Furthermore, these results confirm the previously published presumptions that the two main PiCV genes follow different evolutionary pathways and recombination events may occur frequently. All domestic pigeon samples, found positive in this study, originated from diseased birds that subsequently died. This underscores the direct or indirect pathological role and immunosuppressive effect of PiCV.

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## REGULATION, FUNCTION AND STRUCTURE OF VIRULENCE FACTORS OF *CANDIDA PARAPSILOSIS*

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*Candida parapsilosis* is an emerging human pathogen that has dramatically increased in significance and prevalence over the past two decades such that *C. parapsilosis* is now the second most common cause of invasive candidal disease worldwide. Individuals at highest risk for severe infection include neonates and patients in intensive care units. *C. parapsilosis* infections are especially associated with the contamination of hyperalimentation solutions, prosthetic devices and indwelling catheters, as well as nosocomial spread of disease through the hands of healthcare workers. Factors involved in disease pathogenesis include the secretion of hydrolytic enzymes, adhesion to prosthetics, and biofilm formation.

New molecular genetic tools provided the opportunity to gain much needed information regarding *C. parapsilosis* virulence. Recent results opened a new avenue toward a deeper understanding of *C. parapsilosis* pathogenesis that will definitely facilitate the development of new therapeutic approaches for treating *C. parapsilosis* infections.

The lecture will focus on the molecular biology of pathogenesis, virulence factors, genetics, clinical manifestations, epidemiology and antimicrobial susceptibility of *C. parapsilosis* for the goal to give a broad and up to date overview for this key pathogen.

## REVERSAL OF MULTIDRUG RESISTANCE BY SELENOESTER DERIVATIVES

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Multidrug resistance is a major factor in the failure of many forms of chemotherapy. One of the most important mechanisms of MDR is the over-expression of energy-dependent ATP-binding cassette (ABC) transporters. It has been described that compounds with selenium moiety have anticancer and chemopreventive properties. The aim of this study was to investigate novel selenoester derivatives with different physico-chemical characteristics and to evaluate their anticancer activity on multidrug resistant mouse T-lymphoma cells. The cytotoxic activity of the compounds was assessed by MTT method, the inhibition of the MDR transporter P-glycoprotein (ABCB1) was studied by rhodamine 123 accumulation assay using flow cytometer. The apoptosis inducing properties of the compounds were determined by Annexin V-FITC detection kit. Out of the eleven compounds investigated in this study, four derivatives showed remarkable anticancer activity in MDR mouse T-lymphoma cells. Compounds EDA-1, EDA-71, EDA-73, and EDA-74 had potent cytotoxic activity, they could inhibit the activity of the ABCB1 transporter by increasing the amount of rhodamine 123 accumulated inside of the cells. Furthermore, these compounds could induce apoptosis and the most potent compound was EDA-1, which induced early apoptosis in 32.2% of the gated cell population. We can conclude that selenoester derivatives are potent anticancer agents based on the type of the functional groups. The most active molecule was benzo[c]selenophen-1,3-dione (EDA-1), which has a cyclic selenoanhydride moiety.
In addition, the compounds which presented a ketone group in the alkyl moiety bounded to the selenium atom also had potent anticancer activity (EDA-71, -73, -74). On the other hand, the replacement of this ketone group by an amide (EDA-117), an ester (EDA-45, -93) or an unsubstitued methyl group eliminates anticancer properties, and the capacity to induce apoptosis.

## INVESTIGATING THE YEAST MICROBIOTA OF THE BADACSONY WINE REGION: ISOLATION AND CHARACTERIZATION OF INDIGENOUS "TERROIR" STRAINS

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For centuries, wine has been produced by the spontaneous fermentation of grape juice carried out by indigenous yeast and bacterial species. In the past two decades, the increasing demand for the mass production of wines with similar characteristics has changed the production technique in large-scale wineries, and spontaneous fermentation has been replaced by pure culture fermentations. In order to avoid the risk of slow or incomplete fermentation, industrial wineries use commercial yeast strains ("starters") to ferment wine, as they guarantee product uniformity. The wide-spread application of a limited number of "starter" cultures, however, led to the loss of complexity in the finished product, and as a result, recently there is a growing market for the production of "organic wines" fermented by indigenous yeast strains that are better adapted to the local wine-producing conditions and techniques. In the present study we collected data about the biodiversity in the selected vineyard (Badacsonytomaj, RIVE-NARIC) and established a collection of indigenous yeast strains that could be implemented in the development of "terroir" starter cultures to be used in the production of "organic wines". Grape berries from cultivars "Kéknyelű" and "Szürkebarát", grape juice, and must samples from different phases of wine fermentation were collected from the vineyard before and during harvest (2014). Environmental yeast flora was also sampled from cane, soil and winery facilities. Different enrichment and selection methods were applied for the isolation of yeasts. The isolated yeast strains were categorized by their morphological, biochemical and physiological characteristics. Classical microbiological taxonomy tests included the examination of colony and cell morphology, utilization of carbon and nitrogen sources. Further metabolic/physiological properties, such as fermentation capacity, tolerance level to ethanol, acetic acid, glucose (osmotolerance) and acid production were also investigated prior to the categorization of the 528 yeast isolates. A representative number of isolates from each category was identified by molecular biology methods. Genomic DNA was extracted from 99 yeast isolates and subjected to PCR specific for the D1/D2 and the internal transcribed spacer (ITS) regions of rRNA gene. The size and nucleotide sequence of ITS4-ITS5 locus, which has been reported to result the highest probability of correct species identification for the broadest range of fungi, allowed the identification of the following genera/species: *Saccharomyces cerevisiae, S. pastorianus, S. uvarum, Candida zemplinina, Hanseniaspora uvarum, Metschnikowia* spp., *Pichia* spp., *Rhodotorula* spp., *Cryptococcus* spp., *Sporidiobolus* spp. The nucleotide sequences of 31 starter strains were also determined in the ITS4-ITS5 region and included in our database. PCR amplifying the inter-delta regions of the genome, which has been shown to be highly variable in terms of number, distribution and size between strains, was applied to identify the isolates below the species level. "Starter" strains were involved in the inter-delta specific PCR as controls. Restriction fragments length polymorphism of mitochondrial DNA, microsatellite specific PCRs, and other molecular typing techniques that generate DNA profiles and could help distinguish isolates are currently under evaluation.

# SILVER-SUSCEPTIBILITY OF MULTIDRUG RESISTANT NOSOCOMIAL GRAM-POSITIVE AND GRAM-NEGATIVE PATHOGENS

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Surfaces contaminated with multidrug resistant bacteria contribute to the transmission of hospital pathogens and can be the sources of nosocomial infections and outbreaks. Many types of antimicrobial finishing agents are available which are able to destroy the microorganisms on abiotic surfaces. Silver compounds are among the most popular ones in hospitals and also in daily life. Silver cations are usually microbicidal at low concentrations without adverse effects for humans. Silver compounds are used to treat burns, wounds and ulcers, to coat indwelling medical devices to inhibit microbial colonisation and biofilm development. Our goal was to investigate the silver susceptibility of the most important multidrug resistant nosocomial pathogens and the effect of continuous exposure to subinhibitory concentrations of silver-ions. We examined 15 strains each of VRE (i.e., vancomycin-resistant *Enterococcus faecium*), MRSA (i.e., methicillin-resistant *Staphylococcus aureus*), MACI (i.e., multidrug-resistant *Acinetobacter baumannii*), and MRKP (i.e., multidrug-resistant *Klebsiella pneumoniae*) collected from different areas of Hungary between 1998 and 2014. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined for $AgNO_3$ solution and Sanitized T27-22 Silver liquid (2 m/m% AgCl and 8 m/m% $TiO_2$) by broth microdilution method. In the case of two selected *Klebsiella pneumoniae* strains (OEK-3 and OEK-4) passage experiments were performed by continuous exposure to increasing concentrations of $AgNO_3$ and Sanitized T27-22 Silver liquid. In case of Sanitized T27-22 Silver liquid average MIC and MBC values were 5.5 and 9.9 mg/L for MRSA; 5.5 and 10.9 mg/L for VRE; 10.0 and 15.1 mg/L for MACI; 9.9 and 16.7 mg/L for MRKP isolates, respectively. Average MIC and MBC of $AgNO_3$ were 5.4 and >947.4 mg/L for MRSA; 4.9 and >298.4 mg/L for VRE; 3.9 and 4.4 mg/L for MACI; 4.9 and 6.6 mg/L for MRKP strains, respectively. The Sanitized T27-22 Silver liquid proved to be bactericide regarding all examined pathogens, but Gram-negative strains can tolerate significantly (P=0.001, One-Way ANOVA model) higher concentration than Gram-positive ones. $AgNO_3$ was bactericide against Gram-negative bacteria, this is consistent with other investigations. Although, the MIC values of Gram-positive strains were similar to Gram-negatives, the agent is proved to be bacteriostatic against 53% and 93% of MRSA and VRE isolates, respectively. We found one VRE, and one MRSA strain, which are able to survive 1700 and 6800 mg/L concentration of $AgNO_3$ without any selection, respectively. After the passage experiments the MIC values of strain OEK-4 were not elevated. The OEK-3 derivative isolates had >32 and >2560 times higher MIC values than the parent strain in case of Sanitized liquid and $AgNO_3$, respectively.

Moreover, OEK-3 derivative isolates retained their ESBL-production during induction of silver resistance. Several studies have reported successful passage experiment to induce silver-resistance for Gram-negative bacteria, but to the best of our knowledge, no such increase of the $AgNO_3$ MIC value of multidrug-resistant *Klebsiella pneumoniae* strain has been described. According to our investigation performed so far, there are multidrug resistant nosocomial pathogens also in Hungarian hospitals, which are capable to resist different silver compounds.

## THE PREVALENCE OF HUMAN PAPILLOMA VIRUS AND TORQUE TENO VIRUS IN HEAD AND NECK CANCER PATIENTS IN SOUTH-EAST HUNGARY

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Fourty saliva- and related tumor samples of patients diagnosed with head and neck cancer (HNCC) as well as samples of healthy individuals attending the University of Szeged Faculty of Medicine and Faculty of Dentistry were collected and tested with PCR method for the presence of Human Papilloma Virus (HPV) and Torque Teno Virus (TTV). Typing of HPV positive samples was done by sequencing of the GP5+/GP6+ primer product. The aim of our work was to evaluate the prevalence rates of these viruses in head and neck cancer patients and compare them to those of healthy individuals. In the same time we wanted to evaluate the feasibility of a non-invasive, saliva-based method for the detection of these viruses. The overall TTV prevalence was higher among patients diagnosed with HNCC than in the control group. In case of HPV the prevalence rate in HNCC patients was low, suggesting that traditional risk factors including smoking and alcohol consumption may play a role in the etiology of HNCC in South-East Hungary.

## FIRST STEP OF SPECIATION OR SIMPLY INTRASPECIES DIVERSITY? TWO PIGMENT-PRODUCING YEAST "SPECIES" SHOW NO DIFFERENCES IN PHYSIOLOGY OR SINGLE-COPY GENE SEQUENCES

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Several studies have dealt with the antagonistic effects of the pulcherrimin-producing members of the genus *Metschnikowia* against filamentous fungi since this effect can be used for bioprotection of fruit against postharvest diseases. Many such strains were identified within this genus. They were found to be variable with respect to nutrient utilisation and other physiological characteristics which are generally used for taxonomic identification of yeasts. Therefore, we analysed the conserved D1/D2 domains of the 26S rDNA of the isolates to determine their taxonomic affiliation. Surprisingly, the analyses could not assign the isolates to species. Subsequent experiments showed that in the genomes of certain isolates and of the type strains of the pulcherrimin-producing *Metschnikowia* species repeats of this domain were highly diverse and the alleles of the strains did not form distinct groups. As these results indicated that the species boundaries are uncertain, it is also not surprising that the type strains can not be distinguished on the basis of three protein-coding genes (ACT1, RPB2, EF2) that are commonly used in fungal phylogenetics either. However, the mtDNA-RFLP profiles of the types were different. The length and number of chromosomes were not different in the type strains of *M. fructicola* and *M. andauensis*. These data suggested that the

isolation boundary between these *Metschnikowia* species is not complete and the species may be able to hybridise with each other. To clarify this, we crossed the type strains by generating mutants auxotrophic for different amino acids. Then we cultured the mutants in mixed cultures to allow their cells to mate. From these cultures we selected prototrophic clones as putative hybrids. The formation of prototrophs confirmed the idea that *Metschnikowia fructicola* and *Metschnikowia andauensis* may not be biologically isolated species. The hybrids were characterised by RAPD analysis and tested for sporulation ability. Our results on this species group can be interpreted in two ways: either the species boundaries are arbitrary due to incipient speciation and incomplete isolation, or the whole *Metschnikowia pulcherrima* group is a single biological species with ongoing gene flow across a variety of its strains.

## COMBATING *MYCOBACTERIUM TUBERCULOSIS* WITH NOVEL COMPOUNDS: HOST CELL TARGETING STRATEGIES IN A SERIES OF *IN VITRO* AND *IN VIVO* MODELS

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Mycobacteria are intrinsically resistant to most commonly used chemotherapeutic agents. Due to its specific composition, the mycobacterial cell wall is an effective permeability barrier, generally considered to be a major factor in promoting this natural resistance. As several drugs in combination have to be used against tuberculosis (TB), and as the number of effective drugs is limited, any additional drug having also different mechanism of action to combat *Mycobacterium tuberculosis* (MTB) would be welcome. MTA-ELTE Research Group of Peptide Chemistry and our collaborators have a productive history on MTB related projects: (i) synthesis, structural and functional characterisation of peptides spanning the sequences of immunodominant proteins of MTB (ii) exploring various approaches to design and test new compounds with antimycobacterial activity [1-6]. Our strategy was mainly oriented toward (i) the synthesis of prodrug forms, combination of two active compounds in order to achieve their possible synergic interaction and preparation of new type of potential active compounds with possible new mechanismus of action (ii) *in silico* docking methods and structure-based drug design which can be used to analyze the structure of crucial bacterial enzymes for binding sites, generate candidate molecules, dock these molecules with the target and rank them according to their binding affinities (iii) increasing bioavailability through application of host cell specific carrier systems for clinically used and new antimycobacterial compounds. The *in vitro* antibacterial effect of the novel agents was determined on MTB H37Rv

and MTB MDR cultures. Agents with the lowest minimal inhibitory concentration (MIC) were optimised to get lead molecules. Considering that mycobacteria can survive in host phagocytes their elimination could be more efficient with macrophage-selective delivery/penetration of active compounds by endocytosis or via specific receptors. In order to enhance the efficacy of active agents a novel lipopeptide carrier was invented based on the sequence of tuftsin, which has been reported as a macrophage-targeting molecule. The new conjugates directly killed intracellular MTB and showed much greater efficacy than the free antimycobacterial compounds themselves. To further improve bioavailability, the conjugates were encapsulated into poly(lactide-co-glycolide) (PLGA) nanoparticles and tested *in vivo* in a guinea pig infection model. External clinical signs, detectable mycobacterial colonies in the organs and the histopathological findings substantiate the potent chemotherapeutic effect of orally administered conjugate-loaded nanoparticles.

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[1] Horváti K. et al. (2014) Bioconjug Chem 25, 2260-2268.
[2] Horváti K. et al. (2015) Tuberculosis (Edinb) doi: 10.1016/j.tube.2015.02.026.
[3] Kiss É. et al. (2014) Colloids Surfaces A 458, 178-186.
[4] Krátký M. et al. (2015) Bioorg Med Chemistry 23, 868-875.
[5] Baranyai Zs. et al. (submitted, Ref. No.: EJMECH-D-15-00060).
[6] Horváti K. et al. (2012) Bioconjug Chem 23, 900-907.

## DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* SENSITIZATION USING A MODIFIED INTERFERON-GAMMA RELEASE ASSAY

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Despite the worldwide availability of vaccination, diagnosis and drugs, tuberculosis (TB) is still a major global health problem. It is estimated that more than one-third of the world's population is infected with the causative agent, *Mycobacterium tuberculosis* (Mtb) [1]. Exposure to Mtb bacteria results in a silent, yet persistent infection (called latent TB infection, LTBI) in the vast majority [2]. Each case of LTBI carries about 5-10% lifetime risk of developing active TB and comprises a significant reservoir of future epidemics, particularly in countries with high HIV burdens. Currently applied BCG vaccination renders the TB diagnosis more difficult as the most commonly used tuberculin skin test (TST) may give false-positive result due to previous BCG immunization. Since the whole genome of Mtb [3] and several related mycobacteria has been described [4, 5], genome-wide search for loci missing from strains other than Mtb became a great tool to find specific antigens for TB diagnosis. Previous studies have demonstrated that Rv2654 protein had great potential in specific T-cell based IFN-γ release assays (IGRA) [6]. Rv2654 protein is highly specific for Mtb and absent from most of the atypical mycobacteria. In this project, detailed epitope mapping of immunodominant Rv2654 was evaluated and the p51-65 region was described as the most determinant part of the protein. Synthetic peptide, representing this region, provokes high percentage positivity in Mtb sensitized individuals in both LTBI and active TB patients. These results suggest the addition of p51-65 peptide as a boost of commercially available IGRA test

(QuantiFERON-TB Gold In-Tube, QFT) to increase diagnostic efficacy. The boosting effect of p51-65 peptide on QFT test was evaluated in HIV infected (n=50) and HIV uninfected (n=55) groups from the South African Xhosa population, where TB is endemic. In the HIV uninfected cohort, the quantitative performance of the QFT test increased significantly from median IFN-γ response of 1.8 (IQR 0.3, 7.4) to 2.8 (IQR 0.3, 12). In the case of HIV infected group, the boosting effect was not translated into a change in the responder's percentage. However, after the initiation of antiretroviral treatment, p51-65 peptide boost results a change in the frequency of persons scoring negative to positive responses (from 26 positive / 45 all tested to 29/45).

This may account for the significant increase of CD4+ T-cell number, which cell type plays a key role in antigen presentation and cytokine production.

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[1] WHO Global tuberculosis report (2013) http://wwwncbinlmnihgov/pubmed/24176622.
[2] Kaufmann, S.H. (2010) Immunity 33, 567-577.
[3] Cole, S.T. et al. (1998) Nature 393, 537-544.
[4] Garnier, T. et al. (2003) PNAS 100, 7877-7882.
[5] Li, L.L. et al. (2005) PNAS 102, 12344-12349.
[6] Aagaard, C. et al. (2004) J Infect Dis 189, 812-819.

## HIGH HYDROSTATIC PRESSURE TREATMENT OF REDUCED SALT CONTENT DRY PRODUCTS FOR REASONS OF FOOD SAFETY

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For thousands of years, humans have been consuming meat. For the same amount of time, they have also been racking their brains for the most suitable way to store and preserve meat products. The brining/drying processes, developed as a result of food industry needs, stand available to all. There is just one problem: to this day, no one has found out how to treat meat so it is at once tasty, healthy, and storage-friendly. Here salt must be mentioned, both for its positive food safety attributes, and for its numerous "bad habits", first of all, it is undesirable from the health standpoint. The goal of our activities was to discover a preservation method which allows the use of salt in reduced quantities on dry food products. Specifically, we searched for technology that ensures microbiological stabilisation of dry food products containing a reduced amount of salt. In the course of our investigations we used dry, traditionally cured sausage, a favorite product type in Hungary. The study involved the effect of high hydrostatic pressure treatment (HHP) on low salt content, traditional-method cured dry sausage. The parameters were food safety and sensory/physical characteristics. Both 450 and 600 MPa levels of pressure were applied during treatment. The results prove that with HHP technology, *Listeria monocytogenes, Salmonella* Typhimurium, and Enterobacteriaceae bacteria counts may be significantly reduced. At the same time, however, at 450 MPa pressure, the dry sausage used in the study suffered significant damage in sensory value. Judges noticed negative changes in color, shape, smell, taste, and density.

In conclusion, it may be said that reducing salt content in traditionally cured sausages leads to increased risk to food safety, while high hydrostatic pressure technology is not applicable in view of the parameters we examined. Based on positive microbiological results, we do, however, recommend tests of HHP's effect at lower pressure values.

## MOLECULAR EVENTS DETERMINING PRIMARY HIV INFECTIONS: NEW APPROACH FOR INHIBITION OF HIV ENTRY

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The initial molecular events of HIV infection, determined by gp120 and gp41 viral surface glycoproteins, and cellular receptors are with primary significance. Redox changes in these proteins are also required for successful HIV entry and infection. Redox processes active in viral entry could be potential target for treatment of HIV infection. Earlier we demonstrated significant *in vitro* anti-HIV effect of thiolated pyrimidine nucleotides (TPN) and derivatives. Our aim was to determine selective effect of these compounds on monolayer and suspension cells, non-infected and infected acutely by HIV-1. Six chemically modified derivatives of TPNs were used in concentrations ranged 5-100 micromole. Compounds were added for 24h or 48h to H9 human lymphoid cells, and on HeLaCD4+β-gal monolayer cells non-infected, or infected with HIV-1IIIB 30 min after adding compounds. Viral infectivity was demonstrated by syncytium induction assay on MT-4 T cells, and MAGI assay on HeLaCD4+β-gal cells. Cytotoxicity was determined quantitatively by XTT Assay, based on mitochondrial dehydrogenase activity in living cell. All but one compounds exhibited a certain degree of cytotoxicity. On HIV infected cells however cytotoxicity were much more pronounced: on H9 cells 23% to 50%, on HeLa cells 47% to 72%. Cytotoxic effect was dose-dependent. TPNs inhibit the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as they may interfere with the function of the essential –SH groups of CD4 cells and –SH groups in viral envelope. In our study the compounds induced a selective cytotoxic effect on HIV infected cells. HIV infection activate cells, and cellular components – including cell membrane lipid rafts – expressing increased number of –SH groups.
Mod-2 may function as an effective, new generation entry inhibitor for HIV.
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## A DEFICIENCY OF MANGANESE IONS IN THE PRESENCE OF HIGH SUGAR CONCENTRATIONS IS CRITICAL TO ACHIEVE HIGH ITACONIC ACID YIELDS BY *ASPERGILLUS TERREUS*

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Itaconic acid (IA), an unsaturated dicarboxylic acid with a high potential as a platform for chemicals derived from sugars, is industrially produced by large-scale submerged fermentation by *Aspergillus terreus*. Although the biochemical pathway and the physiology leading to IA is almost the same as that leading to citric acid (CA) production in *A. niger*, published data for the volumetric (g/L) and the specific yield (mol/mol carbon source) of IA are significantly lower than for CA. CA is known to accumulate to high levels only when a number of nutritional parameters are carefully adjusted, of which the concentration of the carbon source and of manganese ions in the medium are particularly

important. We have therefore investigated whether a variation in these two parameters may enhance IA production and yield by *A. terreus*. We show that Mn concentrations < 3 ppb are necessary to obtain highest yields. Highest yields were also dependent on the concentration of the carbon source (D-glucose), and highest yields (0.9) were only obtained at concentrations of 12 – 20% (w/v) thus allowing the accumulation of up to 130 g/L IA.

These findings show that applying the fermentation technology established for CA production by *A. niger* citric acid production to *A. terreus* should lead to high yields of IA too.

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## *STREPTOCOCCUS AGALACTIAE* ISOLATES FROM THE DERMATOLOGY CLINIC OF SEMMELWEIS UNIVERSITY, BUDAPEST

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*Streptococcus agalactiae* (GBS, group B *Streptococcus*) is one of the most important causes of neonatal sepsis and meningitis, however, it often colonises the genital mucosa of adults. Its role as a causative agent in patients with STDs is questionable, but it is interesting to see how frequently is it isolated from these patients. 96 GBS isolates were collected over a 1-year period (10.2010-09.2011) at the Dermatology Clinic of Semmelweis University, 76 deriving from the STD ambulance. The mean age of the patients was 42.4 years (ranging between 15 and 77), and the genders were equalised: 50 females and 46 males. After the thorough identification of the isolates, we determined their antibiotic sensitivity with agar dilution, and in the case of macrolide resistant strains, we also detected the erm, mef and *lin*B resistance genes. We identified the serotypes of the strains with antisera and PCR, and we detected the presence of the major surface proteins (AlpC, Rib, Alp2, Alp3 and Epsilon). The ST-17 and ST-23 hypervirulent clones were detected by PCR (*hvg*A for ST-17 and *bib*A for ST-23). MLST was performed for 4 isolates. All isolates were fully penicillin sensitive (max. MIC=0.06 mg/L). On the other hand, macrolide and tetracycline resistance proved to be a major problem. Forty isolates (41.7%) were resistant to erythromycin; 33 of these carried the *erm*B and 7 the *mef* gene, and 1 strain had *erm*B + *lin*B together. Moreover, 8 isolates carried the ermB gene, but were phenotypically macrolide sensitive. 82.3% of the isolates (n=79) were resistant to tetracycline. The majority of the isolates belonged to serotype V (28.1%), III (27.1%) and Ia (22.9%). 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, and AlpC with types Ib and II. Based on the PCR results, the vast majority (78.1%) of the strains were *bib*A positive, i.e. theoretically ST-23, however, MLST detected different ST-s in the examined 3 isolates. In one strain a 44-nucleotide deletion was present within the *atr* allele. ST-17 was identified in 21.9%, these were all serotype III, except for one case (serotype IV). For this latter isolate the MLST analysis identified ST-91; however, there is only a single nucleotide difference in the *phe*S allele compared to ST-17. Although according to the literature GBS seems to be rarely directly associated with STDs, but a heavier colonisation was sometimes detected in case of infections. If this bacterium should be eliminated, penicillin should be the drug of choice, and macrolide resistance should be checked before used as alternative therapy.

Compared to our previous findings in pregnant women, where serotype III was most prevalent, here serotype V was equally frequent. Although based on the PCR results, all the strains belonged to either ST-23 (*bib*A +) or ST-17 (*hvg*A +), MLST showed different results in case of *bib*A + strains, so this ST-specific PCR must be re-evaluated.

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## MIXED CULTURE BIOFILMS: INHIBITION WITH ESSENTIAL OILS AND THEIR MAIN COMPONENTS

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The first step of bacterial biofilm formation is the attachment of food spoilage bacteria and food-borne pathogens to different surfaces. These structures are protected by an extracellular polysaccharide-based matrix (EPS) due to which they are more resistant to antimicrobial agents and are difficult to eradicate. In the food industry they represent a continuous source of infection and can cause major economical losses by lowering the shelf life of foods. In food processing environments biofilms are commonly present in mixed cultures that are more stable than those which are formed by a single species. In a mixed-culture biofilm, complex processes like intraspecific communication, competition for resources or in some cases synergism can take place; all these can be beneficial for the formation of biofilms. *Listeria monocytogenes* in food products is a potentially dangerous pathogen responsible for large number of food-borne outbreaks. Studies show that *Listeria* can occur within a mixed culture and in some cases its growth can be induced by other species present in the biofilm matrix. For prevention of food spoilage, synthetic additives have been widely used but this practice has negative feedback from consumers. Studies have started to shift towards natural antimicrobial agents like essential oils (EOs) that are well known for their antiseptic qualities and are widely used in the food industry as spices. Based on this, our research focused on the inhibitory effect of some essential oils against the development of mixed culture biofilms formed by *Listeria monocytogenes* and *Pseudomonas putida*. After the determination of the MIC values (that was done in a previous study) MIC intervals were used for biofilm inhibition. Scanning electron microscopy (SEM) were applied to observe the structural differences between untreated and treated biofilms. These experiments revealed that EOs have good anti-biofilm forming effect against mixed culture biofilms. Cinnamon EO and its component were the best anti-biofilm forming agents but marjoram and terpinene-4-ol also showed good inhibitory effect. The characteristic structures of biofilms were not observable with SEM after treatment with marjoram; damaged cells could also be observed. From these findings we can conclude that these EOs are good candidates for inhibiting the formation of mixed culture biofilms. Therefore, they could increase shelf life of foods as natural preservatives and could be used as alternatives to chemical additives.

## HUMAN *BORDETELLA BRONCHISEPTICA* ISOLATES FROM HUNGARY

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*Bordetella bronchiseptica* is a widespread bacterial pathogen causing a number of respiratory diseases in mammals. Increasing number of human infections have been reported mostly in immunocompromised patients; occasionally the animal origins of the infections have also been established, therefore *B. bronchiseptica* is regarded as zoonotic agent. Based on latest genetic methods (e.g. MLST, genome sequencing), two distinct lineages have been distinguished within *B. bronchiseptica*. One of them is a human-adapted lineage which contains (mainly, but not only) human strains. The other, ancient lineage is animal-adapted. It includes strains from different animal host species and also a number of strains of human origin. Our previous study demonstrated that signs of host adaptation maybe observed among the strains, and host animal species (dog, cat, pig, etc.) can be determined by investigation of virulence genes. In this work, three human originated strains from Hungary were examined. Strain 1 was isolated in Budapest, 2014 from a 57-year-old, dog-owner female patient with deteriorating chronic obstructive pulmonary disease (COPD). Strain 2 was also isolated in Budapest, 2014 by pre-operative check-up from nasal secretion of a 82-year-old man who did not have any pet. Strain 3 was isolated in Szeged, 2007, but further details are unknown. The presence of four virulence factor genes (dermonecrotoxin [*dnt*], adenylate cyclase-haemolysin [*cya*A], fimbria [*fim*A] and flagellin [*fla*A]) as well as the *cya*A-replacement *ptp* gene was examined by polymerase chain reaction (PCR). PCR products of *cya*A, *fim*A, *fla*A and *ptp* were cleaved by BglI, HincII, NarI and/or SalI restriction endonucleases in restriction fragment length polymorphism (RFLP) method. On the basis of PCR-RFLP of *fim*A, the three human strains were uniform, but the results of the other genetic assays presented differences among the strains. Strain 2 and Strain 3 were identical: both strains were *fla*A- and *cya*A-positive and *dnt*- and *ptp*-negative. PCR-RFLP analysis of *fla*A- and *cya*A revealed the identity of these strains. According to our previous study, the flaA fragment pattern of Strain 2 and Strain 3 are not typical among any animal originated strain, and their *cya*A-type is equal to the *cya*A RFLP-type of the most foreign human strains. Based on these, Strain 2 and Strain 3 could be sorted into the human-adapted lineage of *B. bronchiseptica*.

On the other hand, Strain 1 had *dnt*, *fla*A and *ptp* genes, but did not possess the *cya*A gene. These properties are typical of Hungarian, dog originated *B. bronchiseptica* strains. In RFLP assays, the *fla*A and *ptp* fragment patterns of Strain 1 also showed identity with a profile of strains form dogs. Presumably, the patient carrying Strain 1 was infected by her dog.

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## EPIDEMIOLOGY AND LABORATORY DIAGNOSTICS OF *BLASTOCYSTIS* SPECIES

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*Blastocystis* species are world-wide distributed enteral protozoons capable of parasitizing both animals and humans. Isolates from human samples can be categorised into 9 subtypes (ST) based on the sequence analysis of small subunit rDNA. Mainly ST1-ST4 and ST6 cause human disease. Due to its polymorphic nature, recognition and exact identification of the pathogen require substantial practice. The aims of this study were to optimize the traditional procedures (microscopic examinations and culturing), and to develop a sensitive, specific PCR-based molecular detection method. Based on the result of 100 tested stool samples we can conclude that direct microscopic examination – because of its low sensitivity - should be supplemented by at least one culturing method. For this purpose, the Boeck-Drbohlav-Locke medium containing serum and egg without vaseline is appropriate. For development of the molecular method, DNA was isolated from *Blastocystis*-positive stool samples. A PCR method based on the primers RD5 (5'-ATCTGGTTGATCCTGCCAGT-3') specific for eukaryotes and BhRDr (5'-GAGCTTTTTAACTGCAACAACG-3') specific for the genus *Blastocystis* was developed. Sequence analysis of the amplified fragments confirmed that this optimized process is a specific and sensitive protocol with the potential to be applied for routine laboratory diagnostics. The highest positivity rate (31%) was obtained by this molecular method compared to traditional examinations (3%). Among patients with positive samples, the youngest was 2 years old, and the mean age was 35 years. The positivity rate was the highest among patients between 20 and 40 years, but the difference was not significant between genders. The main symptom was abdominal pain, and this protozoon was detected in 3 patients suffering from colitis ulcerosa.

## DETECTION OF *ASPERGILLUS* GALACTOMANNAN ANTIGEN IN HUMAN SAMPLES WITH DIFFERENT METHODS

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Fungal infections including several forms of aspergillosis have increased over the years among immunocompromised hosts. *Aspergillus* disseminates by means of conidia, which disperse readily throughout the environment because of their lightweight. Airborne conidia enter human host via inhalation or inoculation. Invasive pulmonary aspergillosis is the most severe form, but aspergilloma or allergic forms also may become invasive. As the microorganism is rarely recovered from sputum and may only be a contamination the diagnosis of a pulmonary infection is difficult to establish. Diagnosis may be facilitated by the use of serological examinations such as detection of the *Aspergillus* polysaccharide antigen (galactomannan) in serum or in bronchoalveolar lavage. In our laboratory two techniques have been used from 2004 to 2014. In this era 1655 galactomannan test were made. 66% of the positive samples was from male patiens and 34% was from female patiens. In the first six years a simple latex agglutination technique was used to detect galactomannan antigen in 986 cases. Only 13 tests (1.38%) were positive. In 2010 we introduced a new method: enzyme immunoassay. 669 samples have been tested from then and 35 (5.38%) were positive. In the examined decade both, cultivation from samples of respiratory tract and galactomannan antigen detection were made with samples from 369 patients. 19 of 44 galactomannan positive patients had positive cultivation (43%), but only three (7%) were *Aspergillus* sp. positives. 40 of 325 galactomannan negative patients had *Aspergillus* sp. positive cultivation. Only 0.7% of patients who

had *Aspergillus* sp. positive culture were galactomannan antigen positive. Our laboratory does detections of *Aspergillus* IgG antibody.
To compare to the antigen results: 12% of patients who had *Aspergillus* sp. positive culture were positive for *Aspergillus* IgG antibody. Our data shows that the quick serological methods may facilitate the diagnosis in deed, but they are not sufficient, cultivation is necessary as well.

## PURPLE BACTERIAL BLOOM IN AN ALKALINE SODA PAN

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In April 2014 a spectacular, dual bloom occurred in a shallow alkaline soda pan near the village Fülöpszállás (Danube-Tisza Interfluve, Hungary). The upper three cm water layer was inhabited by a dense population of the planktonic green alga, *Oocystis submarina*; and under that, there was a separate purple layer formed by rod-shaped purple bacteria with flagella and showing phototaxis. Identification of these bacteria was carried out using cultivation and high-throughput DNA-sequencing methods. As a result of next-generation sequencing (Roche GS Junior platform) 3131 sequences were obtained, of which more than half were identified as members of the genera *Ectothiorhodospira* and *Rhodobaca*. Members from many dominant groups were successfully cultivated. Three different solid media were used during aerobic and anaerobic cultivation, two of them were designed particularly for this work. During aerobic cultivation, $1.7\text{-}35\times10^6$ CFU/mL plate counts were obtained after 19 days of incubation, while under anaerobic conditions highly dissimilar values were recorded on different culture media. 63 strains were isolated in pure cultures (12 grew under anaerobic incubation and 51 under aerobic conditions). Half of the isolated bacterial strains had reddish coloration, a few of them showed irisation.
All strains belong to the phyla Proteobacteria and Bacteroidetes, with the highest similarity to the genera *Halomonas, Pseudomonas, Nitrincola, Vibrio, Alkalimonas, Porphyrobacter, Loktanella, Paracoccus, Roseicitreum, Roseinatronobacter, Rhodobaca, Belliella, Aquiflexum* and *Ectothiorhodospira*. Several strains represent potentially new species, their taxonomic description will be carried out in the near future.
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## NOVEL BACTERIA ISOLATED FROM THE WATER OF A THERMAL BATH, BUDAPEST

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The well and pool waters of Gellért thermal bath were studied with cultivation techniques using 10% R2A and minimal synthetic media (with “bath water”). It was combined with polyuretane foam (PUF) enrichments and with prolonged incubation time. These techiques revealed the appearance of novel bacterial taxa from the water samples. The full 16S rDNA sequence analysis and the complete phenotypic characterisation of the isolated strains ensured that these bacteria belong to new species

or genus of bacteria. The similarity of the dominant bacterium in the well water to *Hartmannibacter diazotrophicus* as closest relative was 93.93%. The isolated strains are strongly oligotrophic, mesophillic, neutrophillic and positive only for some enzyme activities (esterase, lipase, tripsin, acidic phosphatase and naphtol-AS-BI-phosphohydrolase). Our results show that these bacteria represent a novel genus, its proposed name is *Lavacria oligotropha*. The closest relatives of strain FDRGB2b are the type strains of *Brevundimonas nasdae* and *B. vesicularis* (both 97.24%). Our strain is very inactive in carbon source utilisations, mesophillic and based on our results it represents a novel species within the genus *Brevundimonas*. Its proposed name is *Brevundimonas balneaticum*. Bacterial strain RAM11 is a relative of *Mycoplana dimorpha* (96.83%) and *Shinorhizobium americanum* (96.69%).
This bacterium is also oligotrophic, but has strong esterase, lipase, leucin arylamidase, tripsin and acidic phosphatase activity. Its proposed name is *Shinorhizobium hungaricum*.

## BIOCONVERSION OF OILSEED RESIDUES BY *RHIZOMUCOR MIEHEI* FOR PRODUCTION OF PHENOLIC ANTIOXIDANTS

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High amount of plant-derived waste materials arise from the seed processing technologies annually. These by-products, especially oilseed residues, may contain phenolic antioxidants, which are well-known as natural antitumor, anti-inflammatory, anti-thrombosis and antihypertensive agents. Most of these compounds are in glycosylated forms. As these glycosylated phenolics are unable to diffuse across the intestinal wall, their physiological antioxidative effect is reduced. Beta-glucosidase enzymes can hydrolyze these glycosidic bonds; thus, the liberated aglycon is able to get across the cell membrane. Beta-glucosidases are important part of the cellulase enzyme system as they split the cellobiose units formed during cellulose degradation. Consequently, fermentation with filamentous fungi with high cellulase activity may enable the liberation of antioxidant phenolics. In the group Mucoromycotina there are good cellulase-producer filamentous fungi. Recently, many researches focus on the cellulase production of these microorganisms, and even more importantly, on their fermentative properties and their potential application in the eco-friendly utilization of plant-waste residues. In previous screening tests, a *Rhizomucor miehei* isolate has been identified as high-yield beta-glucosidase producer under wheat bran-based solid conditions. As beta-glucosidases may able to digest the phenolic glycosides of pressed hempen-, line-, poppy- and pumpkin seed residues, our present study investigates the antioxidative effect of these substrates after solid-state fermentation with *R. miehei*. Extraction of the plant-seed oils results seed residues as a by-product. For fermentation, solid oilseed medium was supplemented with soy flour as nitrogen source. During incubation, samples were taken every second day and extracted with distilled water or ethanol:water (50:50) solution. Total phenolic content and antioxidant activity were determined, including radical scavenging capacity and ferric reducing capacity. In addition, beta-glucosidase activity was also measured to analyze the correlation between the deglycosylation and the antioxidant capacity. Experiments revealed an increase in the amount of soluble free phenolic components during the fermentation process. The antioxidant capacities of the crude extracts were also enhanced, especially when line- and hempen seed were used as substrates. Fermentation on poppy-, line- and

pumpkin seed residues showed correlation between beta-glucosidase activity and radical scavenging/ferric reducing capacities; however, despite the high antioxidant power determined, low beta-glucosidase activity was found on hempen seed.

Our results indicate the promising applicability of *R. miehei* fermentation to enhance and release health-protective extractable phenolics from seed by-products.

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## ENDOPHYTIC FUNGI ISOLATED FROM GTD SYMPTOMATIC GRAPEVINES AND TESTING POTENTIAL BIOCONTROL AGENTS FOR TREATMENT

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Grapevine trunk diseases (GTD), caused by different phytopathogenic fungi threaten the vineyards worldwide and are one of the most important diseases in the Hungarian vineyards as well. They are caused by several fungal pathogens and the environmental factors has probable role as well. GTD pathogens can be detected only from the woody tissues of the plants. These fungi can often be found on asymptomatic plants as well. Currently there is no adequate and environmentally friendly protection against GTD pathogens. The aim of our work was to detect the pathogenic and non-GTD pathogenic endosymbiotic fungi from the grapevines, and to search for potential biocontrol agents from the healthy, asymptomatic plants. Pure fungal cultures isolated from wood tissues were used for morphological and molecular identifications. Different endophytic and GTD pathogen fungi could be isolated from the grapevines from the Tokaj wine region between 2013 and 2014. *Diplodia seriata* and *Diaporthe* species were identified as GTD pathogen fungi from the diseased woody tissue. The presence of *D. seriata* pathogen was 72%, in 2013, while in 2014 it was only 48%. The *Diaporthe* sp. pathogens had identified in 4% and 3% in 2013 and 2014 in the diseased trunks. The identified non-GTD pathogen endophytic fungi were *Alternaria* sp., *Mucor* sp., *Penicillium* sp., *Epicoccum* sp., *Fusarium* sp., *Botrytis* and *Aspergillus* sp. The rate of these fungi was 72% in 2013, while in 52% in 2014. Several *Trichoderma* species were also isolated from the asymptomatic grapevines. Endophytic, mycoparasitic fungi, like *Trichoderma* species have huge potential against grapevine plant pathogens colonizing woody tissues. Testing new isolates with biocontrol potential and ability to grow under different environmental conditions may provide effective biopesticide to control GTD pathogens. The *Trichoderma* isolates were identified based on their ITS1,2 and *tef1* marker sequences. The ten isolates belonged to three species: *Trichoderma harzianum* (eight isolates), *Trichoderma orientalis* (one isolate) and *Trichoderma viride* (one isolate). Their mycelial growth was determined from average colony diameters on PDA at different temperatures between 5 and 37 °C. *T. orientalis* and one of *T. harzianum* isolates showed the highest growth rates within the whole temperature range, and their growth rates were especially high at 30 and 37 °C. The mycoparasitic ability was tested *in vitro* against GTD pathogens (*D. seriata* and *Neofusicoccum parvum*). All *Trichoderma* overgrew the GTD pathogens, and sporulated on their colonies, therefore their Biocontrol Index was 100%. Some GTD symptomatic plants were treated with *Trichoderma* spore suspension in Tokaj wineyard in 2014. The treated trunks were checked monthly. The symptoms decreased on all treated samples, except one. Moreover the *Trichoderma* strains could be isolate again from their woody tissues 10 month following the treatment.

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## LACTOSE UTILIZING ASCOMYCETOUS AND BASIDIOMYCETOUS YEASTS: CELLULAR LOCATION OF ß-GALACTOSIDASE AND RELATION BETWEEN ENZYME ACTIVITY AND BIOMASS YIELD

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Based on the recent classification of yeasts [1] nearly 100 species of 17 different genera belonging to the ascomycetes possess lactose utilization characteristics. Majority of them have the ability for the aerobic assimilation, only the *Kluyveromyces* genus contains efficient and well-documented lactose fermenting species such as *K. lactis* var. *lactis* and *K. marxianus*. Three other species of this genus (*K. aestuarii, K. nonfermentans* and *K. wickerhamii*) can only assimilate but not ferment lactose. Lactose metabolism is much more frequent in the basidiomycetous yeasts since 236 species representing 43 genera have this characteristic but any of them has the ability for lactose fermentation. "*In silico*" screening of lactose utilizing yeasts has been followed by testing the selected strains for β-galactosidase activity on X-gal chromogenic medium, which allowed not only the indication of the enzyme activity but also checking its sensitivity for glucose repression. Location of the enzyme β-galactosidase (i.e. intracellular or extracellular) has been determined by measuring the β-galactosidase activity of the cell-free supernatants before and after adding the membrane permeabilizing agent digitonin to the cells [2]. Highest enzyme activity was measured in the case of *Candida auringiensis, Dekkera anomala, Kluyveromyces lactis* var. *lactis* and *K. marxianus*, although big differences were found occasionally among the strains belonging to the same species. Biomass yield of *D. anomala, K. lactis* var. *lactis* and *K. marxianus* strains was determined on lactose containing culture media or cheese whey in aerated (shaken) cultures, which indicated strong catabolite repression of growth above 2% lactose. Surprisingly no correlation was found between the β-galactosidase activity and biomass yield of the strains studied.

[1] Kurtzman, C.P. et al. (eds) (2011) The yeasts, a taxonomic study. 5th ed. Vol. I-III. Elsevier.
[2] Bacci Junior, M. et al. (1996) Antonie van Leeuwenhoek 69, 357-361.

## MYCORRHIZAL STATUS AND MICROBIAL FUNCTIONALITY OF MYCORRHIZOSPHERE IN RELATION TO VEGETATION TYPES OF SOLONCHAK GRASSLAND IN APAJPUSZTA

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Arbuscular mycorrhizal fungi (AMF) can help their host plants in water and nutrient uptake and to cope with all kinds of stress, and therefore they have a strong influence on the species composition

and dominance relations in plant communities. For conservation treatments and to preserve the natural environment in the Hungarian steppe temporal and spatial changes of the plant communities and the influencing factors behind this were examined. Extreme water supply is the most important factor influencing the microbial activity in the saline soils. Our knowledge is very limited on the arbuscular mycorrhizal community of saline soils, as well as on their role in drought and salt stress. The goal of our work was to examine the effect of the drying-wetting cycles and salinity of the soils on the AMF communities in the saline solonchak grassland of the Kiskunsági National Park. Chemical and physical properties of soils in Apajpuszta were analysed. The indigenous plant composition of the communities was investigated and functional and status analyses were performed to characterize the biological properties of the mycorrhizosphere. The mycorrhizal status of the most abundant plants and the diversity of AMF communities were examined with PCR-RFLP and DGGE methods used taxon specific primer systems for the ribosomal RNA gene sequences of the fungi. Four typical plant communities, such as *Lepidio crassifolii-Puccinellietum limosae* ("mézpázsitos"), *Artemisio santonici-Festucetum pseudovinae* ("ürmöspuszta"), *Lepidio crassifolii-Camphorosma annuae* ("vakszik") and *Achilleo-Festucetum pseudovinae* ("sziki legelő") were examined, where the salt concentration and the pH of the soils showed a descending gradient and the humus content showed a growing gradient in the order of "vakszik"-"mézpázsitos"-"ürmöspuszta"-"sziki legelő" communities. In "vakszik" plant community mostly non-host plants was found. In "ürmöspuszta" and "sziki legelő" communities mycotroph plants were in a higher ratio and in "mézpázsitos" the mycotroph and less mycorrhiza dependent *Puccinellia limosa* could be found in more than 90% abundance. Very similar tendencies of microbial functionality were shown by the fluorescein diacetate (FDA) test, the soil catabolic activity pattern analysed by MicroRespTM, and the measurement of glomalin-related soil proteins (GRSP). The "vakszik" community always had the lowest values; while the other three variously differed significantly from each other. AMF root colonization and GRSP were used for characterization of the intra- and extraradical spread of AMF. Density of AMF infective propagules were estimated by the amount of mycorrhizal colonization in roots and by an MPN test where separated soil samples were used as inoculums. We observed remarkable differences between the four plant communities. The "vakszik" plants had very low root colonization and a barely measurable GRSP. In "ürmöspuszta" and "sziki legelő" 40-70% (M% Trouvelot et al. 1986) colonization intensities were found and they had the highest GRSP content. In "mézpázsitos" colonization intensity was under 10% and it had GRSP content between those measured at "vakszik" and "ürmöspuszta". In the MPN test no colonization were found in "mézpázsitos" and "vakszik"; "ürmöspuszta" and "sziki legelő" soils contained low density of infective propagules. The "mézpázsitos" was flooded which blocked infection.

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## THE EFFECTS OF BIOCHAR ON RHIZOBACTERIA

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Biochar is charcoal that is used for agricultural purposes. It is produced through pyrolysis – the thermal degradation of organic materials in the absence of air. It is distinguished from charcoal by its use as a soil amendment, which means to improve soil fertility to increase crop yields, to limit fertilizer rainout, to hold moisture, and to replace exhausted soils with organic carbon. In addition, the biochar fosters the growth of soil microbes that are essential for nutrient absorption. Another

important effect is to sequester carbon to mitigate climate change. Application of biochar in soil offers many agricultural benefits, but the effects of biochar on soil biota have received little attention. The pH value of biochar can vary widely, depending on feedstock type, pyrolysis temperature and degree of oxidation, which generates very different living conditions for microorganisms in biochar. In most studies, microbial biomass has been found to increase with biochar additions. The significant changes induced in the soil microbial community composition and enzyme activities may explain the biogeochemical effects of biochar. The reasons for changes in microbial abundance may differ for different groups of microorganisms. After biochar administration, the pH of soils may increase or decrease, depending on the pH and liming value of the biochar. The pH of biochars may therefore have a very important influence on total microbial population [1]. The Biofil Ltd.'s researchers – in collaboration with strategic partners – select and examine some rhizospheric bacteria which are capable to grow in the presence of several biochar types. Plant growth promotion (PGP) is a complex phenomenon, including biological nitrogen fixation, biocontrol effect on phytopathogens in the rhizosphere, nutrient solubilization in the root zone, and the phytostimulation by production of plant growth regulating phytohormones.

In our work we isolated hundreds of rhizobacteria from soil samples. Thereafter we selected the PGP rhizobacteria and we examined their pH stress tolerance. Tolerant strains were investigated for growth in the presence of biochar. Bacterial growth was detected by spectrophotometry. We have found that the pH condition is an important but not the only selective factor.

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[1] Lehmann, J. et al. (2011) Soil Biol Biochem 43, 1812-1836.

## AFLATOXIN B1 PRODUCTION OF ASPERGILLUS FLAVUS UNDER DIFFERENT MEDIUM COMPOSITION

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Aflatoxins (AFs) are a group of polyketide metabolites produced by several toxigenic species of *Aspergillus* such as *A. parasiticus* and *A. flavus* after infections of seeds with high protein and lipid contents, e.g. peanut, corn and walnut. AFs are toxic and carcinogenic, posing serious threats to both animal and human health. The environmental factors (e.g. light, temperature, pH, calcium, and nutrients) regulate secondary metabolite aflatoxin production in a concerted way. Under laboratory condition, there are many different media to cultivate fungi; however, not all of them are suitable for investigation of toxin production of *Aspergillus flavus*. Therefore, we compared the growth and aflatoxin production of *Aspergillus flavus* on well-known *Aspergillus* media like Czapek-Dox medium, malt extract medium and corn meal agar at a constant temperature (30 °C). The fungal colonies and the medium together were homogenized with extraction solvent, separated with filtration, and concentrated on Rota-Dest before HPLC detection of aflatoxin B1. Our results revealed that Czapek-Dox medium was good the growth but not for the aflatoxin B1 production. The corn meal agar was inhibitory for the growth and supported sclerotium production.

In contrast, inoculation on malt extract medium supported the fungal growth, and the aflatoxin B1 production as well; however, in a spore density dependent way. In higher spore density inoculation lower aflatoxin B1 production was detected.

## TEMPERATURE-, PH- AND WATER ACTIVITY DEPENDENCE OF *PHOTORHABDUS LUMINESCENS* STRAINS AND THEIR *IN VITRO* INHIBITORY EFFECT TO TRICHODERMA SPECIES CAUSING MUSHROOM GREEN MOULD DISEASE

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*Agaricus bisporus, Lentinula edodes* and *Pleurotus ostratus* are the three most abundant cultivated mushrooms in the world. Their production is getting increasingly affected by green mould infections causing great crop losses. The fungi responsible for the green mould disease of *Pleurotus* have been described as *Trichoderma pleurotum* and *T. pleuroticola. T. aggressivum* is the main causative agent of *Agaricus* green mould disease, while *T. pleuroticola* was reported from infected Shiitake mushroom. As the application of chemical compounds against *Trichoderma* species in mushroom production is not allowed or very limited, there is an increasing need for biological control agent. *Photorhabdus luminescens* strains can be promising candidates for this purpose. The aim of our study was to determine the *in vitro* inhibitory effect of 2 *P. luminescens* (SZMC 22400, SZMC 22401) and 2 *P. luminescens* subsp. *kayaii* (SZMC 22402, SZMC 22403) strains against four *Trichoderma* strains causing mushroom green mould disease (*T. aggressivum* f. *aggressivum*, *T. aggressivum* f. *europaeum*, *T. pleuroti* and *T. pleuroticola*). We used an image analysis based method for the quantification of the inhibitory effect which is a simple method to compare the activities of different strains. After this image analysis we defined the Antibiosis Index (AbI) values of the *Photorhabdus* isolates in comparison with the growth of control Trichoderma strains. All of the tested *P. luminescens* strains were sustantially inhibiting the growth of the green mould strains, with *P. limunescens* subsp. *kayaii* SZMC 22403 showing the highest inhibition of all tested *Trichoderma* strains. We also examined the temperature-, pH- and water activity dependence of *P. luminescens* strains on microtiter plates. In the case of *P. luminescens* SZMC 22400 and SZMC 22401 the optimum temperature value were 25 °C and 20 °C, respectively, they proved to be higher (30 °C) in the case of the two *P. luminescens* subsp. *kayaii* strains. All of the tested *P. luminescens* strains showed an increased growth at pH values between 5 and 8 while none of them were able to grow under the water activity value of 0.980. The knowledge about the temperature-, pH- and water activity-dependence of *P. luminescens* strains and about their inhibitory effects against *Trichoderma* species is very important when planning the development of an appropriate control method against *Trichoderma* green mould in mushroom production.

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## WIDENING SPECTRUM OF FILAMENTOUS FUNGI CAUSING MYCOTIC KERATITIS

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Mycotic keratitis is known as a serious suppurative, usually ulcerative disease among eye infections, which may cause blindness or reduced vision. We studied the fungal spectrum of keratitis based on the data recorded in the 10-years-period of 2005-2014 at the Aravind Eye Hospital and Postgraduate Institute of Ophthalmology, Coimbatore, Tamilnadu, South India. Fungal isolates were collected from corneal scrapings of mycotic keratitis patients. Species-level identification of the isolates was performed by morphological examination followed by nucleotide sequence-based confirmation. *Fusarium* proved to be the genus most frequently occurring in the corneal samples. Members of five *Fusarium* species complexes could be detected. The *Fusarium solani* species complex (FSSC) was predominant, followed by the species complexes *Fusarium dimerum* (FDSC), *Fusarium fujikuroi* (FFSC), *Fusarium oxysporum* (FOSC) and *Fusarium incarnatum-equiseti* (FIESC). The sexual fungus *Neocosmospora vasinfecta* from FOSC and *Fusarium napiforme* from FFSC were firstly detected from keratitis. The genus *Aspergillus* was the second most frequent taxon with *Aspergillus flavus* as the predominant species, followed by *Aspergillus fumigatus* and *Aspergillus terreus*. *Aspergillus amstelodami* from section Aspergillus, *A. melleus* from section Circumdati, *Aspergillus tamarii*, *A. pseudotamarii* and *A. nomius* from section Flavi, *A. lentulus* from section Fumigati, *A. variecolor* and *A. sydowii* from section Nidulantes as well as *A. brasiliensis, A. tubingensis, A. welwitschiae* and *A. neoniger* from section Nigri of the genus *Aspergillus* were firstly recognized as causative agents of mycotic keratitis.

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## TEMPORAL AND SPATIAL FLUCTUATIONS OF PLANKTONIC BACTERIAL COMMUNITY STRUCTURES OF LAKE HÉVÍZ REVEALED BY DGGE

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Lake Hévíz is a unique thermal spa located in Hungary. Owing to the thermal springs nourishing the lake, it has a relatively rapid water turnover. In spring 2011 a comprehensive embankment reconstruction was performed to preserve the water supply of the surrounding wetland habitats. The aim of the present study was to gain information about the temporal and spatial changes of planktonic bacterial communities using denaturing gradient gel electrophoresis (DGGE) method together with the changes in the physical and chemical parameters of the water of Lake Hévíz with special respect on the effect of the disturbance caused by the embankment reconstruction. Samples were taken in April, July, October 2010 and 2011 from four different locations of the lake. According to the abiotic components, both temporal and minor spatial differences were revealed with the exception of autumn samples. The reconstruction resulted in a short term but dramatic alteration of the total planktonic bacterial and cyanobacterial community structures as revealed by DGGE. In addition greater seasonal than spatial differences of bacterial communities were also

observed. Planktonic bacterial community composition of Lake Hévíz included mainly typical freshwater species within phylum Actinobacteria, Chloroflexi, Cyanobacteria and class Alpha-, Beta- and Gammaproteobacteria according to the identified DGGE bands.

## COMPARISON OF GENOTOXICITY TESTS USING DIFFERENT CYTOCHROME P450 ENZYMES AND REPORTERS FOR DETECTING AFLATOXINS AND ITS DERIVATIVES

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Aflatoxins are arguably the most potent carcinogen thus most harmful mycotoxins known. It is generally accepted that aflatoxin-B1 (AFB1) causes genotoxic effects by a cytochrome P450 (CYP)-mediated reaction yielding AFB1 8,9-epoxide. The reactive AFB1 8,9-epoxide interacts with nucleic acid causing severe DNA damages and mutation effects. Therefore, detection of aflatoxin-B1 and its derivatives, as well as precursors by analytical immunochemical and biological methods have huge importance on human health. Bio-monitoring of mycotoxins has an increasing importance nowadays. Our aim is to monitor aflatoxin-B1, aflatoxin-M1 and sterigmatocystin by different bacterial bio-tests and identify the most appropriate tool for their detection. First, colorimetric assays based on *Escherichia coli* were conducted. The principle of the SOS-Chromotest is the SOS response that is induced by DNA-damaging agents. The test takes advantage of an operon fusion placing structural gene of β-galactosidase under the control of *sfi*A gene responsible for SOS error-prone.For colour development X-Gal and para-nitrophenyl phosphate (pNPP) substrates for β-galactosidase and alkaline-phosphatase (indicative for cytoxicity) are used. SOS-Chromotest based on the *E. coli* PQ37 makes use of S9 rat liver homogenate to examine those compounds which have indirect genotoxic activity and need metabolic activation to actuate their genotoxic effect. SOS-ExpressTestTM strains are unique compared to the SOS-Chromotest PQ37 strains, as they express human P450 1A2 liver enzymes internally, which promotes bio-activation of xenobiotics into DNA reactive species in the absence of the S9 mix. This system acts as an *in vitro* mimic for the uptake and breakdown of xenobiotic compounds by human liver enzymes, which generate mutagenic products. Additionally, a bioluminescence assay for testing genotoxicity, the SWITCH (*Salmonella* Weighting of Induced Toxicity Cyto/GenoTox for Human Health) test, based on *Salmonella typhimurium* TA1535 strain was applied. The test bears the pSWITCH plasmid that carries the promoterless lux operon of *Photobacterium leiognathi* as reporter element under the control of the DNA-damage-dependent SOS promoter of ColD as sensor element. According to our results by the help of external rat liver extract all of the tested mycotoxins have lower observed effect concentration. Interestingly the *E. coli* based colorimetric test is the most sensitive for detecting aflatoxin-B1; aflatoxin M1 and sterigmatocystin were detectable at lower concentration in the *S. typhimurium* based bioluminescence test.

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## HUMAN *DIROFILARIA REPENS* INFECTION WITH UNUSUAL LOCALIZATION – CASE REPORT

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*Dirofilaria* infections are vector-borne parasitic infections mainly of dogs and cats. In Europe, they are caused by *Dirofilaria immitis* and *D. repens*. *D. immitis* causes heartworm disease in dogs and cats, while *D. repens* is found mainly in subcutaneous tissues. The intermediate hosts and vectors are mosquitoes of the family Culicidae. Dirofilariosis is an endemic zoonosis in the Mediterranean area and it seems to be an emerging disease in Central Europe. Humans become infected with these worms through mosquito bites. *D. immitis* causes pulmonary disease in humans. *D. repens* infection in humans manifests as a subcutaneous or ocular nodule, but there were several reports of presentation in unusual body sites, like pulmonary, hepatic, orbital, spermatic cord, abdominal cavity etc. In this report we describe a case of pulmonary dirofilariosis in a female patient. The 57 year old female patient had her regular pulmonary X-ray screening in July 2014. A suspect nodule was seen in the lower lobe of the left lung, which corresponded to single nodular shadow measuring 10-15 mm on the anteroposterior chest X-ray. The other parts of the lungs were clear. The contrast enhanced chest CT examination showed a well circumscribed homogeneous 18 x 8 mm size mass in 4th segment of the left lung. The lung was emphysematous, without other pathological densities. No pleural effusion or enlarged mediastinal lymph-nodes were seen. In September, the surgical removal of the left lung lesion was performed by VATS technique. The patient was monitored at the Division for Subcritical Care postoperatively, and as her condition become stable, she was emitted from hospital. Examination of the histopathological sections of the nodule revealed cross sectional aspects of helminth. *D. repens* was identified on the basis of the morphological characteristics in the histopathological sections. In conclusion: *D. repens* dirofilariosis may have unusual presentation, including pulmonary localization which can be easily confused with benign / malignant tumors in the lung, resulting in surgical intervention.
With this presentation the authors would like to call the attention to this possibility.

## INTESTINAL CACO-2 CELLS SUPPORT *CHLAMYDIA* REPLICATION AND PRODUCE HUMAN BETA-DEFENSIN-2

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Chlamydiae are obligate intracellular bacteria that cause subclinical and persistent infections, and symptoms at different body sites in humans. *Chlamydia* species in animals, domestic mammals, rodents and birds target the gastrointestinal tract (GI) which is shown to be the site of persistent infection. It is likely that during human infection the GI tract become infected too, and there is clinical evidence to support this. LGV and non-LGV serovars of *C. trachomatis* can cause proctitis.

Clinical investigations screening patients for *C. trachomatis* urogenital tract infection found DNA in the urogenital tract and in a significant percentage of anorectal samples too. It has been suggested that the gastrointestinal tract might act as a reservoir and might play a role in repeat urogenital infections. The respiratory tract pathogen *C. pneumoniae* has been detected in intestinal mucosal biopsies from IBD and non-IBD patients. Our first aim was to use a human intestinal epithelial cell line Caco-2 to investigate whether this cell type is able to support the replication of different *C. trachomatis* serovars (D and L2) and *C. pneumoniae*. The next aim was to test whether chlamydial infection induces secretion of antimicrobial peptide human beta-defensin-2 by Caco-2 cells. Semipurified *C. trachomatis* serovar D and L2, and *C. pneumoniae* were used to infect monolayers of Caco-2 cells. The infected cells were tested for the presence of chlamydial inclusion by immunofluorescence. The infected cells with the supernatants were harvested at different time points post-infection. The infectious chlamydia was isolated by inoculation of the samples onto permissive host cell lines, HeLa 229 and HEp-2 and quantitated by LPS-specific immunofluorescence staining. For detection of human beta-defensin-2 production by this cell line the supernatant of the cells were tested by HBD-2 ELISA. Active replication was detected with all chlamydia strains tested as shown by the presence of fully developed chlamydial inclusions with active phenotype and recoverable infective chlamydiae. *C. trachomatis* serovar L and *C. pneumoniae* replication continued to produce increasing number of infective elementary bodies for upto 72 h and reached high titer of infectious bacteria. *C. trachomatis* serovar D was recoverable from Caco-2 cells with moderate titer. Replication efficiency will be compared to the yield in the usual host cell line used for propagation. *Chlamydia* strains induced HBD-2 production in Caco-2 cells at later stage of infection except for L serovar which prompted the cells to produce HBD-2 early and with higher level compared to the *E. coli* Nissle strain used as positive control of HBD-2 induction. Caco-2 cell line proved to be a highly permissive cell type for chlamydial replication, suggesting the gastrointestinal tract as a site of replication. We observed an increase in the production of HBD-2 by these cells upon exposure to *C. trachomatis* D and *C. pneumoniae* that can contribute to antibacterial activity in the epithelium.
Interestingly, L serovar with known intestinal pathology is a potent inducer of HBD-2 but this effect does not influence its vigorous replication in these cells.

## MODULATING MITOCHONDRIAL FUNCTION AND MORPHOLOGY IN *ASPERGILLUS NIDULANS* HAD LOW IMPACT ON CELLULAR PHYSIOLOGY AND AGEING

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Mitochondria play a determinative role in the organismal lifespan and apoptotic cell death of the eukaryotes. For example, the filamentous fungus *Podospora anserina* has a definite lifespan, which can be explained with age-related instabilities and rearrangements of its mitochondrial DNA. Another ascomycete model organism, *Aspergillus nidulans*, does not display a *P. anserina*-like senescence, and elimination or overexpression of some genes related to mitochondrial function and morphology like *aod*A, *mn*SOD, *dnm*A and *pim*A encoding alternative oxidase, manganase

superoxide dismutase, dynamin related protein and Lon protease, respectively, affected cellular physiology only sporadically in the vegetative tissue of the fungus. Namely, although both the deletion of *dnm*A and overexpression of *pim*A increased the size and volumetric ratio of mitochondria in mid-aged mycelia these morphological changes did not result in any delay in the onset of programmed cell death elicited by the small molecular mass antifungal protein of *Penicillium chrysogenum* (PAF). Importantly, the elimination and overexpression of the selected genes did not have a large impact on either fungal growth or carbon starvation dependent autolysis in liquid cultures. Nevertheless, reduced autolytic proteinase and no sterigmatocystin productions were observed in submerged cultures of the *Δaod*A mutant, which may be significant in future industrial strain development projects. Furthermore, overexpression of *pim*A increased the Cd2+ tolerance of *A. nidulans*, which may lead to the construction of heavy metal tolerant *Aspergillus* strains. On the other hand, the manipulation of the mitochondria-related genes affected more profoundly the developmental processes of the fungus. The most remarkable observation was that all genes studied were necessary for conidiospore production and vitality, and the deletion of the *pim*A gene inhibited the ascopore production even after long-term incubation. In conclusion, these genes seem to have less impact on cellular physiology and ageing than their orthologs in senescent *P. anserina* cultures. Furthermore, some interesting new phenotypes arose, which may be exploitable in the future in the biotechnology industry.

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## EFFECT OF APPLE ON QUANTITATIVE CHANGES OF PROBIOTICS BACTERIA

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Different varieties of apples are widespread cultivated in Szabolcs-Szatmár-Bereg County. It is believed that apple consumption is healthy. Apple consumption associated with consumption of probiotic is similarly suggested. We hypothesized that nutritional properties of apple may be probiotic bacteria proliferation-promoting agents. In our study we supposed that different apple sorts cannot be equal in helping the proliferation of the bacteria. Yogurts and pharmaceutical probiotic formulations with live probiotic bacteria were grown in juices made from different apple sorts. Three of the examined eight varieties of apple significantly increased the growth of bacterial strains signed DLZS (Jonagold > Golden > Mutsu) and four apple varieties the strains signed SPA (Mutsu > Idared > Golden > Florina). Apples significantly differ in fibres and other nutritional properties but their effect is primarily due to the content of antioxidant and of citric acid, which affect the growth of the probiotic bacteria. We recommend the incorporation of the Golden and Mutsu apple varieties into yogurt with live probiotic cultures before consumption. We do not recommend the immediate consumption of apples following the consumption pharmaceutical probiotic products. In the future we want to determine nutritional properties of apple varieties used in the experiment, furthermore, we wish to model the effects of digestive enzymes in the gastrointestinal tract.

## INVESTIGATION OF BIODEGRADATION KINETICS OF DIFFERENT SUBSTRATES IN MICROBIAL FUEL CELLS (MFCS)

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Over the past decade the headway of the MFC (Microbial Fuel Cell) in biotechnical research offers a brand new technology for producing electricity from renewable sources ("bioelectricity"). Under strictly anaerobic conditions a group of bacteria, called exoelectrogens (e.g. species of the *Shewanella, Geobacter* and *Pseudomonas* genera), are capable of transporting the electrons gained by the oxidation of organic matter to a solid conductive surface providing high electrode potential. The exoelectrogenic electron transport can be carried out by three different mechanisms: direct contact with the surface; through chemical mediators; contact with the surface with conductive nanopili. By the cultivation of exoelectrogenic biofilm on the anode surface of the MFCs, this special electron transporting capability can be utilized by harvesting electric power in a circuit and a resistance. Thus, chemical energy stored in organic matter can be converted directly to electric energy in an MFC. The applications of microbiological fuel cells are very diverse. Most studies focus on producing renewable electricity via utilization/biodegradation of organic compounds. The most significant costs in wastewater treatment are those of aeration and solids handling. A well designed MFC needs no aeration at all, and its biomass yield is up to one order of magnitude lower that of aerobic treatment. Direct oxidation of these organic compounds may not be the only way to obtain energy in an MFC. With an additional voltage (0.11 V, based on calculations), an MFC can be easily modified to produce "biohydrogen" gas. Furthermore, an MFC can be used as a biosensor, utilizing the relationship between the obtained voltage and the concentration of biodegradable organic matter. As a first step of the research, experiments have been conducted in order to have a better understanding of the effects of the physical, architectural and chemical parameters of the cell on the efficiency of the MFC. Based on the results an MFC appropriate for the investigation of the biodegradation kinetics (carried out by the exoelectrogenic biofilm) has been designed and constructed. The main goal of the study was to investigate the kinetics of biodegradation of different substrates with this nouvelle technology. Graphite felt supported with Pt/C catalyst layer and cathode without catalyst layer has been used as cathode. Our results suggest that the biodegradation of organic matter can be effective in MFCs even with very low concentrations (TOC < 20 mg/l), without a major setback of the power produced by the fuel cell. Thus, MFCs can be used more effectively in the elimination of low concentrations than we ever thought before.

## UPDATING THE CONTROLS OF ANAEROBIC HYDROCARBON DEGRADATION IN GROUNDWATER: A MICROBIAL COMMUNITY PERSPECTIVE

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Contamination with petroleum hydrocarbons is widespread in nature and a notorious threat to our water resources. Two central paradigms are currently understood to control biodegradation in groundwater and sediments: (i) Redox gradients and interphases between compartments are 'hot-spots' of key importance for contaminant breakdown, and (ii) that biodegradation is primarily

limited by local electron acceptor availability, in particular that of oxygen. However, while the physiology and biochemistry of biodegradation are relatively well understood for a number of laboratory enrichments and degrader cultures, researchers are only beginning to unravel the ecology of the microbes truly responsible for these processes in situ. Here I report on our recent advances on the identity and diversity of anaerobic aromatic hydrocarbon degraders in contaminated aquifers. Using a combination of molecular methods and isotopic labelling, we show that at a number of sites, biodegradation seems dominated by a rather limited diversity of degraders within the Deltaproteobacteria and Clostridia. We show that a highly specialised, low-evenness degrader microbiome can be established and active at the fringes of contaminant plumes, and provide evidence that such populations may be subject to ecological disturbance by hydraulic feedbacks in groundwater. Finally, work recently started within my group aims at again reconsidering the perspective of the stratification of microbial groups and biodegradation processes at plume redox gradients. Based on unexpected own field observations as well as recent advances in the fields of microbial long-distance electron transfer and oxygenic physiology, we tackle new hypotheses on the role of molecular oxygen in biodegradation in groundwater. This targeted dissection of degrader community functioning at contaminated sited may help to establish a more ecological understanding of the controls of biodegradation in groundwater systems.

## *XYLANOBACILLUS XYLANOLYTICUS* GEN. NOV., SP. NOV., A MODERATELY THERMOPHILIC SPECIES WITHIN THE FAMILY PAENIBACILLACEAE

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Strain K13 is a Gram-positive, motile, rod-shaped bacterium, which was isolated on xylan containing agar-plate from overheated (70 °C) horse manure compost near Gödöllő, Hungary. This organism grew optimally at 45 °C and pH 9.0. The strain shows strong xylan-degrading activity, and expresses multiple endoxylanase enzymes according to xylan-zymography. K13 cells are rod shaped (2.5-0.7 μm) and bear two polar flagella according to the transmission EM morphology. Phylogenetic analyses revealed that the new isolate belong to the family Paenibacillaceae, exhibiting low 16S rRNA gene sequence similarities (92.12-91.32%) to the nearest type strains *Paenibacillus polymyxa* DSM36T and *Fontibacillus aquaticus* DSM 17643T thus formed a well-supported lineage that is clearly distinguished from all currently described genera in this family (*Paenibacillus, Saccharibacillus, Fontibacillus, Cohnella, Thermobacillus*). The G+C content of the genome is 52.25%. Chemotaxonomic data (meso-DAP, menaquinone: MK7, polar lipids: PG, DPG, PSer, PE and APL) also supported affiliation of the isolate with the family Paenibacillaceae.

Based on our polyphasic taxonomic characterization, we propose that strain K13 represents a novel genus and species, for which we propose the name *Xylanobacillus xylanolyticus* gen. nov., sp. nov. (type strain K13T = NCAIM B.02605T), within the family Paenibacillaceae.

## COMPARATIVE STUDY OF ANTIBODY LEVELS DEVELOPED BY VACCINATION AGAINST POLIO VIRUS IN POPULATION AFTER VACCINE TYPE ALTERATION

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During clinical trials performed in 2014, samples from Hungarian patients of different age groups were tested for antibodies against all 3 serotypes of poliovirus, a member of Picornaviridae family. During the virus neutralization serological test, blood samples were titrated using permanent virus concentration. Based on the cythopathic effect observed under a light microscope, the antibody level of the patient was assessed. The 100 people examined were classified into 5 groups based on age and type of original vaccine: I. Newborns, no vaccination given; II. Immunosuppressed patients; III. Born before 1986, received only OPV vaccine; IV. Born between 1992– 2005, received a combination of OPV and IPV vaccines; V. Born after 2006, received only IPV vaccine. Results show that vaccination coverage meets all the criteria. None of the immunized persons was seronegative to all three polioviruses. Both IPV and OPV vaccines are effective against poliovirus. Blood samples from newborn babies with no immunization were also examined.

Results show that most newborns have maternal antibodies in their blood. Results of group II show that immunosuppression does not have a negative influence on blood antibody levels against polioviruses. In spite of the low number of samples, our results show that seroconversion after immunization in the Hungarian population is adequate. For more accurate results about vaccineation coverage in the population, further trials would be necessary.

## SEQUENTIAL DIVERSITY OF PEPTAIBOL PROFILES OF *TRICHODERMA* SPECIES CAUSING GREEN MOULD DISEASE OF CULTIVATED MUSHROOMS

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The green mould disease of cultivated mushrooms firstly emerged at an epidemic level in champignon (*Agaricus bisporus*) production during the 1980's in Northern Ireland. Later, the disease caused large yield losses in several other countries including Hungary. Initially the species *Trichoderma harzianum* was suspected as the causal agent of this epidemic disease, but later the morphological and DNA sequence differences resulted in the description of the virulent species as *T. aggressivum*. The infections were also observed in North-America, where it turned out that the infectious agent is another biotype of the same species *T. aggressivum*. The European biotype was named as *T. aggressivum* f. *europaeum* while the American one as *T. aggressivum* f. *aggressivum*. Later, green mould diseases were also observed in oyster mushroom (*Pleurotus ostreatus*) production. Morphological, physiological and molecular analyses revealed that the causal agents of *Pleurotus* green mould disease are different from *T. aggressivum*, they were described as the new species *T. pleurotum* and *T. pleuroticola*. In our present work, four strains of the genus *Trichoderma, T. aggressivum* f. *europaeum* SZMC 1811, *T. aggressivum* f. *aggressivum* SZMC 1813, *T. pleurotum* SZMC 12454 and *T. pleuroticola* SZMC 12727 were investigated for peptaibol

production on malt extract-, champignon powder- and oyster mushroom powder-containing media. For analysis, on-line reversed-phase high performance liquid chromatography (HPLC) coupled to electrospray ionization ion trap mass spectrometry (ESI-IT-MS) was used after the solid phase clean-up of the culture extracts. Differences were detected in the produced groups of peptaibols on various media and new peptaibol compounds could also be identified in the groups. Several known peptides belonging to the group of trichorzianins were detected, e.g. trichorzianin TA IIIb/IIIc, TA/TB IIa, TB IVb, TA II a, TA/TB Vb, TAP-14a, TA VII and TA/TB VI. Some new, previously unknown members of the trichorzianins and trichorzins were also detected. Furthermore, a completely new group was also observed and described.

The highest amounts of the total produced peptaibols were observed in the extracts of strains cultivated on malt extract medium. In conclusion, the diversity of the peptaibols proved to be different in the extracts and showed dependence from the media.

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## TAXONOMIC COMPOSITION OF PICOCYANOBACTERIAL COMMUNITIES IN CENTRAL EUROPEAN LAKES

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Members of the picocyanobacterial genus *Synechococcus* are important planktonic components in open ocean ecosystems, in continental freshwaters and also in saline aquatic habitats. Freshwater phylotypes form phylogenetically completely distinct clades apart from those typically occur in the open ocean. Taxonomic identification of these small cyanobacteria is based on molecular biological techniques, because their distinctive morphological features are limited. Based on microscopic enumeration, picocyanobacteria are predominant members of the plankton in various aquatic habitats of Central Europe. Our aim was to determine the taxonomic composition of picocyanobacterial communities in freshwater (Lake Balaton, Hungary), soda (Zab-szék, Hungary) and saline lakes (Lake Tarzan, Lake Cabdic and Lake Ursu, Romania) on the basis of the comparative sequence analysis of the ribosomal ITS region and the 16S rRNA gene.

It seems that salinity defines the ratio of marine to non-marine picocyanobacterial clades in these aquatic environments, since non-marine clades of the genus *Synechococcus* appeared in freshwater, soda lakes and in salt lakes having lower salt concentrations, but these taxa were absent in saline lakes having higher salt concentration. Interestingly, the *Synechococcus* community of all studied salt lakes was dominated by phylotypes characteristic to oceans and seas. The dominance of marine picocyanobacteria in continental waters was not reported previously.

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## TOWARDS "UNNATURAL PRODUCTS": COMBINATORIAL BIOSYNTHESIS OF BIOACTIVE BENZENEDIOL LACTONE POLYKETIDES

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Polyketides are one of the largest families of small molecule natural products with pharmaceutically important activities. These metabolites have provided lead compounds and structural inspiration for multibillion-dollar drugs that are used every day to save lives. The biosynthesis of polyketides in fungi involves multi-domain iterative polyketide synthase enzymes (iPKS) of >300 kDa that catalyze the recursive condensation of malonyl-CoA units in a manner conceptually similar to fatty acid biosynthesis. However, these iPKS enzymes follow a complex and currently not well understood biosynthetic program to create structural diversity. Benzenediol lactones (BDL) are a growing family of polyketide natural products biosynthesized by various Ascomycete fungi. BDLs are privileged structures whose various members bind to distinct receptors, or modulate the heat-shock response and the immune system. Unusually amongst fungal polyketides, BDL biosynthesis involves collaborating and sequentially acting iPKS enzyme pairs that can be viewed as subunits of a BDL synthase. Combinatorial biosynthesis aspires to exploit the promiscuity of microbial anabolic pathways to engineer the synthesis of new chemical entities in an environmentally sustainable, economical, and inherently scalable manner. Our group has used BDL synthase iPKSs that produce distinct BDLs to investigate various methods of combinatorial biosynthesis. Upon heterologous expression in the model host organism *Saccharomyces cerevisiae*, engineered BDL synthases were used to produce known and novel BDLs whose structures were elucidated. These structures informed us on the idiosyncratic programming rules operating in the iPKS enzymes.
In this presentation, I will review our work on engineering BDL biosynthesis by: A) Structure-guided rational mutagenesis to edit product specificity; B) Domain replacements to engineer novel biosynthetic programs; and C) Subunit combinations to create product structural variety. Broadening the medicinally relevant chemical space of polyketides by such methods will provide "unnatural products" as valuable entry points for drug discovery and development.

## NEW PERSPECTIVES IN THE TREATMENT OF *CHLAMYDIA TRACHOMATIS* INFECTIONS: PHENOTHIAZINES AND DISILOXANE DERIVATIVES AS POTENTIAL ANTI-CHLAMYDIAL AGENTS

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Chlamydiae are Gram-negative obligate intracellular bacterial pathogens responsible for a range of diseases of clinical and public health importance. The obligate intracellular bacterium *Chlamydia trachomatis* invades into host cells to replicate inside a membrane-bound vacuole called inclusion. *C. trachomatis* D is of great public health significance because of the impacts of untreated diseases on reproductive outcomes. Ascending uterine infections can lead to pelvic inflammatory disease

(PID), tubal scarring, ectopic pregnancies, and infertility. *C. trachomatis* infection is easily and effectively treated with antibiotics. The current recommended treatments for genital tract infections caused by *C. trachomatis* are azithromycin or doxycycline. There is emerging evidence that *C. trachomatis* is developing antibiotic resistance, with some clinical isolates having single and multidrug resistance when cultured *in vitro*. Development of new antibiotics is required to overcome this problem. *C. trachomatis* D (UW-3/Cx) was propagated on HeLa cells. The partially purified and concentrated elementary bodies (EBs) were aliquoted and stored at -80 °C until use. The titre of the infectious EBs was determined by indirect immunofluorescence assay. EBs of *C. trachomatis* D ($4x10^4$ IFU/ml) were incubated with phenothiazines and disiloxane compounds at various concentrations in sucrose-phosphate-glutamic acid buffer (SPG) for 2 h at 37 °C. As a control, *C. trachomatis* D was also incubated in SPG alone. To quantify the anti-chlamydial effects of compounds, HeLa cells were seeded in 24-well tissue culture plates with 13-mm cover glasses. After 24 h, the confluent cells were infected with compounds-treated *C. trachomatis* D or the non-treated controls. After 48 h, the cells were fixed on cover glasses with acetone at -20 °C for 10 min. and stained with monoclonal anti-*Chlamydia* LPS antibody and FITC-labelled anti-mouse IgG. The number of *C. trachomatis* D inclusions was counted under a UV microscope, and the titre was expressed in inclusion forming units/ml (IFU/ml). After the determination of non-toxic concentrations of phenothiazines and disiloxane derivatives on HeLa cells, the anti-chlamydial effect of the compounds were determined on HeLa cells. Promethazine (PMZ) could inhibit completely the formation of inclusions at 2.5, 5, and 10 μg/ml, furthermore chlorpromazine was effective at 0.5 μg/ml concentration showing 94% of inhibition of inclusion formation compared to the controls. In addition, the previously patented disiloxane derivative SILA-421 showed 100% inhibition at 5 μg/ml. It can be concluded that phenothiazines and disiloxanes could be used as effective antimicrobial agents against *C. trachomatis*. Since thioridazine has been shown to be effective against *Mycobacterium tuberculosis*, the further studies could support the use of phenothiazines as potential candidates in anti-chlamydial therapy.

## CORRELATION BETWEEN CARRIAGE OF VIRULENCE-ASSOCIATED GENES AND LETHALITY IN A NEUTROPENIC MOUSE MODEL AMONG DIFFERENT *PSEUDOMONAS AERUGINOSA* CLONES

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Murine virulence of endemic *Pseudomonas aeruginosa* clones and sporadic isolates was compared with virulence gene composition. Two endemic clones of *Pseudomonas aeruginosa* as determined by pulsed field gel electrophoresis as well as 22 independent isolates were screened for 15 virulence-associated genes (*exo*S, *exo*Y, *exo*U, *exo*T, *fpv*A-I, *fpv*A-III, *phz*I, *phz*II, *phz*S, *phz*M, *las*B, *pil*A, *apr*, *tox*A, *alg*D, *plc*H, *plc*N). A neutropenic mouse model was used to determine the correlation between virulence-assocatiod genes carriage of different cluster and lethality. Female mice weighing approximately 25 g were rendered neutropenic by intraperitoneal administration of cyclophosphamide (200 mg/kg of body weight/day) on days −4, +1 and +4 post-infection. Disseminated infection was produced by injection of an inoculum of $1.8x10^5$ CFU/mouse in 0.2 mL volume via the lateral tail vein. The mice were followed up for two weeks. All isolates were tested

in triplicates. Survival was analysed using Kaplan-Meyer test. Cluster A included eight isolates. The cluster was characterized by presence of *fpv*A-I and all other virulence genes were found. Cluster B contained 49 isolates. All of the isolates carried *fpv*A-III, *phz*I, *phz*II, *apr*, *las*B, *exo*S and *exo*Y, however the genes *pil*A and *exo*U were absent. Virulence gene distribution was very heterogeneous among the 22 unique isolates; only *phz*II, *apr*, *exo*T, and *plc*H genes were present in all isolates; however, the other genes, except *pil*A and *exo*U, were found in 16 of 22 isolates. Only a single isolate harboured all 15 tested virulence determinants. Highest lethality rate was found in case of a sporadic isolate, which was more virulent than any of the clones or of other isolates ($p=0.02$-$p<0.001$); its virulence gene set lacked only *pil*A and *exo*U genes. This was followed in virulence by two further sporadic isolates with virulence gene set identical to the former isolate. All other four isolates tested, including those representing cluster A and B showed similar virulence, which was markedly lower than that of the former three isolates ($p<0.001$ in all comparisons). Notably, the virulence gene set of isolate representing cluster B was identical to the more virulent isolates excepting ferripyoverdin receptor type (type III vs. type I). In conclusion, the examined virulence genes do not fully explain the differences in virulence between isolates and the virulence may have played little role in the maintenance of the endemic clones.

## GENETIC DIVERSITY AND CATABOLIC ACTIVITY PROFILES OF RHIZOSPHERE BACTERIAL COMMUNITIES FROM SOLONCHAK GRASSLANDS IN APAJPUSZTA, KISKUNSÁG NP, HUNGARY

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Conservation of saline-alkaline soils of the Great Hungarian Plain are of great importance because they provide habitat for a unique halo- and alkaliphilic flora and fauna. We conducted a preliminary study in the Kiskunság National Park, nearby the village Apaj, to reveal the differences in the soil microbiota of four solonchak soils with differing vegetation types. Soil samples were taken twice in 2014: in June which is the dry season, and in September, when the area was wet or even waterlogged. The soil physical and chemical properties (texture, pH, water, salt and humus content) were examined and were find to be quite different at the four sites: the *Lepidio crassifolii-Camphorosmetum annuae* site "vakszik" had the highest salt content and pH and the lowest humus content followed by *Lepidio crassifolii-Puccinellietum limosae* "mézpázsitos" then the *Artemisio santonici-Festucetum pseudovinae* "ürmöspuszta" and the highly diverse *Achilleo-Festucetum pseudovinae* shortgrass pasture "sziki legelő". A genetic fingerprinting method (denaturing gradient gel electrophoresis, DGGE) and community level physiological profiles (CLPP) assessed by MicroRespTM method were used to compare the microbial communities of each sites. Multivariate statistics (PCA and UPGMA) revealed that the four communities were well separated from each other both in June and in September samples according to the genetic profile as well as the catabolic activity profile which can be attributed to the differences in soil properties.

Seasonal changes were also detected. Soil chemical properties, mainly the salt content, alkalinity and humus content were strong selective factors not only for plant species distribution but also to the belowground bacterial communities.

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## HORIZONTAL GENE TRANSFER BY STX PHAGES AND THE EMERGENCE OF PATHOGENIC *ESCHERICHIA COLI*: THE WHOLE PICTURE

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Shiga toxin-encoding phages (Stx phages) are highly diverse entities responsible for the acquisition of the most dangerous virulence trait of pathogenic *E. coli* strains, the Shiga toxin. Stx phages are temperate phages that after infection of the bacteria can remain as prophages inserted in preferred specific sites of the bacterial chromosome in a latent state. The presence of one or more Stx phages in a lysogenic bacteria induce phenotypic changes in their bacterial hosts and regulate other characters of the wild type strains. Bacteria have however developed mechanisms that should prevent lysogeny by Stx phages, although wild type pathogenic *E. coli* seem to have a predisposition to incorporate prophages in their genome. In a Stx phage lysogen, the prophage induces its lytic cycle when some environmental factor affects the cell, usually by activating the bacterial SOS response. When they activate their lytic cycle, the propagation of the phage genome also multiplies the number of stx copies, increasing Stx production and making the strain more virulent. The Stx production is therefore highly dependent on the phage regulation, particularly the subtype Stx2, that also uses the phage-mediated lysis to release the cell. On the contrary, for Stx1 subtype the toxin production could be dependent or independent of phage induction, since Stx1 possesses and independent promoter regulated by the presence of iron. Under conditions that activate the phage lytic cycle, the benefit of lysogeny by Stx phages becomes a paradox that poses a threat for bacterial population survival. Indeed, to express Stx and increase its pathogenicity, the cell should activate an element that will ultimately cause its death. Single-cell analysis could demonstrate that the population overcomes this paradox by showing heterogeneity on phage induction. Some intrinsic elements of the host cell also regulate the heterogeneity on phage induction of the population.
Finally, once released, Stx phages can be found free in different extraintestinal environments, where they can be abundant and able to persist for some period of time until they encounter a suitable host and transduction could take place. Recent examples can be found on the emergence of new and dangerous serotypes, highlighting the role of the environment in the horizontal gene transfer and the generation and evolution of *E. coli*. Here, the whole picture on Stx phages and the emergence of pathogenic E. coli and the most recent contributions on this subject are presented.

## EXCISION AND CONJUGAL TRANSFER OF A GENOMIC ISLAND: INVESTIGATIONS OF *SGI*1-ENCODED ORFS OF UNKNOWN FUNCTIONS

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The multidrug resistant *Salmonella* Genomic Island 1 (SGI1) emerged in the 80's in *Salmonella enterica* serovar Typhimurium. This multiresistant pandemic clone with supposedly enhanced virulence spread quickly worldwide in the 90's. SGI1 belongs to the class of integrative mobilizable genetic elements. It contains a complex In104 integron encoding the resistance for ampicillin,

chloramphenicol, florphenicol, streptomycin, spectinomycin, sulphonamides and tetracycline. Many variants sharing common backbone, but encoding different resistance pattern have been found in several pathogen *Salmonella* serovars and a few *Proteus mirabilis* strains, which represent serious health risk for humans and livestocks. Some ORFs of the conserved backbone of SGI1 have been shown to participate in the site-specific excision/integration of the island, nevertheless majority of ORFs have unknown functions. The large conjugative plasmids of IncA/C family take part exclusively in the transfer of SGI1 as specific helpers, although the genetic basis of this connection is mostly unexplored. Aims of this work are to identify the relevant ORFs and non coding sequences involved in the horizontal transfer of SGI1 and to investigate the molecular mechanisms of this process and the genetic background of connection between SGI1 and IncA/C helper plasmids. We have generated directed deletions by the one-step gene-inactivation method using λ Red recombinase mediated homologous recombination in the conserved backbone of SGI1. This set of deletion mutants enable to study the role of SGI1 regions in the conjugal transfer, and in the specific functions such as excision which is triggered by an flhDC-like regulator encoded by the IncA/C helper plasmids. We found an flhDC homologue on SGI1 backbone which also appears to have a role in the conjugal transfer. By comparing the conjugation frequency of deletion mutant and wild type islands and by detecting excision activities with PCR, we aim to map all SGI1 genes involved in excision and transfer. Based on these results we would like to deduce and create the fully transfer competent and also simplified SGI1 model system for further investigations.

## THE HISTORY OF AN ENIGMATIC CELL ORGANELLE, THE HYDROGENOSOME

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Cell biology in the 1950s, 1960s underwent many changes through the deeper structural analysis by electron microscopy and biochemical analysis by cellular fractionation. Structure, enzymatic composition and function of major organelles of eukaryotes were largely elucidated. It became also increasingly accepted that two organelles, mitochondria and chloroplasts, arose from bacteria and cyanobacteria that were transformed into cell organelles of eukaryotic cells. The origin of the eukaryotic cell and its organelles has been much debated ever since. Mitochondria in most cells are site of respiration accompanied by ATP generation. Some unicellular eukaryotic organisms, however, do not exhibit mitochondrial type respiration. Some of these contain organelles of mitochondrion size, some do not. Starting in the 1970s we were engaged in studying parasitic anaerobic flagellates. *Trichomonas vaginalis*, a parasite of the human genitourinary tract, and other trichomonads were known to produce $H_2$, an unusual metabolic end-product for an eukaryotic organism. Cell fractionation and electron microscopy have shown this process localized in mitochondrion-sized organelles, which we named hydrogenosomes. No such organelles were found in another anaerobic parasitic flagellate, *Giardia lamblia*. However, some enzymes homologous to hydrogenosomal enzymes. Electron transport linked ATP-formation is found in neither of these organisms. The biological relationship of these organisms and that of the hydrogenosomes attracted much theoretical and experimental interest. For a while they were regarded as “amitochondriate” and in some phylogenetic hypotheses they represented ancestral eukaryotes, separated in evolution before the mitochondrial endosymbiosis occurred, they were regarded as premitochondrial. Recent studies led to a drastic revision of this problem. More and more free-living and parasitic “anaerobic” eukaryotes have been shown to follow the pattern of trichomonads and *Giardia*. Phylogenetic

reconstruction revealed that such organisms are present in numerous separate eukaryotic lineages, indicating their separate origins. Mitochondrial functions unrelated to energy metabolism were found in these organisms, indicating their origin from mitochondrion containing ancestors. Extensive sequence information on these organisms indicates that many of the "unusual" functions have been acquired by lateral gene transfer.
Our present view of the eukaryotic world is that consisting of organisms that all derive from an ancestor that contained mitochondria but reached a great morphological and never before expected biochemical diversity, already before multicellularity arose in some lineages.

## DETECTION OF WEST NILE VIRUS RNA FROM HUMAN URINE AND SERUM SAMPLES DURING THE 2014 SEASONAL PERIOD

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West Nile virus belongs to the *Flavivirus* genus, Flaviviridae family. This virus is one of the most important endemic viral zoonoses in Hungary, that regularly causes human infections. The first neuroinvasive human West Nile virus infection is dated back to 2003. Therefore, we have information about human infections since that year: the average number of cases is between 10-20 per year. However many of the cases are asymptomatic, West Nile virus infections could be associated with milder symptoms characterized by fever and rash or even with neurological diseases such as meningitis or encephalitis. In case of flaviviruses currently, mainly serological methods are applied in the human differential diagnostic laboratory, because at the time of the onset of specific clinical symptomes the detection of viral nucleic acid is difficult. Due to the nucleic acid amplification or virus isolation from living people so far was not succeeded, we have lack of information about exactly which genetic variants cause the human flavivirus infections in Hungary. However, in some cases of human West Nile virus infections, long-term viral nucleic acid-shedding with urine was published eralier. During the 2014 seasonal period our reference laboratory monitored the serologically confirmed West Nile virus infected patients and the molecular biological analysis of their serum, urine and cerebrospinal fluid samples was performed. In case of 3 patients the West Nile virus RNA detection from urine sample was successful.
This was the first time in Hungary that virus nucleic acid detection and sequence determination was possible directly from living patients.

## LOCALIZATION OF DIFFERENT ISOPRENOID BIOSYNTHETIC PATHWAYS IN *MUCOR CIRCINELLOIDES*

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Isoprenoids are one of the most diverse groups of natural chemical compounds. They have different biological functions in the cell, among others as pigments (e.g. carotenoids), cell membrane

components (e.g. ergosterol, cholesterol) or functional groups of certain proteins (e.g. RAS). Isoprenoids are synthesized via the mevalonate pathway in *Mucor circinelloides*. The central step of this pathway is the conversion of HMG-CoA to mevalonic acid catalysed by HMG-CoA reductase. *M. circinelloides* is a carotene producing filamentous fungus used as a model organism of the microbial carotenoid biosynthesis. In fungi, it is suspected that carotenoids and other isoprenoid derivatives are synthesized in different subcellular compartments. In the present study, genes of four isoprene biosynthetic enzymes (three *hmg*R and a *car*RP) of *M. circinelloides* were fused with the *gfp* gene to analyse the subcellular localization of the encoded proteins. *M. circinelloides* has three *hmg*R genes (*hmg*R1, *hmg*R2 and *hmg*R3) encoding HMG-CoA reductases. CarRP is a key enzyme of the carotenoid specific biosynthetic pathway and has phytoene synthase and lycopene cyclase activities. We have started to investigate the localization of the different isoprenoid biosynthetic pathways in the *M. circinelloides*.

Our results suggest that HmgR3 is an endoplasmic reticulum associated protein, while HmgR2 is localized to the membrane of the endoplasmic reticulum and other small compartments. Similarly to HmgR2, CarRP is localized to different subcellular compartments.

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## PREPARATION OF SCALED-UP DECHLORINATING INOCULUM IN THREE-PHASE ANAEROBIC MICROCOSMS AND MONITORING OF DECHLORINATION PROCESSES

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Halogenated aliphatic hydrocarbons were used as solvents and degreasing agents in industry, agriculture and even in households in the past few decades all over the world. Aromatic (petroleum derivatives) and aliphatic hydrocarbons and their halogenated derivatives can penetrate to soil and groundwater, and accumulate in the environment causing serious damages. 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. The key microorganisms in anaerobic, reductive dechlorination belong to the genus *Dehalococcoides*, which are strictly anaerobic bacteria and can grow well in mixed culture. The aim of our project was to enhance the complete dehalogenation processes in pilot field project. The first step was the scale up to 5L volume. A new technique was developed to set up and increase the volume of the anaerobic microcosms avoiding dissolution of oxygen in the system. The initial anaerobic three-phase microcosms were prepared using trichloroethene contaminated groundwater as inoculum. Biodegradation of the halogenated hydrocarbons were monitored by using gas chromatography and by detecting the reductive dehalogenase (trichloroethene-reductase – *tce*A and vinyl-chloride reductases - *vcr*A, *bvc*A) genes. The initial bacterial community composition and its change in the microcosms over time were assessed by Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis. The proportion of genera *Dehalococcoides*, other Bacteria and methanogenic Archaea comparing the total cell count were determined by Fluorescence In Situ Hybridization (FISH) technique. At first, optimal co-substrates for enhance dechlorination processes were defined. Twelve microcosms, containing the appropriate co-substrates, were inoculated with

contaminated groundwater as inoculum from three different sources. The presence of the genus *Dehalococcoides* was correlated with dechlorination processes observed in the groundwater. In four out of the twelve microcosms the degradation of TCE was observed in line with the increase of cis-dichloroethene. Moreover, reductive-dehalogenase enzymes were present in these microcosms simultaneously with the TCE degradation. Microcosms inoculated with distinct groundwater showed remarkable differences due to the dehalogenation process present. Even the total bacterial community structure of the groundwater was altered in the microcosms. The initial cell counts increased in the microcosms over time. FISH was optimized to detect genus *Dehalococcoides* and methanogenic Archaea, which had significant proportion to the total cell count.
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## MONITORING THE SURVIVAL AND EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA APPLIED ON ACIDIC AGRICULTURAL SOIL

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Experimental mixtures of different plant growth promoting rhizobacterium (PGPR) strains were applied for inoculating agricultural fields where soil had acidic characteristics to enhance corn plant growth and crop yield. Three different combination of certain PGPR-s were used in two different quantities and the effect of inoculation was being monitored for 16 weeks. The changes in total bacterial community structure of rhizosphere were examined by Terminal Restriction Fragment Length Polymorphism (T-RFLP) and prior to that three restriction endonucleases had been selected *in silico* for specific detection of the applied strains. All the applied combinations contained two strains which may be capable for nitrogen-fixation and auxin production to help plant growth. Both of them showed a 2-4% increase in relative abundance in the 1st week in all cases of treatments compared to untreated control. Another strain which was used in every combination belongs to the species which can help nickel translocation of the plant; furthermore it can have antagonistic effect against phytopathogen organisms. Its ratio increased by 2-14% in the 8th week in all cases of treatments compared to control soil. Four of the applied six treatments contained at least one of those two strains which so may have antagonistic effect and additionally can produce siderophores to help plant iron-uptake. The relative abundance of these strains began to increase slightly in the 8th week or later but only in three cases of the combinations compared to untreated control. One of the applied strains belongs to a species whose members are capable for nitrogen-fixation and to produce antimicrobial compounds against the broad spectrum of phytopathogen bacteria and fungi. Another used strain can solubilize phosphate and zinc; the plant needs the latter for chlorophyll synthesis. Additionally, the members of this species can induce systemic resistance of the plant. If a treatment was applied which contained any but only one of these two strains, then its relative abundance increased by 1-2% in the 1-2nd week compared to untreated control.
If both were present in the used combination then they showed increase in relative abundance (~2% compared to untreated control) in the 8-12th week.

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## ESTABLISHING EPSTEIN-BARR VIRUS LATENCY IN MEMORY B CELLS - IMPLICATIONS FOR TUMORIGENESIS

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Ground-breaking work has established the blood memory B cell as the site of permanent EBV latency *in vivo*. However, there is some uncertainty on which path permanent viral latency is established. The answer to this question is important, because it has deep implications for the molecular mechanisms of EBV-associated tumorigenesis, especially endemic Burkitt lymphoma (BL). *In vitro* experiments have shown that upon infection of isolated peripheral blood B cells, linear EBV genomes are circularized to become covalently closed circular (ccc) extrachromosomal episomes as early as 16 hours post infection. Infected G0 cells do not even need to pass through G1 or to enter S phase for EBV genomes to become circularized, however, a single G0 to early-G1 transition is sufficient for circularization. Contrary, in already activated or cycling cells, the incoming EBV genome is subject to a linear chromosomal integration into the host cell genome, but not to circularization. The possibility of quickly establishing viral latency before cellular activation or proliferation take place reflects on the situation *in vivo* during primary infection and on the rise of BL. Different pathways for BL oncogenesis have been proposed. A traditional view is that upon EBV infection of naïve cells in the tonsils, expanded and growth-transformed infected B cells undergo a germinal center passage, upon which they end up as EBV-infected memory B cells. According to this model, the viral latency gene expression pattern would change in concordance with the B cell developmental stage on its path through the germinal center (GC) reaction. An implication of this model is that the EBV-transformed lymphoblastoid cell (LC) is the direct precursor of the BL cell. However, considering the possibility of a quick circularization and chromatinization of EBV genomes in non-cycling cells, a prior LC stage is not really required for the establishment of latency in memory B cells. Considering also that the methylomes of the EBV-transformed LC and the physiologically antigen-activated B cell which undergoes a GC reaction have been shown to be fundamentally different, a different scenario for BL oncogenesis seems more likely: EBV-transformed LCs exhibiting a latency class III expression pattern are entirely extinguished by the vigorous immune response in the course of infectious mononucleosis. Under conditions of GC hyperstimulation, e.g. in patients with malaria tropica or early stages of HIV disease, an occasional EBV-infected memory B cell may suffer a c-Myc translocation while undergoing a GC passage. Then, BL oncogenesis depends on c-Myc binding to its recognition site in the locus control region of the EBV genome which expresses latency type I or II functions, but not EBNA2. Due to the anti-apoptotic functions of the EBV genome, such a "crashed" B cell may have a bigger than remote chance to survive the GC reaction and become the founder cell of a hematological malignancy, like a BL or a Hodgkin lymphoma.

## INVESTIGATION OF Δ9 AND Δ6 FATTY ACID DESATURASE GENE EXPRESSION IN POLYUNSATURATED FATTY ACID PRODUCING *MORTIERELLA* AND *UMBELOPSIS* STRAINS

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*Mortierella* and *Umbelopsis* species are oleaginous microorganisms belonging to Zygomycetes, which are particularly active in polyunsaturated fatty acid (PUFA) synthesis. PUFAs are elemental structural components of biological membranes and they are also precursors of a wide variety of metabolites regulating critical biological functions, such as prostaglandins, leukotrienes and hydroxy-fatty acids. They have beneficial effects on human health as ω-3 PUFAs have significant role in the prevention of the development of cardiovascular diseases, whilst ω-6 PUFAs are important in inflammation processes. Fatty acid desaturases are important in PUFA biosynthesis, which can form double bonds in different sites of the carbon chains. The aim of our work was to investigate the expression of the genes encoding Δ9 and Δ6 fatty acid desaturases in some *Mortierella* and *Umbelopsis* strains under different culturing conditions. Seven strains with different PUFA yield and profile were investigated. *Mortierella* strains produced mainly arachidonic acid, whilst *Umbelopsis* strains produced γ-linolenic acid, linoleic acid and oleic acid. The isolates of *Mortierella wolfii* and *Mortierella simplex* produced linoleic acid instead of arachidonic acid. The partial sequence of the Δ9 and Δ6 desaturase encoding genes as well as the actin gene, which are used as control in the expression studies, have been identified in these strains. The effect of different culturing conditions, such as cultivation time, temperature and medium composition on the expression of desaturase encoding genes was investigated. The highest fatty acid production was observed after 7 or 8 day incubation, whereas the expression of desaturase genes was maximal on the 4th or 5th day. When the effect of temperature was investigated, the fatty acid production was the highest on 20 and 25 °C, and PUFAs containing two or three double bonds are preferentially produced on lower temperature. The expression of the Δ9 and ω9 desaturase genes was the highest on 20 and 25 °C as the fatty acid yield, whilst the expression of the Δ6 desaturase gene increased with the increasing temperature. The effect of medium composition on the PUFA production was also investigated. However, the biomasses were the highest in MEA and GY, the PUFA production and the expression of desaturase genes was the highest in YNB and PDB medium. The investigation of external fatty acids and different plant oils on fatty acid desaturase gene expression is also investigated. Oleic acid enhanced the expression of Δ6 desaturase gene in *M. alpina*, however in *M. antarctica* all external fatty acid resulted in lower desaturase gene expression. Plant oils containing mainly polyunsaturated fatty acids resulted in also lower desaturase gene expression, whilst olive oil, which contained monosaturated fatty acids, slightly enhanced the desaturase gene expression.

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## ACCUMULATING EVIDENCE FOR THE ECOPARALOGOUS WAY OF MICROBIAL ADAPTATION

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Microbes inhabit spaces on Earth that often seem extreme and unpleasant for our eyes and are well adapted to wide ranges of various environmental conditions like temperature, pH or salinity. These conditions describe an ecological niche a set of optimal parameters for the organism but in real life environmental conditions might fluctuate drastically even in the same spot or habitat. Recent advantages in DNA sequencing and proteomics gave us an insight into the genomic content of microorganisms enlisting genes and functioning proteins making this adaptation possible. Genomic and metagenomic analyses showed how new genes arise via duplication and radiation during evolution securing adaptation of the microorganisms all still fitted in a limited small genome [1, 2]. In the pioneering paper of Sanchez-Perez et al. in 2008 the concept of ecoparalogs was suggested [3]. Ecoparalogs are paralogous gene copies (two or more) coding for the same molecular function in one genome or organism to provide adaptation to different ranges of environmental factors such as salinity or temperature. At the formulation of this concept mainly data from in depth genome analyses of *Salinibacter ruber* (Bacteriodetes, Bacteria) corroborated the idea. Since the first publication new examples from comparative genome analyses and experimental evidence accumulated to affirm this concept [4]. Novel findings will be reviewed and evaluated here.

[1] Hooper, S.D. and Berg, O.G. (2003) Mol Biol Evol 20, 945–954.
[2] Francino, M.P. (2005) Nature Genet 37, 573–577.
[3] Sanchez-Perez, G. et al. (2008) Trends in Genet 24, 154–158.
[4] Syutkin, A. S., et al. (2014) Extremophiles 18, 341-349.

## DRUG TARGETING STRATEGY WITH POLYEPTIDE BASED METHOTREXATE CONJUGATES AGAINST *LEISHMANIA* INFECTION

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A promising approach for killing intracellular parasites could be the influence of folate metabolism by using the specific dihydrofolate reductase inhibitor methotrexate (MTX) [1]. We have demonstrated that MTX conjugates with poly[L-lysine] based macromolecular carrier exhibit efficient against-leishmanial effect under both *in vitro* and *in vivo* conditions. Conjugates against *Leishmania donovani* infection contained amfoteric or cationic, branched chain macromolecular carrier (poly[Lys(Xi-DL-Alam)], XAK or poly[Lys(DL-Alam-Xi)], AXK). The most efficient of these compounds was the MTX-ALK (poly[Lys(MTXj-DL-Alam-Leui)]) conjugate, which decreased pronouncedly the parasite number in *L. donovani* infected peritoneal macrophages *in vitro*, as well as in the liver of Balb/c mice infected with *L. donovani* amastigotes *in vivo* [2]. Other MTX-polypeptide conjugates with similar structure containing a GFLGC (Gly-Phe-Leu-Gly-Cys) pentapeptide spacer and a- or g-amide linkage like (SAK(aL-MTX-GFLGC) and SAK(aD-MTX-GFLGC) initiated a chemoattractant or neutral response on *Leishmania brasiliensis* promastigotes in contrast with free MTX that elicited a repellent chemotactic response. [3]. Pattern recognition receptors like scavenger receptor could also be appropriate targets for selective drug delivery into

macrophages, as several scavenger receptors including SR-A1 expression is increased following to Leishmania infection [4]. Our investigations on J774 monocyte-macrophage cell line and murine bone marrow derived macrophages proved that polyanionic (succinylated and maleylated) branched chain polypeptides with poly[L-lysine] and their daunomicin conjugates were taken up by the cells via scavenger receptors [5, 6]. Here we report on the recent results of on the cellular uptake mechanism of ALK, the carrier of MTX conjugate with marked anti-leishmanial effect and structurally related polypeptides on murine bone marrow derived macrophages.

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[1] Schüttlekopf, A.W., et al. (2005) J Mol Biol 352, 105–116.
[2] Kóczán, G. et al. (2002) Bioconjugate Chem 13, 518-524.
[3] Díaz, E., et al. (2013) Exp Parasitol 135, 134-141.
[4] Tempone, G. A. et al. (2004) J Antimicrob Chemother 54, 60–68.
[5] Szabó, R. et al. (2005) Bioconjug Chem 16, 1442-1450.

## MOBILE GENOMIC ISLANDS: THE KEY ROLE OF EXTRACHROMOSOMAL AND INTEGRATIVE MOBILE ELEMENTS IN HORIZONTAL GENE TRANSFER

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The plasticity of bacterial genomes enables the rapid adaptation to the changing environmental conditions. Horizontal gene transfer (HGT), by which bacteria can acquire and disseminate many beneficial traits, is mainly responsible for this plasticity. Conjugative elements such as plasmids, some genomic islands (GIs) and transposons have a key role in HGT since their mobility can lead to the rapid acquisition of virulence, pathogenicity or resistance factors and/or complete metabolic pathways of bacteria. Recent investigations of bacterial genomes put the mobile GIs in the spotlight of scientific interest. Mobile GIs are classified into two groups: integrative/conjugative elements (ICEs) and mobilizable genomic islands (MGIs). Unlike ICEs, MGIs are not self-transferable, so they require helper elements (ICEs or conjugative plasmids) that can provide the missing conjugative functions. GIs are stable part of the bacterial chromosome. They can not maintain extrachromosomally, but can be excised by site-specific recombination, transferred to other bacteria by conjugation and integrated into the chromosome of the recipient cell. These maintenance and transfer functions are encoded by the "backbone" of GIs, while another group of genes coding for antibiotic resistance, pathogenicity, catabolic pathways etc. confers adaptive functions to the host. The structure of mobile GIs shows remarkable flexibility: they evolve by acquisition, deletion and exchange of genes or gene clusters via homologous and/or site-specific recombination or transposition. One of the most studied MGIs is the Salmonella genomic island 1 (SGI1), which contains several antibiotic resistance genes embedded in the complex In104 integron segment. Prototype of SGI was detected in multiresistant *Salmonella enterica* serovar Typhimurium DT104 isolates, but its variants have also been identified in many other *Salmonella* serovars and in *Proteus mirabilis* isolates. Interestingly, SGI1 has never been found in natural *Escherichia coli* isolates even though it can easily be transferred into *E. coli* under laboratory conditions. SGI1 is a typical MGI, which is mobilized exclusively by the conjugative helper plasmids of IncA/C family. The first step of SGI1 transfer is the excision from the bacterial chromosome, which is carried out by the SGI1-

encoded site-specific recombinase Int and Xis. The induction of excision and the transfer process also requires helper plasmid-encoded functions.
Beside the conjugation apparatus (T4SS) of IncA/C plasmids, SGI1 also exploits regulatory functions of the helper plasmid. The plasmid-encoded FlhDC-family master regulator controls all conjugation genes of the plasmid including the relaxase, a key factor in the transfer initiation step, the operons of pilus assembly and perhaps some other functions. The master regulator triggers the excision of SGI1, i.e. signals the presence of a helper plasmid for SGI1, thus SGI1 hijacks both the regulation and the conjugation apparatus of IncA/C plasmids for its horizontal transfer. On the contrary, only SGI1 encoded functions are required for the integration into the recipient chromosome. SGI1 represents a good example how MGIs ensure their vertical transmission in absence of a helper and how they can exploit their helpers for horizontal spread.

## XMRV – A NEW RETROVIRUS

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Recently the presence of a new retrovirus has been detected in prostate cancer (PCa) specimens and permanent PCa cell lines, exclusively in US laboratories. Sequencing showed its close relationship to Gammaretroviruses, especially mouse retroviruses. It was designated as "xenotropic murine retrovirus-related virus, XMRV". Infectious particles could be cultured in DU145 and LNCaP cells, the receptor (XPR1) also was identified. Electron microscopy showed typical C-type particles resembling Moloney murine leukemia virus (MoMuL). Viral polypeptides were visualised by using anti-MuLV antibodies. A unique 24 nucleotide deletion was shown in all isolates as compared to MuLV DG75. Isolates showed 98% nucletide, and 99% amino acid homology, and high homology to MoMuL. Sites and other characteristics of integration were demonstrated in several PCa cultures and specimens, especially obtained in the hereditary forms with RNaseL and APOBEC A3G deficiency. Patients homozygous for RNaseL deficiency carried XMRV at the highest ratio (40%). Neutralising antibodies were shown in their serum. The rate of virus detection increased with the severity of Gleason score. Androgens, dexamethasone activate, IFN-b and some HIV integrase inhibitors block XMRV replication. XMRV was also detected in RNaseL deficient immune cells of patients with chronic fatigue syndrome. A publication error shed light on the problems in XMRV research. This virus was not detected outside US. Reagents used for other murine retrovirus studies resulted in false positivity in XMRV research. As retrospective studies showed, through maintenance of an XMRV negative prostate cancer specimen by serial passages in nude mice between 1992 and 1996, cells were contaminated by two endogenous retroviruses, subsequently their recombination occured. The established permanent cell line, 22Rv1 already contained XMRV. Its wide distribution among oncology and virology laboratories transmitted contamination to many other cell cultures. This case is an extremely important warning sign regarding biosafety practicised in laboratories working with cell cultures and reagents of murine origin.
As XMRV can replicate in human cells, there is a high risk that through accidental infection or even a criminal case, it spreads to the human population causing an unknown disease.

## TOXICITY INVESTIGATION OF AIR SAMPLES WITH MICROTITER PLATE METHODS

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Toxicological investigations were performed with air samples collected in the central part of the town Szeged, Hungary. From the air filters water extracts were made by a gentle sample pre-processing method; this ensured that toxic compounds were dissolved from the filter but the extracts become germfree. The filter extracts were processed in Eppendorf-tubes with sterile beads in a high frequency Eppendorf-tube shaker. An important task was the removal of the heat- and radiation-resistant *Bacillus* spores which were present in substantial amount on the surfaces of the air filters. Instead of heat or radiation treatments, the extracts were centrifuged through spin columns containing cellulose acetate membrane (0.22 micron pore size). Cytotoxicity investigations were performed with these germfree extracts applying the *Pseudomonas putida* growth inhibition test in a microplate-based cultivation system. The growths of *Pseudomonas* cultures were followed via turbidimetry with a microtiter plate photometer. We worked out and evaluated a simple Ames test system for the genotoxicological measurement of air samples. Two tester *Salmonella typhimurium* strains were employed: TA98 detects mutagens causing frameshift mutations while TA1535 detects base substitution mutations. In our new approach of Ames test, we washed the cells in minimal liquid medium, resuspended them in the same medium and from this suspension $10^7$ cells were pipetted in 150 µl volume to the wells of a sterile microtiter plate (with lid). To the cell suspensions, 50-50 µl of the sterile filtered environmental samples were added. The optical density of the mini-cultures was measured at 620 nm immediately and after 48 hours of incubation at 35 °C. The increase in absorbance at 620 nm reflects the mutagenicity of the samples. Positive correlation was found between the cytotoxicity and genotoxicity data series.
In the case of some samples the adding of S9 enzyme fraction of homogenized rat liver to the samples significantly elevated their mutagenicity.
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## CAPILLARY BLOOD OR VENOUS BLOOD? AN ALTERNATIVE SPECIMEN COLLECTION FOR THE RAPID DIAGNOSIS OF EBOLA VIRUS INFECTION DURING AN OUTBREAK EMERGENCY

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The largest Ebola virus outbreak in West Africa was first described in March 2014 in Guinea. Until the end of April, 2015, the World Health Organisation (WHO) reported a total of 26 312 cases of Ebola virus disease (EVD) with 10 899 deaths, making this outbreak in West Africa the most widespread and the most complex Ebola outbreak since Ebola virus was first discovered in 1976. Currently the reliable laboratory diagnosis of Ebola virus infection based on polymerase chain reaction using whole blood, plasma, or serum collected by venipuncture. Venous blood sampling has

a high risk for needle stick injuries, requires trained and skilled medical personnel and difficult to perform with newborns and infants. Moreover, due to cultural and religious beliefs the patients in African often refuse venous venipuncture. To overcome these problems, we evaluated the suitability of capillary blood sampling from fingersticks in the diagnosis of EVD. A total of 120 venous and capillary blood samples were collected from 53 patients suspected of having EVD in Guinea between July and August 2014. All sample specimens were analyzed by RT-PCR using the RealStar Filovirus Screen RT-PCR Kit 1.0 from Altona Diagnostics (Germany). Our main objective was to compare the sensitivity and specificity of the RT-PCR tests that used capillary blood samples with samples obtained by venous puncture during an outbreak emergency. Furthermore, we assessed the stability of capillary blood swab samples in a limited study on the field.
Our data suggest that capillary blood samples could serve as an alternative to venous blood samples for the diagnosis of EVD in resource-limited settings during an emergency crisis.

## ANDROID SMARTPHONE APPLICATION FOR THE AUTOMATIC COUNTING OF BACTERIAL COLONIES

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Counting bacterial colonies on agar plates is a basis of the diagnosis of significant bacteriuria and also used for determining the effect of known and novel antibiotics on bacterial growth. The manual counting is labour-intensive and subjective, while the available hardwares for automatic counting are expensive and not mobile. To solve these problems, we are developing a user-friendly and accurate smartphone application for the automatic counting of bacterial colonies. Images taken with the smartphone is being analyzed by a custom Android software, built on Matlab and ImageJ applications, written in Java. For image manipulation OpenCV library functions and classes were used. At this point the software is on a developmental phase, however preliminary measurements have already been performed. To test the accuracy of the software, serial dilutions were made of *Escherichia coli, Enterococcus faecalis* and plated on 8 cm diameter Chrom agars. Bacterial colony counts were enumerated by manual and automatic colony counting. The software was able to detect various types of the colonies with high sensitivity and dynamic range up to 1500 colonies/ plate. The approximate processing time from taking the image to the end of enumeration was 20-60 seconds, depending on the number of colonies. The comparison of manual and automated colony countings showed high correlation when bacteria were grown on Chrom agar: *Escherichia coli* (R2:0.96), *Enterococcus faecalis* (R2:0.98). Mixed *Escherichia coli* and *Enterococcus faecalis* colonies modelling multibacterial urine infections were also counted accurately, independently from each other. Modern smartphones equipped with quality cameras and efficient processors are proper choices for the colony counting task. Our smartphone application was able to detect colonies of various bacteria with good accuracy and dynamic range. We are currently optimizing the software using other bacterial strains and agars, and also optimizing the processing time.
Our application combined with the readily available smartphones could provide an affordable, easy to use and mobile solution of the colony counting task.

## INTERSPECIFIC COMPLEMENTATION ANALYSIS BETWEEN *SCHIZOSACCHAROMYCES POMBE* AND *CANDIDA ALBICANS*

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*Candida albicans* is the most common dimorphic human fungal pathogen. The pathogenesis of its infection is poorly understood. A recent study showed that Cas5 played an important role in the pathogenesis of *Candida albicans* infections and it revealed that Cas5 is required for expression of caspofungin-inducible genes, many of which had connection to cell wall integrity. Since defense of the host immune system based on the recognition of conserved molecular patterns of the fungal cell wall, deficiencies in the cell wall structure might promote the recognition and elimination of *Candida albicans* by host immune cells. *Cas*5+ encodes a zink-finger transcription factor that is partially homologous with *rsv*1+ gene of *Schizosaccharomyces* group. Based on the sequence similarity it is presumable, that *rsv*1 and *Cas*5 have the same function. To prove their functional homology, we started the interspecific complementation analysis. Namely, we amplified the *Cas*5+ gene by PCR and cloned into *S. pombe* nmt promoter containing overexpression vector. This DNA construction was transformed into the *S. pombe rsv*1 mutant strain. The characterization and the caspofungin sensitivity of the transformants are in progress.

## *FHL*1 CONTROLS TRANSCRIPTION OF THE NITROGEN STARVATION INDUCED GENES IN COOPERATION WITH TORC1 IN *SCHIZOSACCHAROMYCES POMBE*

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One of the most important regulators of cellular processes is the protein kinase TOR (Target of Rapamycin), which is conserved from yeast to humans. TOR is a protein kinase that was isolated as the target of the immunosuppressive and anticancer drug rapamycin. It regulates the response to environmental changes and cellular energy status, cell growth, cell proliferation, cell survival, protein synthesis, and transcription. In the fission yeast, *Schizosaccharomyces pombe* there are two TOR homologs. Tor1 is part of a protein complex known as TORC2, while Tor2 is part of a protein complex known as TORC1. These complexes were found to regulate oppositely the response to nutrient depletion, in particular the response to nitrogen starvation. TORC1 is essential and plays major role in the control of cellular growth, while TORC2 regulates cell survival under stress conditions. Here we demonstrate that overexpression of *fhl*1+ gene suppresses the mRNA level of the nitrogen starvation response genes: *mei*2, *ste*4, *ste*11 and decreases the mating efficiency of *tor*2-ts mutant. Moreover overexpression of *fhl*1+ modifies the stress response of *tor*2-ts mutant, among others in the presence of rapamycin.

Based on these findings we can conclude that *fhl*1 plays a role in the TOR pathway and *tor*2 controls the response to nitrogen starvation via *fhl*1 transcription factor.

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## UNRAVELING THE ABILITIES OF *AGARICUS BISPORUS* TO DEGRADE PLANT BIOMASS THROUGHOUT ITS LIFE CYCLE

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The common edible mushroom *Agaricus bisporus* is a basidiomycete that thrives on decaying plant material in the forests and grasslands of North America and Europe. It is adapted to forest litter and contributes to global carbon recycling, degrading cellulose, hemicellulose and lignin in plant biomass to oligomers and monomers. *A. bisporus* is also an edible mushroom that is widely cultivated and economically important. But the process of growing *A. bisporus* in compost and utilization of this substrate is poorly understood. In this study, we performed a wide analysis of genes encoding plant biomass degrading enzymes using high throughput sequencing (RNA-Seq) and lignocellulolytic enzymes secretion by *A. bisporus* grown in compost under commercial conditions to understand the carbon nutritive needs of the fungus and its capabilities to degrade plant biomass. Clear correlations were observed between secreted extracellular polysaccharide degrading enzymes, the expression of the corresponding genes and the composition of compost, which is rich in plant material such as cellulose and hemicellulose.

Differences in the expression of genes from different stages of development were detected between spawning, pinning and harvesting stages, suggesting that as soon as the monosaccharides that were released during composting are depleted, *A. bisporus* starts producing enzymes to degrade plant biomass components to satisfy its nutritive needs. Interestingly, our results also uncovered differences in gene expression and secreted proteins between two different harvesting flushes that might be corresponded to the loss in number of mushrooms during second harvesting flush.

## EXPLORING THE MICROBIOTA OF SAMPLES FROM TRADITIONAL DAIRY PRODUCTS DERIVED FROM A TRANSYLVANIAN FARM

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Microbial diversity of factory made dairy products is rather low due to standardized technology and starter cultures used for their production. Natural microbiota can be found where still traditional ways are used to produce dairy products. Studies on the microbial diversity of samples, collected on a Transylvanian farm from curd, kefir, fresh and ripened cheeses as well as a bovine maw prepared for milk inoculation, were initiated. About 2000 culturable microorganisms were isolated from the samples and they were grouped according to their viability in different growth conditions such as different media, aerobic- and anaerobic conditions. Representatives of the groups were selected for

identification by determination of the 16S RNA gene sequences. *rpo*B gene sequences were used to differentiate between strains of some species. Although we are still in the early stage of our studies, so far we have identified 185 isolates. According to differences already known in their sequences, they belong to at least 44 strains representing 31 species of 15 genera. Further characterizations of the isolates are planned including the determination of their metabolic profiles, interference with growth of other microorganisms as well as features important for dairy industry. Determination of genome sequences of strains with valuable features is also planned.

## STRESS RESPONSES IN THE ASPERGILLI – OMICS-BASED APPROACHES

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It is difficult to overestimate the environmental, industrial and biomedical significance of the aspergilli, a versatile group of ascomycetous fungi that occupy various ecological niches. Reflecting their importance, a number of *Aspergillus* genomes have been sequenced and annotated. Omics-based technologies are now targeting many understudied areas of *Aspergillus* biology, including stress responses. Comparative genomics approaches have illuminated the remarkable complexity and robustness of the stress response systems operating in the aspergilli. These systems incorporate many elements also found in the budding yeast *Saccharomyces cerevisiae* and in the fission yeast *Schizosaccharomyces pombe*. Although yeast-based models are widely used for the *in silico* reconstruction of the stress response systems of various filamentous fungi, this approach has obvious limitations considering the large evolutionary distances between these species. In this lecture we summarize our current knowledge of the stress response systems of yeasts and filamentous fungi. We also provide an overview of the Fungal Stress Response Database, a collection of fungal stress response proteins with verified physiological functions and their orthologues found in other completely sequenced fungal species. We also describe the Fungal Stress Database that incorporates stress tolerance data acquired for 17 *Aspergillus* species. Our current work aims to combine comparative genomic and stress physiology datasets in a single database. In addition, we participate in several genome annotation projects including further *Aspergillus* species and the nematophagous fungus *Drechmeria coniospora*. We identified numerous elements of the stress response systems of *Aspergillus nidulans* by large-scale transcriptomic studies in stress-exposed cultures of this model organism. At first, the sizes of gene groups solely responsive to peroxide, superoxide radical, and glutathione/glutathione disulfide (GSH/GSSG) redox imbalance were estimated. More recently, stress-responsive genes responding to three different oxidative stress conditions in the same manner (Core Oxidative Stress Response genes) were also identified in our laboratory. Importantly, our data did not support the existence of a budding yeast-like general environmental stress response (ESR) in *A. nidulans*. In a separate study, the remarkable complexity of carbon starvation stress response (CSSR) was mapped using whole-genome-based DNA microarrays. Elements of the menadione-elicited oxidative stress response of *A. nidulans* were also identified in proteomics studies, and the functional analyses of some stress-responsive genes/proteins of unknown function are now in progress. Considering future omics studies, we would like to (i) expand and improve our databases to make it easier to find fungal stress response data; (ii) include more fungal species with outstanding significance, e.g. fusaria, and also more stress signaling and regulatory mutants in stress-related omics studies, and (iii) analyze the currently available and future datasets in depth to

initiate further research in the stress biology of fungi. A deeper understanding of the organization and regulation of fungal stress response systems may lead to the development of new, stress-tolerant strains for a number of biotechnological applications. It will also facilitate the discovery of novel antifungal drugs and strategies to combat fungal infections.

## SEQUENCING AND ANNOTATION OF THE GENOME OF THE NEMATOPHAGOUS FUNGUS *DRECHMERIA CONIOSPORA* REVEALS THE ARSENAL OF AN ENDOPARASITOID FUNGUS FOR THE INVASION OF ITS HOST ORGANISMS

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The genome of the fungus *Drechmeria coniospora* exemplifies the evolutionary changes required for the successful invasion and exploitation of a nematode host organism by a near-obligatory endoparasitoid. While key elements of the basic metabolic and signaling pathways remained mostly unaffected, many gene families necessary for saprophytic competence or plant pathogenesis were found to be deflated in this fungus. *D. coniospora* relies on adhesive conidiospores to attach to and to penetrate the cuticle of the nematode. The contingent of regulatory genes for asexual sporulation were found to match those in the fusaria that feature no phialide-bearing structures, but were distinct from those of the aspergilli that develop conidiophores with a vesicle and metulae. Reconstruction of the central regulatory pathway of conidiogenesis shows that neither *D. coniospora* nor the fusaria harbor an ortholog of *brl*A, the primary transcriptional regulator initiating the formation of conidiophore vesicle in the aspergilli. After breaching the nematode integument, stress sensing, signaling and regulatory pathways are of paramount importance to facilitate the adaptation and survival of *D. coniospora* inside the host. Importantly, a number of stress-response-related transcription factors present in other filamentous fungi like the aspergilli were found to be missing, or have undergone rapid and significant evolutionary changes in *D. coniospora*. At the same time, some stress response-related regulator families underwent specific radiation. Thus, *D. coniospora* harbors four orthologues of the Mak1/2/3-type oxidative stress sensor kinases of the fission yeast, and feature two orthologues of the *Aspargillus nidulans* HogA mitogen-activated protein kinase, which is involved in various environmental stress responses. Effective iron acquisition is a crucial factor when animal hosts are invaded by fungi.

Consequently, *D. coniospora* has a complex and delicately regulated iron acquisition system, which is reminiscent of that of the opportunistic human pathogenic fungus *Aspergillus fumigatus*. The *D. coniospora* system enables this endoparasitoid to acquire iron from its prey through (i) production of a coprogen-type siderophore; (ii) reductive iron assimilation; and (iii) uptake and degradation of heme after extracellular proteolysis of host hemoproteins. Further elements of the remarkable arsenal of this nematophagous endoparasitoid fungus evolved for the invasion of nematodes have also been elucidated, but these observations are outside of the scope of this report. These findings have helped us to answer the intriguing question: What kind of genome level evolutionary changes make parasite fungi successful to invade animal hosts, even humans?

# TAXONOMY PROPOSAL FOR OLD WORLD MONKEY ADENOVIRUSES − EXISTENCE OF SEVERAL NON-HUMAN, NON-APE PRIMATE ADENOVIRUS LINEAGES

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Adenoviruses (AdVs) are icosahedral, non-enveloped viruses with double stranded, linear DNA genome. Because of their widespread occurrence in a variety of vertebrate hosts, AdVs make an ideal model for the study of viral evolution. More than 50 human AdV (HAdV) types are classified into seven species (Human mastadenovirus A to G, HAdV-A to G) within the genus *Mastadenovirus*. Certain HAdV species do contain also chimpanzee and/or other ape AdVs; HAdV-G contains HAdV-52 and several monkey AdVs. The first classification of simian AdVs (SAdVs) was based on hemagglutination-inhibition test as a tool of taxon demarcation. Nowadays, the recognized diversity of SAdVs is approaching that of the HAdVs. SAdVs are also members of the genus Mastadenovirus in family Adenoviridae. Species Simian mastadenovirus A (SAdV-A) is so far the only species officially approved for monkey AdVs exclusively. Comparative molecular analysis of the 25 recognized SAdV serotypes, SAdV-1 to 20, isolated from Old World monkeys (OWM), and SAdV-21 to 25 from chimpanzees has been performed by PCR amplification and sequencing of the virus-associated RNA (VA RNA) gene(s). However, while ape AdVs are well characterized and fully classified, most monkey AdVs still await classification, and only very short or no sequence is published from their genome. The short sequences acquired from the VA RNA are inappropriate for comparative analysis. Therefore we targeted multiple genes of (serotyped) OWM AdV prototypes, hoping that these new sequences will help the better understanding of the phylogenetic relationships of SAdV types suspected to have several distinct lineages. Based on partial sequences of the IVa2, DNA dependent DNA polymerase, penton base and hexon genes, acquired by consensus PCR, we found most of the SAdVs to belong to one or the other of the two accepted species into which some monkey AdV serotypes had already been classified. Human mastadenovirus G has been established for HAdV-52, but SAdV-1, -2, -7, -11, -12 and -15 belong to it as well. The species Simian mastadenovirus A includes SAdV-3, -4, -6, -9, -10, -14 and -48. Several SAdVs (SAdV-5, -8, -49 and -50) seemed to be members of the earlier proposed species Simian mastadenovirus B, together with baboon AdV-1, and rhesus monkey AdV strains A1139, A1163, A1173, A1258, A1285, A1296, A1312, A1327 and A1335. Simian mastadenovirus C should contain SAdV-19, together with baboon AdV-2/4 and -3. Our study revealed the existence of four further virus lineages. These candidate species are Simian mastadenovirus D (SAdV-13), Simian mastadenovirus E (SAdV 16), Simian mastadenovirus F (SAdV-17 and -18), and Simian mastadenovirus G (SAdV-20). Several biological and genomic properties, such as host origin, hemagglutination panel, number of fiber genes, and GC content of the genome, support this classification. Three prototype SAdV strains, originating from the American Type Culture Collection, turned out to be mixtures of at least two virus types, either of the same species (SAdV 12 and -15 from HAdV-G) or even of two different species (SAdV-5 from HAdV-G and SAdV-B). We assume that our proposal for SAdV species assignment will be confirmed by the recognition of further AdVs and by phylogeny and full genome analyses of a number of SAdVs in the near future.
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## BIODEGRADATION ACTIVITY OF CELLULOLYTIC FUNGI ISOLATED FROM COMPOSTING AGRICULTURAL WASTES

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Composting is a complex biological process in which microorganisms are in dynamic interactions. Filamentous fungi play important roles in the degradation of cellulosic material because they have comprehensive enzyme systems, such as cellulases (endoglucanases, cellobiohydrolases, β-glucosidases), hemicellulases and xilanases. Better understanding of fungal diversity in compost is crucial for predicting the degradation activity of the isolated strains. Inoculation of agricultural wastes with selected fungi could provide the possibility of improving the efficiency of biodegradation of lignocellulosic materials. The aims of the present work were the isolation of thermotolerant/thermophilic cellulolytic fungal strains from agricultural wastes and compost, determination of their cellulolytic activity and physiological properties. With the application of the selected isolates we wanted to specify the optimal conditions for the degradation of agricultural wastes of high cellulose content. The applied screening system proved to be suitable for the selection of fungal isolates with good cellulose-degrading ability in high temperature composting environments. More than 50 fungal strains were isolated from tall wheatgrass (Elymus elongatus subsp. ponticus), corn stalk and mixed composts. Twenty-four isolated thermophilic fungal strains had remarkable cellulolytic activity and their cellulases had an activity up to 50 °C. The cellulolytic fungal strains were identified by morphological characterisation and D1-D2 domain sequences of the LSU. *Absidia corymbifera* was the dominating species on the tall wheatgrass compost, while the species spectra were different throughout the seasons. Biodiversity of fungi in the compost was higher in spring than in autumn. The dominant species during spring was *H. insolens*, but other well-known thermophilic species, such as *Thermomyces lanuginosus, Aspergillus fumigatus, Melanocarpus albomyces*, were also present. Laboratory scale experiments were carried out for optimising the solid phase degradation of tall wheatgrass and corn stalk. Eight fungal isolates, belonging to *Absidia corymbifera, Chaetomium* sp., *Melanocarpus albomyces, Rhizopus oryzae*, and *Phoma* sp. as well as two authentic *Trichoderma* strains were selected for detailed enzymatic analysis and composting studies. Four of the ten examined strains possess a complete cellulase system. The most efficient composting fungal strains were *A. corymbifera* K3-EF1 and *M. albomyces* EFP5, because in co-cultures their hyphae extensively penetrated the cellulosic materials and high cellulolytic enzyme activity was detected in the extracts. Presumably, cellulases and the other decomposting enzymes of these two strains could act synergistically, resulting in faster decomposition of the substrates and accelerated growth of the fungal strains in a mixed culture. As such, they may be suitable as potential inoculated starters for degradation of agricultural wastes.

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## CHALLENGES IN TRANSPLANTATION VIROLOGY

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Virus infections in the immunosuppressed patient may lead to severe and letal infections. In the transplanted host especially infections with members of the herpesvirus family, especially with

Cytomegalovirus or Epstein Barr virus, may be critical. In addition, also polyomaviruses may lead to severe clinical complications, and also infections with hepatitis E Virus have gained increasing attention in transplant recipients. Different approaches have been developed within the past years to limit virus complications. Among these the use of antiviral prophylaxis, the development of methods for early specific viral diagnosis and quantification, the use of pre-emptive therapy schemes as well as the rapid initiation of therapeutic antiviral treatment have become routine components of the clinical follow up of transplant recipients worldwide.
In addition, in the last few years the measurement of the cellular immune response against different viruses has gained increasing attention. Asides the pathogenic viruses, however, also other, non-pathogenic viruses are present in the human host, and recent data provide evidence that anelloviruses, which are considered to be commensal pathogens, account for the by far highest part of the virus population present in persons after transplantation. Especially the Torque Teno Virus (TTV) load in blood seems to be closely associated with the patients' immunosuppression.

## DEMONSTRATION OF PLANT-GROWTH PROMOTION BY STRESS TOLERANT SOIL BACTERIA IN POT AND FIELD SOIL INOCULATION TESTS

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Plant growth-promoting rhizobacteria (PGPR) live in the rhizospere and have positive effects on plant growth. The aim of our work was to utilize PGP effects and abiotic stress tolerance ability of bacteria to develop "new generation" soil inoculants especially for deteriorated soils (of low fertility, low or high pH, high salt content). Developed new inoculants contain abiotic stress tolerant (temperature, osmotic and pH stress), and PGP effective bacteria which were isolated from the target soils. The complex microbiological methodology for obtaining this strain collection, is called "Soil Bacteria Screening System" (patent pending). Previously we had selected more than 1000 strains from many different deteriorated soil types from Hungary. We tested our strains in abiotic stress conditions (extreme pH and high salt content) and for the most important PGP effects, namely N-fixation, indole-acetic acid (IAA) -production, siderophore production, potassium mobilization and phosphate solubilization. Although these strains are promising in laboratory experiments, it is neccessary to prove the effect of our strains and strain combinations directly on plants. Consequently we carried out pot and field experiments in cooperation with authorized institutes, and these results will be presented here. From our strain collection we selected 20 strains, wich were compiled in 7 different strain combinations for soil inoculation. Each combination was able to provide all the tested PGP features. Commercial soil inoculant was applied as a positive control. Pot and field experiments were carried out simultanously with different types of soils, different levels of soil pH and different plant species (Sweet pepper: Solonchaks – Carbonate soil /pHKCl 8.04/; Tomato: Solonchaks – Carbonate soil /pHKCl 8.04/; Cucumber: highly acidic non-podzolic Brown forest soil /pHKCl 3,64/; Soybeen: acidic soil /pHKCl 5.79/; Sunflower: Brown forest soil with clay illuviation /pHKCl: 7.23/). The examined parameters were plant height, number of flowers, yield, leaf area, quantity of root biomass and soil dehydrogenase enzyme activity.
By these experiments we were able to obtain comprehensive information concerning the utility of our strain combinations as effective soil inoculants. The economically most informative result was

the increased crop yield, while the other examined parameters provided additional useful information in terms of productivity. The larger root biomass obtained may enhance water and nutrient uptake, drought tolerance of test plants. Increased leaf surface by more intensive photosyntesis may also improve crop quality and quantity.
Soil dehydrogenase enzyme activity (DEA) is often used as a direct measure of soil microflora activity. So increased DEA suggests soil life ameliorated by the inoculants.
Supported by the project "Development of an effective soil bacteria screening system and production of an instant powder soil inoculant formula" (KMR_12-1-2012-0200) Research and Technology Innovation Fund.

## EXPRESSION OF SRRA STRESS RESPONSE REGULATOR FACTOR AFFECTED BY BOTH CELL WALL INTEGRITY STRESS AND DELTARLMA MUTATION IN *ASPERGILLUS NIDULANS* FILAMENTOUS FUNGUS

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The cell wall integrity (CWI) signaling pathway is responsible for cell wall remodeling and reinforcement upon cell wall stress, which is proposed to be universal in fungal cultures. In *Aspergillus nidulans*, both the deletion of *rlm*A encoding the RlmA transcription factor in CWI signaling and low concentrations of the cell wall polymer intercalating agent Congo Red caused significant physiological changes. The gene deletion mutant *rlm*A strain showed decreased CWI and oxidative stress resistances, which indicated the connection between the CWI pathway and the oxidative stress response system. CWI stress down-regulated the expression of *brl*A encoding the early conidiophore development regulator transcription factor BrlA in the *Δrlm*A strain and, as a consequence, the formation of conidiophores was strongly hindered in submerged cultures. The *in silico* analysis of genes putatively regulated by RlmA and the CWI transcription factors AnSwi4/AnSwi6 in the SBF complex revealed only a few jointly regulated genes, including *srr*A coding for SrrA stress response regulator. In this study, we present data on the regulatory connection of BrlA-SrrA-RlmA in an *Δrlm*A deletion strain of *A. nidulans* and under CWI stress condition. In addition, possible connections among the stress and asexual sporulation signaling pathways and sterigmatocystin production are also discussed here.

## THE PLACE OF OUR EARTH IN THE UNIVERSE AND TURNING-POINTS IN ITS LIFE (THOUGHTS INDUCED BY THE CLIMATE CHANGE)

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The Life is a biological phenomenon which is connected to a structure of a living unit/entity bordered from its environment. Appearance of the Life on the Earth is a result of the general processes of the Universe. The Life on the Earth in the endless Universe has consisted of different systems of living biological units which have been in mutual dynamic connections with each other.

The Bio-sphere in respect of the Life is a closed system. The living units including the conscious human beings, too, have significantly influence on the condition of the environment/the Earth. The Life can only persist when the conditionsfor life are in accordance with the biological laws and requirements. Efforts have performed to stop the unpleasant changes of qualities of the earthly environment verified by exact data have been ineffective. The author has urged convenient and effective actions to preserve the Life.

## SYSTEMS GENETICS TO CHARACTERIZE CLASSICAL MUTANTS IN THE INDUSTRIAL HOST *ASPERGILLUS NIGER*: IDENTIFICATION OF A GLOBAL REGULATORY GENE TO BE INVOLVED IN METABOLITE AND PROTEIN PRODUCTION

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Rapid acidification of the culture medium by the production of organic acids is a key characteristic of *Aspergillus niger*. The D15 mutant of *A. niger* is classical non-acidifying mutant and used often for the expression of recombinant proteins in *A. niger*, because of its reduced production of extracellular proteases under non-acidic conditions. To identify the mutation in the D15 mutant responsible for the non-acidifying phenotype, we successfully combined high-throughput sequencing (Illumina) with bulk segregant analysis. Because of the lack of a sexual cycle for *A. niger*, the parasexual cycle was used to generate a pool of segregants. From the 52 single nucleotide polymorphisms (SNPs) between the parental strains, three SNPs were homozygous in the genomic DNA of pool of segregants. These three SNPs mapped to all the right arm of chromosome II, indicating that this region contains the genetic locus affecting the phenotype related to acid production. Of the three SNPs, one mutation resulted in a missense mutation in the gene encoding the *A. niger* homologue of the *A. nidulans* methyltransferase gene *laeA*. Complementation analysis of the original mutant with the *laeA* gene and targeted disruption of *laeA* further confirmed that LaeA is involved to oxalic and citric acid production in *A. niger*.

Analysis of the secondary metabolite profile of the *laeA* mutants indicate that LaeA is required for the production of several SMs, but deletion of *laeA* also resulted in the presence of SMs that are not detected in the wild type strain.

## MICROBIAL COLONIZATION OF REMOTE AND NEWLY EMERGENT HOT SPRINGS IN HUNGARY AND WESTERN UNITED STATES

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Following the isolation of *Thermus aquaticus* and other "new" thermophilic isolates such as *Thermomicrobium roseum* from Yellowstone National Park, it became obvious that these seemingly hostile thermal environments contained a wide variety of thermophilic microorganisms that had not been previously isolated using conventional microbial media. It also became obvious that these microorganisms are found in a world wide distribution and raised the question of the colonization of these thermophiles from other thermal environments. The present report centers on isolated thermal springs where it is unlikely that surface transfer of an inoculums from another thermal environment would have occurred. Such a spring is Egerszalók Hot Spring which is close to the village of Egerszalók, located 6 km west of the town of Eger in North East Hungary. The spring is an artesian spring that resulted from drilling for oil in 1961, and has continued to flow (temperature of 68-71 $^{o}$C) since that time resulting in the deposit of an extensive travertine terrace. There are no similar high temperature hot springs in Hungary, which has a large number of lower temperature commercial thermal spas. (The nearest similar hot spring and travertine formation to that seen in the Egerszalók Hot Spring is located at Pamukkale in Turkey).
Preliminary DNA sequencing of samples from the runoff channel of Egerszalók Hot Spring by students of the Department of Microbiology at Eötvös Loránd University indicated the presence of a diverse population of microorganisms and the previous isolation of a culture of *Rubritepida flocculans* was reported by da Costa and his associates. Additional cultivation studies of the Egerszalók Hot Spring runoff have shown a microbial population that is similar to the microbial populations from similar temperature hot springs located in Colorado (USA) (e.g. Pinkerton Hot Spring) and in the greater Yellowstone Ecosystem (e.g. Huckleberry Hot Spring) including isolates of *Roseococcus, Roseomonas, Paracaurococcus, Agrobacterium,* Flexibacteria and *Cyclobacterium*. More recently, strains of *Caldindinia aerophilia* and *Schleiferia thermophilia* have been shown to major components of the microbial mat in Yellowstone Hot Spring runoff channels and the total genome sequence of these strains are being determined. There is also some evidence that isolates from Egerszalók may also contain a previously unreported strain of *Meiothermus*.

## EXAMINATION OF THE AFB1-DEGRADING PROFILE OF *RHODOCOCCUS* TYPE STRAINS

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Aflatoxin-B1 (AFB1) is one of the most dangerous mycotoxin due to its carcinogenicity, which mainly infect wheat, corn, rice and seeds. To alleviate negative effects on animals successful

detoxification tools are needed. The application of microorganisms to biodegrade mycotoxins is a novel strategy that indicates potential for application in food and feed processing. Alberts et al. [1] monitored the AFB1 degradation of *Rhodococcus erythropolis* and observed that AFB1 was abolished from the reaction mixture together with mutagenic activities of AFB1 and its by-products. Cserháti et al. [2] concluded *Rhodococcus* species are effective in the degradation of aromatic mycotoxins and their application in mycotoxin biodetoxification processes is a promising field of biotechnology. On the base of this phenomenon the AFB1-degrading ability of the *Rhodococcus* type strains (28) that covers species of the full genome was investigated in this study. The biodegradation experiment with 2 ppm AFB1 was carried out in triplicates. Samples and a microorganism-free control were incubated for 72 hours. 1 ml samples were removed in every 24 hours. Supernatants were separated and analysed by High Performance Liquid Chromatography (Wessling Hungary Ltd., Hungary) for AFB1 and SOS-Chrromotest for remaining genotoxicity. Out of the 28 *Rhodococcus* type strains 11 could eliminate more than 90% of 2 ppm AFB1 and cease the genotoxicity in 72 hours (*R. rhodnii* JCM3203, *R. corynebacterioides* JCM3376, *R. tukisamuensis* JCM11308, *R. imtechensis* JCM13270, *R. pyridinivorans* JCM10940, *R. kroppenstedtii* JCM13011, *R. jialingiae* DSM45257, *R. qingshengii* DSM45222, *R. globerulus* JCM7472, *R. baikonurensis* DSM44587, *R. erythropolis* JCM3201). Furthermore *R. kroppenstedtii* that was isolated from soil (India) could detoxify the toxin within 48 hours. Recently rhodococci isolated from hydrocarbon contaminated sites of Hungary have been screened for their mycotoxin degrading profile in our laboratory [2]. In that previous study 32 strains belonging to 8 species were analysed for their AFB1 degrading ability. The investigated *Rhodococcus* isolates were effective AFB1 degraders and could be ranked on the base of their AFB1 degrading ability as follows: *R. ruber* < *R. aetherivorans* < *R. coprophilus* < *R. gordoniae* < *R. globerulus* < *R. rhodochrous* < *R. pyridinivorans* and < *R. erythropolis*.
However, the intra-species degradation ability varied greatly, too. Comparing results with the degrading ability of type strains, *R. pyridinivorans* and *R. erythropolis* proved to be able to degrade AFB1, while *R. erythropolis* have weaker detoxifying potential. Interestingly, *R. rhodochrous* JCM3202 strain could not weaken the genotoxic potential of AFB1, in contrast *R. rhodochrous* NI2 proved to be an excellent detoxifying strain. This may indicate that different *Rhodococcus* strains may use different routes for AFB1 degradation that varies inter- and intra species.
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[1] Alberts, J.F. et al. (2006) Int J Food Microbiol 109, 121-126.
[2] Cserháti, M., et al. (2013) Int J Food Microbiol 166, 1-5.

## COLLECTION OF STRESS TOLERANT SOIL BACTERIA WITH PLANT GROWTH PROMOTING AND SOIL AMELIORATIVE PROPERTIES

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Soil bacteria are very important in biogeochemical cycles and have been used in crop yield enhancement for decades. Certain soil bacterial species are capable of promoting plant growth (PGPR plant growth promoting rhizobacteria) either living in bulk soil or by colonizing the plant root. Generally, the main functions of these bacteria are to supply nutrients to plants, to stimulate plant growth, e.g. through the production of plant hormones, to control or inhibit the activity of plant pathogens, to improve soil structure and to accumulate or solubilize soil inorganic components. In

Hungary, and also worldwide, economically important food and forage plants are grown mostly on low fertility soil types. In our country, more than 40% of the cropland is low fertility and degraded, "stress"- soils (strongly acidic, alkaline-saline, structureless, sandy soils, etc). We have isolated indigenous bacterial strains from soils which impose extreme growth conditions for bacteria. We collected soil samples from natural habitats and arable lands of Hungary. During the process of isolation different selective parameters - high salt concentration, cold conditions and different pH levels of the culture media - were applied to select "stress" tolerant strains. Our goal was to assemble and preserve a strain collection which contains effective microorganisms isolated from different kinds of "stress" soils, and create a database. The BioFil Ltd. has a collection of about 1500-2000 strains, selected by SBSS – Soil Bacteria Screening System. The successful preservation of reference, type and production strains is of major importance in maintaining a strain collection. We apply lyophilisation and deep freezing at -80 °C for long-term storage which preserve viability and productivity of the strains for years. We have examined plant nourishing and growth promoting effects like nitrogen fixing and siderophore production capacity, the ability of phosphorus and potassium mobilization and phytohormone production (indole-3-acetic acid, gibberellin like substances and cytokinins) etc. Identification of selected strains was carried out by 16S rDNA sequence analysis. We classified the isolated and tested strains based on their PGPR effects. Our collection contains 29 nitrogen-fixing strains, 46 indole-3-acetic acid producing strains, 34 phosphorus mobilizing strains, 94 siderophore producing strains, 155 lipase productive strains, 164 polysacharide producing strains and almost 200 identified reference microorganisms. The 14 most suitable strains were selected to create the BIOFIL® soil inoculant family for acidic, alkaline and neutral soils. BIOFIL® soil inoculant strains hold a prominent place in the BioFil strain collection.

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## COMPARATIVE MIRU-VNTR ANALYSIS OF *MYCOBACTERIUM AVIUM* SUBSPECIES ISOLATES OF VETERINARY ORIGIN

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Subspecies of *Mycobacterium avium* are worldwide distributed potentially zoonotic species. *Mycobacterium avium* ssp. *avium* (MAA) is the infectious agent of avian tuberculosis, while *Mycobacterium avium* ssp. *paratuberculosis* (MAP) causes Johne's disease, mainly in ruminants. "*Mycobacterium avium* ssp. *hominissuis*" (MAH) is frequently isolated from immunocompromised people and *Mycobacterium avium* ssp. *silvaticum* (MAS) was first isolated from wood pigeons. Epidemiological studies which can facilitate disease prevention through revealing infection sources and transmission routes require genotypic discrimination. The aim of our study was the detection of genetic diversity within *Mycobacterium avium* isolates of veterinary origin. For typing Mycobacterial Interspersed Repetitive Units – Variable-Number Tandem Repeat analysis (MIRU-VNTR) was chosen for its easy performance, good reproducibility and high discrimination. Since 2006 over 900 Mycobacterium avium strains were isolated from more than 20 different species of mammals and birds, including domestic and wild animals. Isolates were identified by different molecular biological methods, among others insertion sequence (IS900, IS1245, IS901), large

sequence polymorphism (LSP) and type or subspecies specific PCRs. Within the tested strains 66 different MIRU-VNTR profiles were detected, which proved to be subspecies specific.
In order to determine the genetic relatedness of the different MIRU-VNTR patterns Neighbour-Joining analysis was performed in MEGA6. Our results demonstrate the high diversity of MAH and MAA strains and the relative uniformity of MAS and MAP strains, and contribute greatly to our knowledge about the genetic diversity of *Mycobacterium avium* subspecies.

## GENOME PLASTICITY AND VIRULENCE IN *CLOSTRIDIUM DIFFICILE*

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*Clostridium difficile* is an anaerobic, sporogenic bacterium causing intestinal infections (CDI) ranging from diarrhoea to colitis and pseudomembranous colitis in humans and animals. It is currently among most important pathogens associated with health care associated infections. Approximatelly 11% of *C. difficile* genome is composed by mobile elements. These mobile elements together with other mechanisms contribute to variability within the species and are studied to better understand *C. difficile* virulence. Mobile elements – plasmids have been found, but not associated with any function. Their current most important role is their use for genetic manipulation of *C. difficile*. Specific form of mobile element, ISTron, was described in *C. difficile*. This is a combination of IS element mediating the transposition events and intron element responsible for splicing from mRNA. Bacteriophages are widespread among strains. They were shown to mediate some antibiotic resistances and also contribute to toxin (see below) regulation. Phages do have also potential for use in alternative treatment approaches. But it is very difficult to isolate lytic phages from *C. difficile*, although phage particles were detected in patient stools. A specific, phage like mobile element, SigK is known in *C. difficile* and its splicing has (similar to *B. subtilis*) role during sporulation. The best studied mobile elements in *C. difficile* are transposons mediating antibiotic resistance. CDI is a toxin mediated disease and two large toxins (toxin A and toxin B) are the main virulence factors. Related toxins in *C. novyi* are encoded by phage and in *C. sordellii* were found on large plasmid. In *C. difficile* toxins are encoded on the chromosome, but transfer of the region from toxigenic to nontoxigenic strain was demonstrated recently.
Mobility of this region is also supported by modular structure of variant toxin genes and with unusual integration sites found in a novel *C. difficile* toxinotype.

## HIGH HUMORAL AND POOR CELL-MEDIATED IMMUNE RESPONSES IN MICE IMMUNIZED WITH A SEASONAL TRIVALENT INACTIVATED INFLUENZA VACCINE, FLUVAL AB (2013/14)

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Earlier studies showed that a seasonal trivalent inactivated whole virion aluminum phosphate adjuvanted influenza vaccine (FLUVAL AB) induces high hemagglutination inhibition (HI) antibody levels in humans. However, cell-mediated immune (CMI) responses to the vaccine have not been investigated. We immunized mice with FLUVAL AB (2013/2014), and HI antibody and CMI responses were investigated. For CMI we measured: 1./ serum IFN-γ protein level by ELISA, 2./ mRNA expression of IFN-γ, granzyme B and IL4 in whole blood cells without influenza-specific *in vitro* restimulation of the samples by a real-time quantitative reverse transcription-PCR (qRT-PCR), and 3./ the frequency of T and NK cells containing IFN-γ or granzyme B proteins in splenocytes, after *in vitro* influenza-specific restimulation, as determined by flow cytometry. HI antibodies developed at high titers against all three components of the vaccine. Serum IFN-γ protein level was similar during the course of the immunization. Relative to data obtained before immunization, a reduction in the mRNA expression of IFN-γ ($p<0.05$) and granzyme B ($p<0.05$) was observed but IL4 mRNA expression did not change. As positive controls, a group of mice received the 3rd inoculation with the Fluval AB coupled to complete Freund adjuvant. In these mice mRNA expression of IFN-γ and granzyme B increased ($p=0.046$ and $p>0.05$, respectively) but significant change in IL4 mRNA expression was not seen. In certain subpopulations of the splenocytes, such as CD3+/CD4+ T helper cells, CD3+/CD8+ cytotoxic T lymphocytes or CD3-/CD8+ NK cells, the percentage of IFN-γ+ or granzyme B+ cells was not increased significantly after immunization with FLUVAL AB, while the percentage of such cells was significantly higher in subpopulation obtained from mice receiving the vaccine together with Freund adjuvant ($p<0.05$). Results show high humoral and poor CMI responses for immunization of mice with Fluval AB.

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## KILLER TOXINS AND MYCOVIRUSES IN YEAST: WHAT HAVE WE LEARNED FROM 50 YEARS OF RESEARCH ON KILLER YEASTS?

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The initial discovery in 1963 of toxin-secreting "killer" strains of the yeast *Saccharomyces cerevisiae* as brewery contaminant and its subsequent phenotypic association with the presence of cytoplasmic inherited double-stranded (ds)RNA viruses marked the beginning of intensive research into the field of yeast virology and molecular biology in the mid 60s and early 70s of the last century. At that time, certain yeast strains had been identified which produce and secrete protein toxins that are lethal to different yeast and fungal species. The corresponding toxin-secreting yeasts had been designated 'killer yeasts' and the term 'killer toxin' was created for each cytotoxic protein secreted by the particular yeast. Shortly after this discovery it became evident that killer toxin

secreting strains can be frequently found in yeast and fungal genera and that some of these killer toxins bear a remarkable antimycotic potential by efficiently killing various human and plant pathogenic yeasts and fungi. In the vast majority of killer yeasts, and in contrast to the situation in antibiotic producing bacteria, killer phenotype expression in yeast is usually associated with a specific immunity component, rendering killer cells immune and protected against their own toxin. Meanwhile and after more than 50 years of intensive research in this field, much has been learned about eukaryotic cell biology and virus/host cell interactions by studying virus-infected killer yeasts and investigating and dissecting processes such as killer toxin precursor processing, maturation and toxin secretion. Up to now, an overwhelming number of studies in the field of killer yeast biology fostered our current knowledge and view on important cellular processes, including receptor-mediated endocytosis, retrograde protein transport and translocation from intracellular membranes, protein ubiquitylation and proteasomal degradation up to apoptotic host-cell responses; processes which are not only fundamental to eukaryotic cell biology but also relevant to human diseases. Thus, research on killer yeasts and their dsRNA viruses has proven to be of general importance in understanding eukaryotic cell biology and, in addition, becomes increasingly interesting with respect to biomedical applications and biotechnology. The plenary lecture touches some of these aspects and summarizes most recent advances in this still timely field of research.

## SENSITIVE DETECTION OF PEPTIDOGLYCAN IN GRAM-NEGATIVE BACTERIA BY A GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC APPROACH

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Peptidoglycan (PG) layers in cell envelopes of Gram-negative bacteria are occasionally very thin and hardly detectable. Due to the lack of sensitive detection methods for meso-2,6-diaminopimelic acid (meso-Dpm) as indicator for peptidoglycan and due to the resistance to beta-lactam antibiotics targeting the peptidoglycan synthesis, it had been assumed that peptidoglycan is absent in certain Gram-negatives like e.g. in planctomycetes. Recent bioinformatic evidence of the presence of enzymes essential for PG synthesis and the genomic potential for production of beta-lactamases of planctomycetes challenged this hypothesis [1]. Therefore efforts for detecting low amounts of peptidoglycan by sensitive analytical approaches were undertaken. Cell walls of Gram-negative bacteria are reported to contain directly cross-linked PG either of the type A1γ based on meso-Dpm or of the type A1β based on L-ornithine (L-Orn) [2, 3]. Structural peculiarities that differentiate PG from the hypothetical proteinaceous cell wall of Gram-negatives are the occurrence of D-amino acids, of muramic acid and of the non-proteinogenic diamino acids meso-Dpm or ornithine. Gas chromatography is suited for quantification and enantiomeric analyses of N-perfluoroacyl alkyl esters of amino acids from peptidoglycan hydrolysates. Mass spectrometry allows highly sensitive detection of derivatives of PG indicator components by selected ion monitoring of characteristic fragmentions [4]. A method using gas chromatography and mass spectrometry of volatile muramic-acid and amino-acid derivatives was developed and successfully applied to the detection of PG in low amounts in planctomycetes, spirochaetes and an isolate belonging the class Phycisphaerae [1].

[1] Jeske, O., et al. (2015) Nature Comm in press.
[2] Schleifer, K. H. and Kandler, O. (1972) Bacteriol Rev 36, 407-477.
[3] Schleifer, K. H. and Joseph, R. (1973) FEBS Lett 36, 83-86.
[4] Schumann, P. (2011) Methods Microbiol 38, 101-129.

# TWO-YEAR STUDY OF THE AFLATOXIN-PRODUCING *ASPERGILLUS* STRAINS IN THE MAIZE FIELDS OF HUNGARY

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Aflatoxins are one of the most potent toxic substances that occur naturally. *Aspergillus* section Flavi contains the most common aflatoxin producing moulds, *A. flavus* and *A. parasiticus*. Recently numerous studies proved the presence of aflatoxins and/or the producing fungi in the feed and food in European countries. Thus our aim was to set up a database on the occurrence of the aflatoxin-producing members of *Aspergillus* section Flavi in the maize fields throughout Hungary. Soil and maize samples were collected from 78 and 118 locations in the year 2013 and 2014, respectively. Most samples were collected from the regions of Southern Transdanubia, Southern Great Plain and Northern Great Plain, as most maize is grown there. Classical culturing method was applied with the use of selective medium (PBGA). The *Aspergillus* isolates were identified according to their phenotypic characteristics which was confirmed by the analysis of their partial calmodulin sequence. The strains were tested for the presence of a regulatory (*afl*R) and two structural (*afl*D, *afl*P) genes involved in the aflatoxin B1 (AFB1) biosynthesis. Aflatoxin-producing ability of the strains was also detected in artificially contaminated maize with SOS-Chromotest which can measure the genotoxic effect of AFB1. In 2013, 21 soil samples and 33 maize samples contained propagula of the fungi belonging to *Aspergillus* section Flavi, while these moulds were found in 19 soil samples and 41 maize samples in 2014. The soil samples of the region Southern Transdanubia, Central Hungary and Southern Great Plain were contaminated with the fungi in high proportion (24.1%, 26.7% and 31.1%, respectively), while the lowest proportion (12.8%) was found in the soil samples of Northern Great Plain. The lowest rate of the maize samples containing member(s) of *Aspergillus* section Flavi was experienced in the region Northern Hungary; only 1 of the 21 maize samples proved to be infected with moulds. In contrast, fungi belonging to the *Aspergillus* section Flavi could be isolated from more than the half of the maize samples of the regions Central Hungary and Western Transdanubia. The highest rate of aflatoxinogenic moulds were found in Central Hungary in soil samples (20.0 %), as well as in maize samples (26.7%). Similarly, high rate of aflatoxin-producing fungi was experienced in the maize samples in Western Transdanubia (25.0%) and in Northern Great Plain (23.1%). According to their partial calmodulin sequence, all isolates proved to belong to the species *A. flavus*, with the exception of four strains, which were identified as *A. parasiticus* (3 of them were isolated from soil and one from maize sample).

After comparing the results of the detection of aflatoxin biosynthesis genes and that of SOS Chromotest, it was found that strains whose genome lacks at least one of the three analysed aflatoxin biosynthesis genes did not produce aflatoxin and that strains which were able to produce aflatoxin contained the regulatory and the two structural genes. On the other hand, in spite of the presence of the three genes, some strains were found to be non-aflatoxin producer.

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## SYSTEMS BIOLOGY APPROACHES FOR *TRICHODERMA REESEI* STRAIN ENGINEERING

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*Trichoderma reesei* is one of the most widely used organisms to produce plant cell wall degrading enzymes which find their application in different industries including the emerging biofuel and biorefinery industry. Strain improvement programs have resulted in a number of high performance cellulase producer strains and cellulase non-producer strains. During recent years, the genome of the wild-type strain and genomes of several of these hyper-producing and non-producing mutants have been sequenced and their comparison has provided first insights. Here I will present the result of a comparative analysis of two cellulase non-producing mutants to their ancestor and present our analysis of the molecular basis of the cellulase negativity in these two mutants.

## A TWO-DECADE EXPERIENCE IN EXAMINATION HOW ANTIBIOTIC RESISTANCE GENES ARE INVOLVED IN ANTIBIOTIC RESISTANCE DEVELOPMENT OF *BACTEROIDES* SPP.

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The action of once 'magic' antibiotics is corroborated by the antibiotic resistance mechanisms spreading rapidly just by the frequent and sometimes not prudent use of antibiotics. The antibiotic resistance mechanisms of pathogenic bacteria mean not only the mechanisms of action of the effector proteins but also the regulation of their gene expression and genetic elements which allow their spread. The most significant group of anaerobic bacteria, the *Bacteroides* and related species, with important healthcare issues have this significance also by their high antibiotic resistance rates and their numerous antibiotic resistance mechanisms possessed. Resistance surveys of clinical *Bacteroides* strains have been performed since the anaerobic cultivation methods have been widely applied, and have reached a quite sufficient level in the past three decades in the USA and Europe. For some antibiotics the resistance rates increased parallel with their use (clindamycin, moxifloxacin, beta-lactam/beta-lactamase inhibitor combinations) and for some antibiotics their susceptibilities remained roughly the same (carbapenems and 5-nitroimidazoles). In the antibiotic resistance mechanisms of *Bacteroides* species it is an interesting feature that the resistance genes are often activated by insertion sequence (IS) elements or somewhat rarely by an attenuation mechanism. During our almost two decades investigations we described novel IS and mobile, resistance gene-carrying genetic elements, and made important contributions to the description of the genetic population structure of *B. fragilis*. In our genetic investigations we supplied the most comprehensive data about the antibiotic resistance gene content of the *Bacteroides* species.
Since there are some open questions regarding our knowledge about the antibiotic resistance mechanisms of *Bacteroides* therefore we continue our efforts in solving these problems which might contribute to better therapeutic options.

## PROPHAGES OF AN *ESCHERICHIA COLI* O157:H43 STRAIN

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*Escherichia coli* O157 strains are significant zoonotic pathogens, including several strains of enterohemorrhagic *E. coli* (EHEC) capable of causing life-threatening disease, therefore a large number of genomic studies are focusing on this group of bacteria. Recently we characterized an *E. coli* O157:H43 strain (T22) from cattle with novel genotype with an atypical array of virulence genes. The following assembly of the genome showed that strain T22 harbours a total of 10 prophages, ranging in size from 10 to 45 kb, comprising a total of 337 kb of its 4.9 Mb length of genomic DNA. Four of these prophages carry potential virulence genes, including Bor lipoprotein, lom-like protein, cytolethal distending toxin type V and subunit A of heat-labile enterotoxin type II. Three prophages are P2-like, four are lambdoid, and partial homologues of a Phi27 and a *Shigella* serotype-converting phage could be identified, the remaining one prophage could not be classified. All prophages showed at least partial homologies with known prophages of other E. coli strains representing various pathotypes and also those of *Salmonella* serovar Typhi and *Shigella* strains. Comparison with the prophage pool of EHEC O157:H7 Sakai strain revealed great differences. Only two lambdoid T22 prophages showed partial homologies to Sakai phages (Sp3, Sp8 and Sp10). These results confirm our earlier notion that the atypical *E. coli* O157 strains represent a different lineage when compared to the tipical EHEC O157 strains. The T22 prophages' GC content varied in range of 45.5-52.6%, suggesting that they were acquired at different time points during the evolution of our study strain. The fact that *E. coli* strain T22 carries these relatively large genomic regions encoding temperate prophages underlines the notion that bacteriophages play a significant role in the genome evolution of *E. coli* O157 strains, and could act as vectors of virulence genes.
The financial support of OTKA (K81252) is acknowledged.

## SEASONAL DYNAMICS OF MICROBIAL COMMUNITIES INHABITING TWO DISTINCT TYPES OF ALKALINE SODA PANS

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Astatic soda pans of the Pannonian steppes are unique environments regarding their physical and chemical characteristics (high turbidity, pH, salinity and special ionic composition), thus they provide extreme habitats for aquatic life. However, little is known about the seasonal dynamics of the bacterial communities inhabiting these lakes and the environmental factors which has the main impact on their structure. Based on their turbidity, the two ecologically distinct types are the 'turbid–white' and the 'non-turbid–coloured' pans. Two lakes, representing these main types (the Zab-szék pan near Szabadszállás and the Sósér pan near Dunatetétlen in the Kiskunság National Park, Hungary) were sampled monthly for more than a year. A pyrosequencing approach based on the variability of the bacterial 16S rRNA V3-V4 region was used to reveal the characteristic genera inhabiting these lakes, and major shifts in the community structures of these habitats. Results

showed taxonomically complex microbial communities, and despite the different physical characteristics, several common taxa were detected as major components in both lakes. Some genera (e.g. *Belliella*) were abundant throughout the year, while for others (e.g. '*Candidatus Limnoluna*', *Nitriliruptor*) a seasonal pattern was observed in their relative abundance. Presumably, algal blooms and desiccation have the most remarkable effect on the composition and the diversity of these bacterial communities. Several functional gene components related to these changing environmental conditions were also identified with our previous shotgun metagenomics study.
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## AFLATOXIN M1 ANALYZES OF HUMAN BREAST MILK SAMPLES FROM HUNGARY

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Aflatoxins (AFs) are secondary metabolites that causing risks for human health due to their widespread presence in foods and environment. Moreover *Aspergillus* species produced Aflatoxin B1 (AFB1) has cytotoxic, mutagenic and carcinogenic effects. It is also determined that the final product of AFB1 metabolization in mother's milk results Aflatoxin M1 (AFM1). AFM1 which were identified in cow milk, is a possible human carcinogenic (Group 2b, WHO IARC) and able to damage liver and DNA. AFB1 production of *Aspergillus* species has close connection with climatological features. Several publications from more AFs exposed areas like Far East, Africa, Middle East, Turkey, Italy etc. show that AFM1 is able to be presented in the mother's milk in detectable concentration. As it was published earlier, nowadays contamination risk of AFs in Europe is increasing because of climate change: On the one hand humid weather supports the spreading of microscopic fungi (e.g. AF producing *Aspergillus* sp.) on different agricultural plants (cereals, corn, fruits etc.). On the other hand if it's followed with a long arid period, the climate stress will induce toxin production, as a vantage in competition between the species of the microbial community. If the climate predictions are correct wide range of harmful effects, and consequently health problems, can be expected due to increasing presence of AFM1. It is well known, that some alarms, because of AFM1 contaminated cow milk from Hungary, were signed in the year of 2012 by the Rapid Alert System for Food and Feed (RASSF) of the European Union. By these results and our former publication it can be said Hungary is concerned with AFs problem, but there was no human breast milk examinations before. In our study the presence of AFM1 in the breast milk of more than a hundred nursing Hungarian mothers were investigated by high performance liquid chromatography (HPLC). A modified MSZ EN ISO 14501 standard method was used for measuring AFM1 from these samples. The original standard is for cow milk samples, thus the modification was only because the different matrix, but the applied analytical method was the same. The limit of detection was found to be 8 ng/l. By our results there was no registered sample that contained more AFM1 than detection limit concentration.
In spite of that these results could establish a long term indication system for the estimation of AFs caused effect of climate change on human health (exposure of AFM1 to mothers and neonates).
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## A NOVEL MECHANISM PROVIDES GENOME QUALITY CONTROL FOR PATHOGENICITY ISLAND TRANSFER IN *STAPHYLOCOCCUS*

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In prokaryotes, the bacterial genome may include genetic elements that can be mobilized to form a more or less independent unit of replication (pathogenicity islands (PIs)) involved in spreading virulence factors and toxins among different bacterial strains. In *Staphylococcus aureus* there is an intimate relationship between pathogenicity islands and helper phages as helper phages are essential for excision of Staphylococcus aureus pathogenicity islands (SaPIs) [1]. We have proposed a novel mechanism for control of SaPI de-repression in *Staphylococcus aureus* where upon helper phage infection, phage-encoded dUTPase first sanitizes the nucleotide pool by hydrolyzing cellular dUTP and then binds to the Stl repressor, leading to de-repression and SaPI activation [2]. The phage dUTPase therefore, in addition to its general function to prevent uracil incorporation into DNA, also regulates SaPI expression. Stl, on the other hand, strongly inhibits the enzymatic activity of dUTPases [3]. This discovery connects SaPI replication to DNA repair, and indicates a new role for DNA damage recognition and repair. Despite its general role as the depository of genetic information, the DNA molecule possesses inherent chemical reactivity that results in frequent and various modifications even under normal physiological conditions.

The major pathways of DNA repair are eminently conserved among free-living species from bacteria to multicellular eukaryotes. The appearance of unusual bases in DNA has been traditionally considered as damage sites, however, in recent years, the potential additional significance of these moieties are also being appreciated. We propose that *Staphylococcus aureus* (and potentially also some other prokaryotes) possesses highly unique traits for uracil-DNA metabolism. We will also cover how these may be related to antibiotic resistance.

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[1] Chen, J. and Novick, R. P. (2009) Science 323, 139-41.
[2] Szabó, J.E. et al. (2014) Nucleic Acids Res 42, 11912-20.
[3] Hirmondó, R. et al. (2015) DNA Repair (Amst) 30, 21-27.

## T-HELPER ACTIVITY IN *MYCOBACTERIUM* INFECTION

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Tuberculosis (TB) is most commonly caused by bacterium strains of the *Mycobacterium tuberculosis* complex. T-lymphocytes have a major role in both the pathogenezis and diagnosis of TB. Our objectivs was to evaluate changes in cytokine mRNA levels, furthermore to characterize the composition of T-cell subpopulations, activated during stimulus 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 expression were determined after stimulation. RNA was extracted from IGRA positive blood samples. After reverse

transcription, cytokine mRNA leves were measured with real-time polymerase chainreaction, relative quantity was determined using ddCt method. Our results suggest that Th1 population is activated after stimulation with TB antigens, as mRNA levels of Th1-specific cytokines, IFNγ and IL-2 were elevated. Th2 markers as IL-10 and transciption factor GATA3 mRNA levels were not increased. Heavy increase in IL-17A and IL-22 mRNA imply Th17 subset activation.
Along with these cytokines, we also observed heavy increase in mRNA levels of IL-13, as it is associated with heavy IL-22 increase, it suggests the activation of Th22 cells as well. Treg specific transcription factor Foxp3 mRNA is also increased after stimulation.

## EFFECT OF ELECTRON ACCEPTOR AVAILABILITY ON ANAEROBIC TOLUENE DEGRADER COMMUNITIES IN CONTAMINATED AQUIFERS

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Groundwater is an important resource but often polluted by aromatic hydrocarbons, such as BTEX compounds. To conceive efficient bioremediation technologies, it is crucial to better understand the ecology of degraders and ongoing biodegradation processes in situ, which mostly rely on anaerobic respiration. It is not yet well understood how the availability of different electron acceptors controls the distribution and diversity of anaerobic degraders, both in distinct redox compartments and along transects of contaminant plumes. To address these questions and to assess anaerobic degradation potentials in contaminated environments, we have developed a targeted catabolic marker assay for genes encoding “fumarate-adding enzymes” (FAEs) which catalyze the first step of anaerobic degradation of various hydrocarbon compounds like toluene and n-alkanes [1]. A comprehensive detection pipeline for a wide range of anaerobic degraders harboring diverse FAEs, i.e. benzysuccinate synthases (bss), naphtylmethylsuccinate synthases (nms) or alkylsuccinate synthases (ass) and also a targeted pyrosequencing pipeline for FAE gene markers is now established, allowing for a high-throughput characterization of degrader populations at contaminated sites [2]. Using these tools, we are investigating central links between structure and functioning of anaerobic degrader communities in contaminated groundwater systems. We hypothesize that more diverse degrader communities harbor a greater level of functional redundancy and thus, are more versatile and robust under dynamic habitat fluctuations. We are currently tackling this in BTEX contaminated field sites with natural redox gradients, in batch microcosms under fluctuating electron acceptor availability and also in a toluene contaminated model aquifer. We monitor the net consumption of electron donors and acceptors by classical biogeochemical analyses and link it to the changes within the degrader community assessed via our optimised FAE gene detection assays. At the different sites, we revealed characteristic redox gradients both in longitudinal and vertical plume transects, accompanied by either highly specialized communities with low toluene-degrader diversity or strikingly diverse communities with distinct degraders. In batch microcosms we show that functionally redundant toluene degraders can maintain toluene degrading activity under changing electron acceptor availability, even when the starting degrader community had a very low abundance within the whole community. These ongoing projects will help to better understand the interplay of abiotic and biotic drivers in anaerobic hydrocarbon degradation in groundwater.

[1] von Netzer, F. et al. (2013) Appl Environ Microbiol 79, 543-552.
[2] Lueders, T. and von Netzer, F. (2014) Hydrocarbon and Lipid Microbiology Protocols. Springer..

## *IN VITRO* INVESTIGATION OF THE EFFECT OF DIFFERENT MUSHROOM EXTRACTS ON PROBIOTIC BACTERIA

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At present we have very few information about the impact of edible mushrooms on those probiotic bacteria that live in the human intestines and have beneficial effects on the human health. In the course of the project we investigated the extraction of five mushroom species in combination with probiotic bacteria. Five mushroom species, the *Agaricus bisporus, A. subrufescens, Pleurotus ostreatus, Ganoderma lucidum* and *Trametes versicolor* were involved into the investigations. Each species were cultivated, then the fruiting bodies were dried and grinded; these powders were then used for the making of water, alkalic, acidic and ethanol extractions. The extracts were mixed with carbohydrate-free liquid broth, specific for bacteria, and inoculated with the following probiotic bacteria subsequently: *Lactobacillus acidophilus, Lb. casei, Lb. paracasei, Lb. rhamnosus* GG. Two faecal species, the *Enterococcus faecium* and *Escherichia coli* were investigated, as well. Colony forming units were counted after 24 and 48 hour of incubation. Prebiotics (technical grade and pure inulin) were used as positive controls and distilled water as negative control. We found that the water extract had the strongest positive effect on the proliferation of probiotic bacteria, followed by the acidic, alkaline extracts and the solid fraction of water extracts. E. coli cell numbers did not show significant differences in the various treatments. Effect of the *A. bisporus, A. subrufescens* and *P. ostreatus* extracts were the strongest. Based on these results we think that certain mushroom species have prebiotic effects (e.g. due to glucans) but of course, there might be other compounds that are responsible for the proliferation enhancing effect. In the course of our work we managed to demonstrate that mushrooms may have positive impact on the human health, in addition to their beneficial nourishment physiological effects.

## CHARACTERIZATION OF SOME *PLEUROTUS ERYNGII* ISOLATES IN CULTIVATION TESTS

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The king oyster mushroom (*Pleurotus eryngii*) is a well-known species and has maybe one of the best taste within the *Pleurotus* genus. There are several cultivation technologies and substrates described in the literature, but the improvement of those are always necessary. In these experiments a comparative cultivation of *P. eryngii* isolates of mostly Hungarian origin was performed. Sixteen *P. eryngii* isolates were cultivated on lignocellulose substrate containing soy bean based enrichment. Composition of the substrate: 65% beech sawdust; 17% bran; 9% beech woodchips; 3.5% gypsum; 5.5% soybean supplement (Promycel 480). Water content was adjusted to 60%, and then the mixture was filled into plastic bags, sterilized and spawned. The blocks were cased by peat-based commercial *Agaricus* casing soil. We determined the amount of yield, number of fruiting bodies,

period of flushes, average weight of fruiting bodies, biological efficiency (BE, %) and productivity (P, %) for each strains. The amount of yield was given for 100 kg of substrate. A photo documentation and description of the strains were also prepared. The highest yield was produced by the Ple-4V (41.5 kg/100 kg) and Ple-5V (39.5 kg/100 kg) strains, whereas the lowest yield was found in case of the PEL (9 kg/100 kg) and PEG (11 kg/100 kg) strains. The average yield of the strains was 27.53 kg calculated for 100 kg substrate. The number of fruiting bodies was 1488 pcs, the average weight of fruiting bodies (concerning the species) was 19.95 g.
Very high biological efficiency was produced by the Ple4V (156.2%) and Ple5V (140%) strains. The lowest efficiency was found at the PEL (28.5%) and PEG (37.8%) strains. Our results showed that the average yield was over 25 kg/100 kg spawned substrate, which means that this species might be a successful rival of the oyster mushroom (*P. ostreatus*) hybrids that are very popular amongst the growers nowadays. In the future it is necessary to select the best strains for cultivation, start cross breeding experiments, determine the optimal substrate composition adapted to the local lignocellulose sources and define the most suitable environmental conditions.

## TRIALS FOR DISCRIMINATING *PLEUROTUS ERYNGII* ISOLATES BY PARTIAL *TEF*1A AND *RPB*2 SEQUENCES

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The *P. eryngii* species complex includes several varieties and certain groups with ambiguous taxonomic position. At the moment the following taxa are recognized: var. *eryngii*; var. *ferulae* /syn.: *P. fuscus* var. *ferulae*/; var. *elaeoselini*; var. *nebrodensis*; var. *tingitanus*; var. *tuoliensis*; *P. hadamardii*; *P. fossulatus*. In the course of our studies sequence analysis of the *tef*1α and *rpb*2 genes was performed, in order to distinguish varieties and reveal variability between isolates. Specific regions of the *tef*1α and *rpb*2 genes were amplified in PCR experiments. The fragments were cleaned, sequenced, aligned then BLAST searched against the NCBI GenBank nucleotide database. Some point mutations were detected in the sequences, which were used for selection of differentiating restriction enzymes for subsequent PCR-RFLP experiments. The *tef*1α gene sequences showed 100% identity in each strain. In contrast to that, point mutations were detected in the 21, 372 and 957 positions of the *rpb*2 sequences. *In silico* digestion was performed on the sequences and two restriction endonucleases, the BsmAI and TspDTI were selected for PCR-RFLP experiments. As a result of the digestions, the isolates could be grouped into two groups with both enzymes. At the same time, BLAST search with both amplified sequences did not give reliable information neither on varietas, nor on species level.
The *rpb*2 locus, with its higher level of polymorphism, is a potential candidate for differentiation between varieties or identification on varietas level. We plan to investigate which loci and molecular methods may be suitable for differentiation of isolates.

# THE PRIVATE LIFE OF INDIVIDUAL CELLS OF FUNGI FOLLOWED BY HIGH TEMPORAL RESOLUTION, NEAR-INFRARED TIME-LAPSE MICROSCOPY AND DIGITAL IMAGE ANALYSIS

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The study of filamentous fungi is generally limited to cellular populations as individual cellular events on a minute timescale are usually equalized during longer periods. This kind of approximation of individual cellular behavior leads to an abstraction model that is usually sufficient for the understanding of most biological processes. On the other hand, there are certain situations when changes of an individual cell can lead to dramatic alterations affecting the whole population including, but not limited to the field of *in vivo* / *in vitro* evolution, pathogenicity and resistance. Dynamic morphological/behavioral investigations of these events require a more sophisticated method than the simple monitoring of populations. Treated and control samples need to be held under same constant conditions during the experiment while the imaging method must be as noninvasive as possible. After realizing these challenges we have established a system containing four identical, computerized microscopes located in a $CO_2$ incubator. We have also developed a low-phototoxicity illuminator using 940nm near-infrared light to optimize wavelength-dependent resolution, sample penetration, camera sensitivity, exposure and biological effects. Transmission light microscopic images were taken every minute up to a week. Quantitative methods were developed for image sequence analysis including dynamic morphological properties as motility, adhesion, yeast-to-hyphal transitions, hyphae extension velocity, ramifications and spatial-temporal mapping. Digital image analysis was used to investigate hyphal growth dynamics in different *Candida albicans* strains. The effects of the quorum sensing tyrosol and farnesol molecules, the deletion of the fungus specific protein phosphatase Z1 (CaPPZ1), and the hypha-specific cyclin (HGC1) genes were analyzed. In agreement with the expectations based on published data our method showed that hyphal extension of wild-type SC5314 cells was accelerated by tyrosol and inhibited by farnesol. Hyphal growth rate was moderately lower in cappz1 and strongly reduced in *hgc*1 deletion mutants. In addition, tyrosol treatment caused a firm adherence, while farnesol treatment and *hgc*1 mutation prevented the adherence of yeast cells to the surface of the culture flask. Transition from yeast-to-hyphal state was faster after tyrosol treatment, while it was reduced in farnesol-treated cells as well as in the *cappz*1 and *hgc*1 mutants. Our data confirm the notion that the attachment of yeast cells, the yeast-to-hyphal transition, and hyphal growth rate are closely related processes. Our method can be adapted to a great variety of experiments for the detection of morphological changes elicited by effector molecules or specific mutations. It is known that *Candida* (spp.) can attach to plastic surfaces, intravenous devices, bladder catheters, and other prostheses routinely used in medicine. Anti-adhesive treatments of devices combined with the inhibition of hyphal growth are expected to be suitable to prevent iatrogenic *Candida* infections. In this respect our experimental approach is expected to contribute to the development of more efficient novel strategies in controlling the growth of *Candida* species.

# A FOLLOW UP STUDY OF COLONISATION AND SURVIVAL OF MULTDRUG-RESISTANT STRAINS ON THE INNER AND OUTER SURFACE OF THE TAPS IN AN INTENSIVE CARE UNIT

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Acquisition of multidrug-resistant strains occurs frequently by the patient in intensive care units (ICUs). Mainly Gram-negative strains *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae* can cause nosocomial ventilator associated infection in ICU patients. Oxacillin-resistant coagulase negative staphylococci (CNS) are the other bacteria, which are also acquired and can colonise the skin as the selection pressure result of antimicrobial treatment and may cause cannula sepsis. *Pseudomonas aeruginosa* can multiply and survive in moist parts of the ICU. This single centre one year follow up study examined the tap water, inner and outer surface of taps, and water angle valve as sources of multidrug resistant strains in a 10-bed intensive care unit. In a previous study we demonstrated that metallo-beta-lactamase producing *Pseudomonas aeruginosa* strains were isolated from water and inner surface of the taps in patient's room before and after hydrogen-peroxide vapour disinfection. In this study we checked the water angle valve (hot and cold), the water, and tap surfaces. Samples were collected at the same time and checked for multidrug resistant strains. Sample collections were performed every three month between April 2014 and May 2015. The isolated strains were identified and resistance pattern, genes of carbapenem resistance and integrons were determined. No Gram-negative pathogenic strain was found in the tap water in social rooms before disinfection vapour, but three months after the disinfection and later *Pseudomonas aeruginosa* strains were isolated from the water and inner surface of the tap in one social room. These strains were sensitive to antipseudomonal drugs. Three months before disinfection of the patients rooms metallo-beta-lactamase *P. aeruginosa* was only isolated from the inner surface of one tap and its water. After the disinfection *P. aeruginosa* strains were cultivated from samples of the inner surface of the taps and their water. Only two strains isolated from samples of one tap and its water collected immediately after disinfection were sensitive to antipseudomonal drugs. Other *P. aeruginosa* sample isolated from patients rooms taps and water carry the blaVIM-1 and class 1 integron genes. *Stenotrophomonas maltophilia* were isolated only from one water and tap samples. Samples from inner surface of water angle valve were negative for multidrug resistant bacteria. Oxacillin resistant coagulase negative staphylococci were isolated from each samples of tap handle. These strains carry *mec*A gene. Multidrug- resistant *Pseudomonas aeruginosa* strains can remain for long time on the inner surface of taps, which can become a reservoir of these strains. Using the water from these taps for washing the patients, these strains can be transferred to the patient and can contaminate the health care instruments. Touching the tap handles methicillin resistant coagulase negative staphylococci can be transferred to everywhere in the intensive care unit. As the water angle valves did not yield multidrug resistant strains the source was probably out of the water system.

## FEAST OR FAMINE: NUTRIENT MEDIATED REGULATION OF DIFFERENTATION IN *STREPTOMYCES GRISEUS*

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The genus *Streptomyces* comprises Gram+, soil-dwelling, filamentous bacteria. It shows complex morphological differentiation that relates to the production of secondary metabolites. This ability makes *Streptomyces* biotechnologically significant bacteria since they produce 75% of the known antibiotics. Extracellular autoregulatory molecules play a key role in controlling antibiotic production and morphological differentiation. In *Streptomyces griseus* A-factor (2-isocapryloyl-3R-hydroxymethyl-γ-butyrolactone) proved to be significant, whose synthesis depends on the *afs*A gene. Our aim is to understand the regulation of A-factor mediated differentiation and antibiotic production in *Streptomyces griseus*. We studied an A-factor negative (AFN) mutant to shed light on the genetic and physiological background of the AFN phenotype. Previous results showed that the *afs*A gene is functional and transcribed in the AFN strain. In this study the production of AfsA protein, aerial mycelium, spores, extracellular protease and antibiotics was followed on rich and minimal medium. The AfsA protein was detected both in the control and AFN strains by Western blotting in cultures growing on rich or minimal medium. The presence of AfsA in the AFN strain supposes that AfsA protein is not enough for sufficient A-factor production. Additional elements are also required that might be involved in the synthesis of A-factor or in the regulation of this process. This also repels the hypothesis that A-factor production would be regulated by the expression of *afs*A gene. Moreover our data suggest different regulation of morphological differentiation on rich and minimal medium. On rich medium the AFN strain did not form aerial mycelium due to the lack of A-factor but spores were formed from the substrate mycelium in old cultures. On minimal medium aerial mycelium emergence and sporulation were observed in AFN although the amount of spores was decreased compared to the control strain. These data suggest that on rich medium A-factor is essential for aerial mycelium but not for spore formation. On minimal medium other nutrient stress mediated pathways have primary role in the induction of morphological differentiation. The production of antibiotics and extracellular proteases was lower in AFN than in the control strain on both rich and minimal medium.

This confirms that A-factor is essential for antibiotic production either by direct gene activation or by the induction of enzymes (extracellular proteases) that provide nutrients for antibiotic synthesis. A crosstalk between A-factor and nutrient stress mediated pathways may contribute to sufficient sporulation and antibiotic production in *Streptomyces griseus*.

Understanding the details of this regulation pattern may lead to the development of antibiotic overproducing strains or to the identification of new type of antibiotics.

## CEFOTAXIMASE (CTX-M) AND QUINOLONE RESISTANCE GENES (QNR) WITH ADDITIONAL ANTIMICROBIAL RESISTANCE MECHANISMS IN COMMENSAL *ESCERICHIA COLI* FROM HEALTHY PIGS

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Concerning the importance of food producing animals as potential reservoirs of enteric bacteria with clinically relevant antimicrobial resistance traits, we tested the prevalence of extended-spectrum β-lactamase (ESBL)-producing and fluroquinolone resistant *Escherichia coli* from pigs in order identify multiple resistance mechanisms circulating in pig farms in Hungary and Croatia with special regards to plasmid mediated genes encoding cefotaximases (CTX-M) and quinolone resistance (qnr). For this purpose, faecal samples were collected from pigs representing three farms from Hungary and six farms from Croatia with 45 and 60 samples respectively. Farms were located in separate regions of the countries. Cefotaxime or nalidixic acid resistance were used as prime markers for the isolation of multiresistant *E. coli* strains. A second selection was based on resistance to additional antimicrobials (i.e. gentamicin) aiming to reduce the collection to isolates with representative multiresistance phenotypes. In several cases more than two different multiresistance phenotypes have been isolated from the same pig, which were considered as independent *E. coli* isolates. This collection of multidrug resistant *E. coli* contained 139 strains and was tested for the presence of blaCTX-M and qnr genes by PCR. Selected isolates carrying genes blaCTX-M and/or qnr are being subjected for confirmation and further typing of antimicrobial resistance genes by using the PCR-microarray AMR05. Cefotaxim resistant *E. coli* have been detected in one Hungarian and one Croatian farm representing 17% of all pigs tested. In majority of the strains, the plasmid-related resistance phenotypes such as ampicillin, cefotaxim, gentamicin and tetracycline occurred in multiple combinations. In 11% of the strains the coexistence of Ctx-Nal phenotypes was detected, together with the presence of the cefotaximase gene blaCTX-M.

*E. coli* strains with nalidixin resistance phenotype have been predominantly (70%) characterizing healthy pigs independently from the farm and country of isolation. Ciprofloxacin resistant strains occured on one farm only. The plasmid-mediated fluoroquinolone resistance gene *qnr*S was identified in 11% of the strains, with or without the nalidixin-ciprofloxacin resistant phenotype, while genes *qnr*A and *qnr*B were absent. Our results lead to conclude that multiresistant commensal *E. coli* strains carrying plasmid-mediated CTX-M type cefotaximase and/or quinolone resistance genes in different combinations are widespread on some pig farms but much less on others, most likely reflecting differences in use of antimicrobials.

Ama Szmolka is a holder of János Bolyai Stipend of the Hungarian Academy of Sciences.

## INSIGHT INTO THE MOLECULAR CHARACTERISTICS OF NEW MULTIRESISTANT CLONES AND PLASMIDS OF *SALMONELLA INFANTIS* IN POULTRY AND MAN

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The lack of the regular monitoring of *Salmonella* Infantis together with the effort to reduce prevalence of "top five" serovars lead to a dramatic increase of *S.* Infantis in poultry with reflection in human population. As a background of this study, a clonal change reported previously in Hungarian *S.* Infantis strains is remarkable. Accordingly, in the early 2000s, older pansensitive isolates of S. Infantis have been replaced by new tetraresistant clones carrying a large plasmid conferring the multiresistance phenotype. Based on the above finding, our aim is to characterize antimicrobial resistance pheno-and genotypes of recently isolated *S.* Infantis strains, and to provide the first description of the large multiresistance plasmid in a poultry isolate representing the prevalent tetraresistant Hungarian clone. For this purpose ~300 strains of *S.* Infantis were tested, originating mostly from broilers and from human clinical samples. Strains intended to represent the current status of *S.* Infantis infection in poultry and human between 2011-2013. The antimicrobial resistance phenotype, pulsotype and the plasmid content were determined, based on which representative strains were selected for resistance genotyping by PCR microarray. One poultry strain (SI 54/04), representative of the tetraresistant, plasmid containing strains was subjected to genome sequencing (Olasz et al., 2015), giving a basis for the characterization of the large multiresistance plasmid. Resistance phenotyping and PFGE analysis has shown a constant circulation of the former major multiresistant clones and patterns within the newly isolated strains of *S.* Infantis both in poultry and human. Multiresistance phenotypes were associated mostly with the presence of class 1 integrons (intI1)and the gene *tet*A for tetracycline resistance, being the prime genetic markers for the carriage of the large multiresistance plasmid. However, the coexistence of the *tet*A and other plasmid related genes for β-lactam and fluoroquinolone resistance such as *bla*TEM-1, *bla*CMY-9 or qnr indicate important and divergent plasmid associations in some of the strains. By sequence analysis we provide the characterization of the multiresistance megaplasmid (pSI54/04) of the strain SI 54/04 which can be considered as the first Hungarian reference plasmid of *Salmonella* Infantis. The backbone of this IncI type plasmid is a mosaic of resistance (nickel-, mercury resistance), and virulence regions (encoding siderophore Yersiniabactin, fimbriae) potentially promoting survival not only in the vertebrate hosts but also in their environment.

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## REMOVAL OF *RICKIA WASMANNII* (LABOULBENIALES, ASCOMYCETES) INFESTATION FROM *MYRMICA SCABRINORDIS* ANTS

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Laboulbeniales fungi can be found everywhere around the globe. Among them are more than 2000 obligate ectoparasites, infesting a wide range of agriculturally important insect species, the protection of which is a critical issue for agriculture. We examined *Rickia wasmannii* (Laboulbeniales, Ascomycetes) infestation on *Myrmica scabrinordis* ants. The objective of our research was to determine the degree of infestation and observe its connection to the deterioration of host fitness. Our aim was also to propose long-term therapeutic methods to handle the problem. The communication of ants as eusocial species, can be of vital importance for the survival of an individual ant. As the antennae and the eyes are the most important organs for the communication of ants, we have chosen to examine the effect of *Rickia wasmanii* infestation in these organs. In antennae, the degree of infestation was determined after dividing the anatomical parts of the antennae into 3 regions, and counting the number of hyphae in each region using scanning electron microscopy. We observed that while infested ants who did not clean their antennae, in mechanically more active regions of antennae many hyphae were broken. At anatomically less mobile segments of the antennae more hyphae were found and the number of intact ones was significantly higher. As far as the infestation of the eyes is concerned, the statistical analysis of the frequency of hypha appearance in the compound eye was tested by photography. In most cases there was a heavy infestation around the eyes, but the presence of the hyphae was also observed several times in the compound eye. In the second phase of our experiments we have examined the reaction of *Rickia wasmanii* to silver colloid treatment and how successfully these ants could be reintegrated in the ant population. Infested ants were treated with different concentrations of silver nanoparticle aerosols. The severely infested *Myrmica scabrinordis* ants were to prevent any possible cross-infestation. All experiments were conducted in the same environmental conditions. The effect of silver nanoparticles was followed for a week, photography of specimens was taken daily, and experiments were repeated three times. Our results showed that higher than 20 ppm concentration of silver nanoparticles caused argyiria, and killed the host along with fungi. Lower concentrations between 0.1-1 ppm had neither effect on host, nor on fungi. At around the optimal concentration (1-5 ppm) the coverage of hyphae dropped, and by the end of the 5 ppm silver nanoparticle treatment some of the *M. scabrinordis* ants were completely free of hyphae. We concluded that *Rickia wasmannii* hyphae hinder the function of important communication organs of *M. scabrinordis*, and reduce it's the fitness. In addition we have determined the optimal silver colloid aerosol concentration (5ppm) that helped to get rid of ants from *Rickia wasmannii* infestation.

## DIFFERENTIAL ENRICHMENT OF Fe(III)-REDUCING BACTERIA FROM GROUNDWATER OF THE SIKLÓS BTEX-CONTAMINATED SITE, HUNGARY

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Dissimilatory Fe(III) reduction is a crucial anaerobic respiratory pathway in which the reduction of Fe(III) is coupled to the terminal decomposition of organic matter. Iron reducers have a great impact on global biochemical cycles, while in contaminated subsurface environments they often play key role in the degradation of organic and inorganic contaminants. *Rhodoferax* species and other Fe(III)-

reducing microbes, like members of the genus *Geobacter* are frequent members of oxygen-limited, BTEX contaminated subsurface environments. Some *Geobacter* species harbor benzylsuccinate synthase (*bss*A), the key gene of anaerobic degradation of toluene, while some *Rhodoferax* species may have a role in the microaerobic degradation of aromatic compounds and harbor subfamily I.2.C-type catechol 2,3-dioxygenase (C23O) genes. The main objective of this study was to investigate diversity of *bss*A genes of the "Siklós" BTEX-contaminated groundwater and to link them to Fe(III)-reducing members of the bacterial community. Acetate enrichment media were inoculated with groundwater samples under anaerobic circumstances. Fe(III)NTA was added to the media to provide Fe(III) as sole electron acceptor. Four different enrichments were set up: (i) supplemented with 0.05% (w/v) yeast extract and $NH_4Cl$; (ii) supplemented solely with 0.05% (w/v) yeast extract; (iii) supplemented with $NH_4Cl$ as sole fix nitrogen form; and finally (iv) omitting both yeast extract and $NH_4Cl$. A metagenomic approach was used to reveal the bacterial community composition in the initial groundwater, while the methods of sequence-aided T-RFLP and qPCR were used to follow changes in the community structure during the enrichments. The diversity of the initial bacterial community considerably decreased during the enrichment culturing, and the lowest diversity was observable in case of enrichment cultures omitting yeast extract.
Nevertheless, *bss*A genes, even yet unknown genotypes were detectable in every type of enrichment culture. The selective enrichment of *Geobacter* species was observable in media without yeast extract, while by omitting the fix nitrogen form from the enrichment medium the selective enrichment of *Geobacter*-related bacteria became to be more expressed. However, completely different *Geobacter* species could be observed in the presence or absence of fix nitrogen form. The enriched *Geobacter*-related bacteria of the "Siklós" BTEX-contaminated groundwater were only distantly related to validly described members of the genus *Geobacter* and unfortunately, none of the detected bssA genotypes could be linked to them. Thus, the direct role of the detected *Geobacter* species in the anaerobic degradation of BTEX-compounds is questionable.
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## FULL GENOME SEQUENCE OF NOVEL CIRCOVIRUSES DETECTED IN LOWER VERTEBRATE ANIMALS

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The first complete genome sequences of circoviruses (CVs) of lower vertebrate animals have been published from fish by Hungarian authors recently. We have also reported the PCR-detection of circovirus-like sequences in samples of poikilothermic animals including fish, amphibians and a reptile. In the present study, the full genomic sequence and phylogenetic analyses of four putative CVs, found in the samples of a common bream (*Abramis brama*), a brown toad (*Bufo bufo*), two green tree frogs (*Litoria caerulea*) and a red-eared slider (*Trachemys scripta elegans*), respectively, were performed. Initial detection of the viruses was by a consensus nested PCR targeting the most conserved (~ 350 bp) fragment of the replication-associated protein gene (*rep*). To amplify the missing parts of the supposedly circular genomes, we used inverse nested PCRs with specific primers designed from the respective rep sequences. The Rep protein of all four viruses showed clear homology to its counterparts in previously described CVs, whereas the predicted capsid proteins (Cap) were more diverse in both, aa sequence and size. Accordingly, the lengths of the full

genomes, ranging from 1172 to 1965 nt, also showed considerable differences. Three genomes had an arrangement typical for members of the *Circovirus* genus, i.e. the origin of replication, consisting from a stem-loop structure including the conserved nonanucleotide sequence (TAGTATTAC), was present between the 5' ends of the two, oppositely directed major genes, on the DNA strand containing the rep gene. The virus genome sequenced from the red-eared slider, was not only the shortest, but had a genome organization resembling that of candidate members of the proposed genus *Cyclovirus*. Its cap gene was on the sense DNA strand with a stem-loop structure containing a slightly divergent (CAGTATTAC) nonanucleotide sequence. Moreover, the DNA-binding region, rich in basic amino acid residues (arginine and lysine) close to the N-terminus of the Cap protein was absent. In the ORF coding for the Cap protein in the green tree frog CV, no suitably located ATG triplet was found, however, an alternative start codon could be presumed. On the phylogenetic tree, obtained by maximum likelihood analysis based on the complete Rep aa, the piscine and the two frog CVs clustered in a common group with the approved members of the *Circovirus* genus. The putative slider CV appeared in a separate branch and seemed to be more closely related to members of the most recently proposed genus *Krikovirus* than to *Cyclovirus*. The eventual pathogenic role of these newly characterized viruses is unclear. The bream did not show any signs of disease. One of the captive-bred green tree frogs had died, but identical *rep* sequences were detected by PCR in the remaining, healthy individuals kept in the same terrarium. The red-eared slider had previously been found to shed adenovirus and was diagnosed with unilateral bacterial otitis, while the toad was found dead in the nature without any visible pathologic alterations.

These are the first full genome sequences from putative members of the Circoviridae family detected in amphibian and reptilian hosts.

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## COMPARISON OF THE INCIDENCE RATE OF DIFFERENT IRON ACQUISITION SYSTEMS OF COMMENSAL, CLINICAL AND ENVIRONMENTAL *KLEBSIELLA PNEUMONIAE* ISOLATES

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*Klebsiella pneumoniae* is known as an opportunistic pathogen causing different type of infections in patients with impaired immune system. According to studies 10% of the population carry *K. pneumoniae* in their gastrointestinal (GI) microbiota. Data also suggest that GI tract is frequent source of different infections types that can occur nearly at any body site. The iron acquisition systems (IAS) are the important members of the bacterial virulence factor repertoire. Numerous studies deal with IAS of clinical *K. pneumoniae* isolates, however much less data are available about the presence and distribution of these systems in commensal and environmental isolates. In the present study 120 commensal *K. pneumoniae* (CK) strains were isolated from the stool samples of healthy individuals. Clonality of the isolates was determined with different DNA based methods in order to exclude the multiple representations. The "cross feeding" method with indicator strains specific for the respective siderophore types was used to screen for aerobactin and enterobactin production. The presences of the *kfu*B and *irp*1-*ip*2 genes were determined by polymerase chain reaction to assess the prevalence of Klebsiella ferric iron uptake (KFU) and yersiniabactin systems in the isolates. Gained data were compared to those available from our preceding studies performed on 88 of urinary tract infection (UTI) and same number of blood stream infection isolates (BSI), 110

strains of wound infection (WI) and 113 wastewater (WW) strains. Our results show that enterobactin had the highest prevalence (83-96%), the second most frequent was the KFU system (15-47%) followed by the yersiniabactin system (11-30%), while aerobactin presented with the lowest occurrence (0.9-9%) among the isolates. The frequency of enterobactin production was significantly higher among CK isolates (96%) ($\chi 2$, $p<0,01$) compared to the clinical isolates UTI (73%), BSI (74%), WI (86%) while WW strains showed non-significantly lower value (94%). In case of aerobactin prevalence WW isolates showed the lowest rate (0.88%) followed by CK (2.5%), WI (5.5%), UTI (5.7%) and BSI (9.1 %) strains. The CK isolates possessed the second less frequent KFU system occurrence (19.1 %) but the difference was smaller than 5% and proved to be non-significant compared to the WW strains, however all clinical isolates showed significantly higher ($\chi 2$, $p<0,01$) frequencies WI (35.5%), UTI (46.6%) and BSI (46.6%). The CK isolates indicated the highest (30%) occurrence of yersiniabactin system which was significant ($\chi 2$, $p<0.01$) compared to WW (11.5%) and WI (12.7%) nevertheless, the differences were not significant in cases of BSI (15.9%) and UTI (23.9 %) isolates.

Further data analysis was performed to reveal prevalence of single and multiple iron acquisition systems among the isolates. After the WW (77%) and the WI (60%) isolates the CK isolates showed the third highest value (58%) for carrying single IAS. The CK strains followed the UTI isolates in respect of double IAS carriage (39%, 47%), respectively. With very similar value (cc. 3%) the WW and CK isolates showed the lowest prevalence for triple IAS carriage followed by the clinical strains (7%-10%). Data analysis revealed that the distribution pattern of IAS of CK isolates was between the clinical and environmental strains but more closer to the clinical ones. These data suggest that *K. pneumoniae* may adapt to the iron levels of the milieu.

## FOOD SAFETY RISK OF DEOXYNIVALENOL, ZEARALENONE, T-2 MYCOTOXINS IN SWINE FEED FROM THREE MANUFACTURERS IN HUNGARY

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Mycotoxin contamination of forages is a significant problem in all countries in the temperate zones. In the *Fusarium* genera the so-called trichothecene mycotoxins - typically deoxynivalenol (DON), T-2- and zearalenone (ZEA) occur most often in feed cereal grains. Animals feeded with mycotoxin contaminated feeds represent a significant food safety risk. By consumption of meat consumers are exposed to a regular small dose toxin load. ELISA tests of different Hungarian swine feeds proved that in the feed of these animals DON, ZEA and T-2 mycotoxins are constantly present. Former tests have proved that these mycotoxins, although only in traces, but can also be found in pork. Long-term consumption may result in a serious mycotoxin risk in human. In our experiments 45 feed samples from three significant Hungarian swine feed producer were tested. These were the followings: sow-, boars and piglet feeds. Five samples were tested per type per manufacturer. For representative sampling mycotoxin tests of the feeds were done according to the regulation nr. 519/ 2014/ EC of the European Committee. The experiments were carried out with competitive Ridascreen Fast ELISA test kits (DON, ZEA, T-2 kits) according to the manufacturer's instructions. Our results showed that among Fusarium toxins generally DON was present in the highest concentration, followed by T-2 and finally ZEA in all tested swine feeds. It could be seen that average values of DON concentrations were an order of magnitude greater than the average values of other toxins.

Each of the toxins were detected in all swine feeds. Previous studies revealed that deoxynivalenol, zearalenone, T-2 mycotoxins can be present in pork. Low dose mycotoxin contamination of pork do not result in acute mycotoxicosis in human but they may have chronic effects and health problems probably may only occur after many years. Therefore, there is a need to establish a stricter limit for DON, ZEA, and T-2 mycotoxins in swine feed's raw materials.

## *CANDIDA PARAPSILOSIS* TRIGGERS NLRP3 INFLAMMASOME ACTIVATION – A COMPARISON WIH *CANDIDA ALBICANS*

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*Candida albicans* (Ca) and *C. parapsilosis* (Ca) are major human pathogens causing severe infections in immunocompromised patients. Production of IL-1β by inflammasomes plays an important role in host defense during invasive candidiasis. Our aim was to compare the inflammasome activation in myeloid cells in response to Ca and Cp. We have previously shown that PBMCs stimulated with live Cp produce lower amounts of IL-1β compared to Ca stimulated cells and this also seem to be true for PBMC-DMs. Here, we demonstrate that Cp induces much lower IL-1β production in human monocyte-derived macrophages and PMA-differentiated THP-1 monocytes compared to Ca. In THP-1 cells, Ca induced the release of IL-1β after 24 hours already at an MOI of 0.01, while a 100-times higher dose of Cp cells (MOI of 1) was needed for IL-1β secretion. This difference was species rather than strain specific and IL-1β production was independent of the presence of pseudohyphae. This originated from the differential processing of IL-1β rather than from dissimilar gene expression, as levels of IL-1β mRNA and pro-IL-1β in THP-1 cells revealed. Also, IL-1β production induced by Ca and Cp in THP-1 cells was dependent on caspase-1, caspase-8, TLR4, Syk and NADPH-oxidase, suggesting that both species activate the inflammasome. This was unambiguously corroborated by experiments with NLRP3 and ASC deficient THP-1 cell lines. Using cytochalasin D, we found that rapid and robust IL-1β secretion induced by Ca was highly dependent on phagocytosis. Comparing phagocytosis kinetics, Ca was phagocytosed notably more rapidly than Cp by THP-1 cells. As it has been associated with inflammasome activation, we measured intracellular reactive oxygen species (ROS) amounts and found that while Ca elicited a robust ROS response, Cp was unable to induce ROS production in the first 4 hours of infection. Additionally, we found larger phagolysosome damage induced by Ca than Cp. Our results strongly suggest that Cp is very inefficient in inducing pro-IL-1β processing in comparison to Ca. We have recently begun *in vivo* murine infection studies and some preliminary data are likely to be presented. These findings contribute to the better understanding of *Candida* infections, and highlight the importance of research on different *Candida* species rather than Ca alone when investigating the immunity against these pathogens.

# LIQUID EGG WHITE WITH EXTENDED SHELF LIFE AS A BASE FOR FRUIT DRINKS

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A food engineer who desires optimal heat treatment parameters is faced with a double challenge when deciding how to treat liquid egg. The first consideration is that even low heat is enough to denature the protein content of liquid egg, so it is not an option to set treatment temperature too high or use too long a treatment time. On the other hand, an expert knows that liquid egg is the favorite "feeding ground" of microbes, whose reduction could be achieved by raising the temperature and lengthening heat treatment time. The situation is made even more complicated if the liquid egg (in the present case, liquid egg white) is to be flavored with ingredients consisting of other microbiological and nutrititive materials, such as 100% fruit juices. This study has examined the making of a microbiologically stable egg white base. Liquid egg white samples from the Capriovus Ltd. production line were used for the experiments. The samples were pre-treated with protease in order that the "drink bases" remain liquid during heat treatment (the manufacturer guarantees the material to remain liquid up to 75 °C in heat treatment). Next, the samples were inoculated with Enterobacteriaceae bacteria derived from egg products, which produced approximately $10^9$ CFU/ml live viable cell count. After inoculation, the liquid egg white was packaged in 100 ml heat- and pressure-resistant pouches, and treated first with heat and then with high hydrostatic pressure (HHP), in sequence. Central composite design was applied to 46-74 °C heat treatment and 280-421 MPa pressure treatment ranges. This produced 11 statistically reliable results. A microbe reduction of 2.7 – 9.5 (lgN/N0) was found in the experimental group. The lowest microbe reduction occurred in the lowest heat treat temperature sample (H – heat treatment: 46 °C, p – pressure treatment: 350 MPa), whereas the greatest variation in viable cell count was found in the highest heat treatment temperature sample (H: 74 °C, p: 400 MPa). These values were in agreement with the given model: although temperature ($p=0.000$) and pressure ($p=0.023$) values significantly affect changes in sample viable cell count, the temperature change of 1 °C ($\beta$-coefficient=0.235) influences microbe death much more strongly than a change in pressure ($\beta$-coefficient=0.008). The derived model coincided well with the results, as the correlation between the calculations and the actual measurements was very close ($r^2=0.989$). The useability of our model further confirms that change in viable cell count was nearly the same for the 3 measured samples taken from the middle of the range (H: 60 °C, p: 350 MPa). The lgN/N0 value ranged between 6.7 and 7.1, a very close match. Based on our results, it can be said that although heat treatment and HHP treatment did not create any synergy when applied consecutively, their microbe-reducing effect was successful, and thus we obtained a better treatment using this combined procedure, without seriously damaging the liquid egg white. Our study included treatments of packaged samples only, in order to avoid post-treatment contamination.

The results show that it is possible to obtain, by means of combined heat treatment and HHP technology – under factory conditions – a long-lasting product that retains its quality (stored at 0-5 °C for 30 to 90 days). However, it is necessary to experiment further with storage methods and conditions, as well as to investigate the effects of certain preservatives (e.g. citric acid).

## CLONING, EXPRESSION AND BIOCHEMICAL CHARACTERIZATION OF ENDOMANNANASES FROM THREE DIFFERENT *THERMOBIFIDA* SPECIES

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Thermobifidas are thermotolerant, compost inhabiting actinomycetes which have complex polysaccharide hydrolyzing enzyme systems. The best characterized enzymes of these hydrolyses are cellulases from *T. fusca*, while other important enzymes especially hemicellulases are not deeply explored. To fill this gap we cloned and investigated endomannanases from those reference strains of the *Thermobifida* genus which have published data on other hydrolases (*T. fusca* TM51, *T. alba* CECT3323, *T. cellulosilytica* TB100T and *T. halotolerans* YIM90462T). Our phylogenetic analyses of 16S rDNA and endomannanase sequences revealed that *T. alba* CECT3323 is mis-classified, in the reality it is a *T. fusca* isolate. The cloned and investigated endomannanases belong to the family of glycosyl hydrolases 5 (GH5), their size is around 50 kDa and they are modular enzymes. Their polypeptide chains exhibit high homology, but they have a 23-25 AA long interdomain sequence which don't show homology to each other, but all of them are built up from 3-6 times repeated tetrapeptide motifs (PTDP-Tc, TEEP-Tf, DPGT-Th). Heterologously expressed Man5A enzymes had similar biochemical characteristics regarding pH and temperature optima and substrate specificities. They exhibited activity only on mannan out of 7 investigated substrates.
Kinetic parameters showed that endomannanases of *T. cellulosilytica* and *T. halotolerans* had three times higher activity than the endomannannase of *T. fusca*. We detected great variability in heat stability, which was greatly influenced by buffer composition. Investigated endomannanases might be important subjects for studying molecular mechanisms of heat stability and for industrial applications to produce mannooligosacharide prebiotics.

## INTERACTION OF *CURVULARIA* STRAINS WITH HUMAN THP1 CELLS

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Opportunistic fungal infections represent a continuously increasing problem, because of the growing population with underlying conditions, difficulties of diagnosis and the high antifungal resistance of

certain fungal agents. Members of the genus *Curvularia* (Ascomycota, Pleosporales) are saprotrophic or plant parasitic filamentous fungi. Some members of this genus have been recovered from human infections known as pheaohyphomycoses. These mycotic infections can manifest as fungal keratitis, sinusitis, cutaneous lesions or invasive infections with frequent involvement of the central nervous system. In the present study, we investigated the cytokine and chemokine response of the human monocyte-macrophage cell line THP1 to three clinical *Curvularia* isolates: a strain of *C. lunata* and *C. spicifera* isolated from human eye infections and a strain of *C. hawaiensis* from a systemic infection. As a non-pathogenic strain, the closely related *Cochliobolus carbonum* isolated from plant leaf was also involved in the study. Relative transcription levels of IL10, IL8, IL6, TNF-α and CXCL10 were measured by real-time, quantitative reverse transcription PCR in response to the conidial and mycelial forms of the fungal strains. In case of the conidia, phagocytosis could not be detected. Therefore, we started to examine the role of the melanin content of the conidia during the infection by infecting the cell line with melanin synthesis blocked conidia.

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## INVESTIGATION OF THE BIODEGRADATION AND CYTOTOXICITY OF VARIOUS HERBICIDES

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The use of pesticides has been gradually increased worldwide since the middle of 20th century because of their crop-protective and yield-improver features. Moreover, the utilization of pesticides doubled in Hungary from 2010 up to the present day and herbicides account for about half of all pesticide use. The adverse side effects of pesticides – e.g. persistence, acute and chronic toxicity to non-target species – can often be detected after long-term application. The residues of pesticides in soil, surface and groundwater can change the diversity of microbial populations of the habitat. However, microorganisms are able to adapt to the altered environmental conditions and degrade these contaminants. The aim of our study was to establish a culture collection of environmental bacterial strains isolated from pesticide contaminated soil and groundwater samples and to examine the bacterial degradation and biodetoxification of three active substances (metolachlor, mesotrione, terbuthylazine) of herbicides. The biodegradation potential of the bacterial strains was examined by analytical chemistry. The biodetoxification was analyzed by *Aliivibrio fischeri* bioluminescence inhibition assay adapted to 96-well microplates. The test was used to measure the cytotoxicity of metabolites from biodegradation test based on the bioluminescence inhibition of the testorganism after incubation times of 0, 3.5, 10, 15 and 25 hours. Furthermore, in this study we compared the 25-hour chronic test to the acute ISO 11348-3 standard assay using 30 minute contact time: the acute and chronic bioluminescence inhibition tests with *Aliivibrio fischeri* were used to determine the acute and chronic toxicity of the pure substances and mixtures of herbicides. The EC50 (Effective Concentration) values were calculated from the concentration-response curves. According to the analytical results four strains were able to biodegrade two active substances of the herbicides with varying efficiency (27-86%). In case of two strains the cytotoxicity decreased, and in case of other two the cytotoxicity increased, presumably due to the formation of more toxic metabolites than the

parent compound. In case of the third substance we did not detect degradation at all. The comparison of the acute (standard) and chronic toxicity tests showed that the latter one is more sensitive than the standard assay therefore we consider the chronic test suitable detecting the toxicity of the active substances of herbicides and their metabolites.
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## CHARACTERIZATION OF A NOVEL SHIGA TOXIN CONVERTING BACTERIOPHAGE FROM *SHIGELLA SONNEI*

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Shiga toxin (Stx) was first reported in *Shigella dysenteriae* type 1 more than a century ago. Since then a wide array of Stx-producing strains has been isolated among further Enterobacteriaceae species, but first of all in *E. coli*. The *stx* genes in Shiga toxin producing *E. coli* (STEC) are present in the genome of temperate lambdoid bacteriophages and these Stx phages can be induced and spread by transduction and convert Stx-negative strains into STEC. Most of our knowledge about Stx phages is based on the characterization of Stx phages carried by *E. coli* and far less information is available on Stx phages identified in other species. In the present study we show the sequencing and characterization of a novel transferable Stx1- converting phage from a *S. sonnei* clinical strain that harbors the archetype of the *stxAB* operon. The *Shigella* STX1-converting phage proved to be transferable to *E. coli* K-12 strains, and cytotoxic effect of the lysogenized K-12 strains was demonstrated in tissue cultures. Genomic analysis revealed that the prophage genome is circular and its size is 60 875 nt that corresponds to 76 ORFs. The genome of the *Shigella* STX1-converting phage shows high degree of mosaic structure and its architecture is related to lambdoid phages. The STX1-converting phage uniformly inserted into the *ynfG* gene framed by phage integrase and antirepressor genes in wild type *S. sonnei* and the three lysogenized K12 strains C600, DH5α and MG1655. The STX1 prophage proved to be stable in its bacterial hosts and also remained inducible.
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## CAPILLARY GEL ELECTROPHORESIS-BASED PCR RIBOTYPING OF *CLOSTRIDIUM DIFFICILE* STRAINS COLLECTED AT TWO SITES OF HUNGARY IN 2014

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Nowadays *Clostridum difficile* is the most frequent etiologic agent for health-care–associated diarrhoea. It was reported that 30% of adults who developed health-care–associated diarrhoea were positive for *C. difficile*. Our aim was to investigate the *C. difficile* ribotypes collected in two laboratories using a new method, called capillary gel electrophoresis-based PCR ribotyping.

Altogether 192 *C. difficile* isolates were collected. Cell pellets from overnight cultures of *C. difficile* were boiled in Chelex (SigmaTM), and PCR amplification of DNA was performed according to previous publications. PCR fragments were analysed in an ABI 3130 genetic analyser. We found that ribotype 027 was the most frequent among the isolates collected in Budapest. Ribotype 036 showed the highest rate in Szeged. Interestingly our data showed that in the Hospital at Kecskemét, which is on half way to Budapest from Szeged, the most prevalent ribotype was also the 036 (50% of the strains tested). It was found that the second common ribotype was 0174 among the isolates collected in Budapest. A great variety of other ribotypes (32.8% of the examined strains) were also found in both sites showing the non epidemic nature of the disease in many cases. Awareness on the epidemiology which is detected mainly by ribotyping of *C. difficile* is important for the clinicians mainly because of the differing virulence potential of the strains. In Hungary we found regional differences in PCR ribotypes of *C. difficile* during the examined time period.

## INVESTIGATION OF THE STAGE SPECIFIC PROCESS OF *CANDIDA PARAPSILOSIS* PHAGOCYTOSIS BY MACROPHAGES AND DETECTION OF INTRACELLULAR SURVIVAL STRATEGIES

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During the last decades the number of *Candida* infections caused by non-albicans species has been increasing exponentially. Despite of the rising incidence of infections caused by non-albicans species like *C. parapsilosis*, little is known about their pathomechanisms. Certain aspects of *C. parapsilosis* and host interactions have already been investigated; however we lack information about the innate cellular responses towards this species. In our recent study we compared the phagocytosis of *C. parapsilosis* to *C. albicans* and to *C. glabrata*. We examined phagocyte migration, uptake rates, engulfment of fungal cells, and subsequent host cell damage after challenging murine and human PBMC-derived macrophages with fungal cells. Our results indicated increased macrophage migration towards *C. parapsilosis* and we observed differences during the engulfment processes when comparing the three species. While the rate of host cell killing and the overall uptake of *C. parapsilosis* showed similarities mainly with *C. glabrata*, the engulfment time of this species was comparable to that of *C. albicans* regardless of the pseudohypha length and position relative to phagocytes. However, *C. parapsilosis* cells killed more human macrophages than *C. glabrata*. Furthermore human and murine phagocytes contributed to the uptake of *C. parapsilosis* differently. Intracellular replication has been reported with *C. albicans* and *C. glabrata*, however not when studying *C. parapsilosis*. Live cell imaging videos showed that *C. parapsilosis* has the ability not only to survive during restricted environmental conditions but to replicate and form pseudohyphae after ingestion by macrophages. The videos taken by the spinning disc confocal microscope verify the presence of these events such as intracellular budding, pseudohyphae growth and aborted mitosis of phagocytic cells.

This is the first study demonstrating a thorough examination of *C. parapsilosis* phagocytosis and reporting intracellular survival mechanisms associated with this species.

# DISAPPEARANCE OF PCV13 SEROTYPES (INCLUDING 19A) AMONG CARRIED PNEUMOCOCCI IN CHILDREN AGED 1-3 YEARS, WITHIN THE FIRST FEW YEARS OF VACCINATION

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To prevent invasive pneumococcal infections, conjugated vaccines (PCVs) were developed. PCV7 was inserted in the national immunisation programme in 2009 as a voluntary but free vaccine. PCV13 replaced PCV7 in 2010, and was made a mandatory vaccine in July 2014. After PCV7 vaccination started, a drastic serotype arrangement was observed also in Hungary, with serotype 19A becoming the leading type. In this study, we wanted to evaluate the efficacy of PCV13 on 19A and the additional five PCV13 serotypes. Nasal specimens were collected from 227 children aged 1-3 years, attending 8 different nurseries in Budapest, in March and April 2013. At least 85.0% of the children were vaccinated, already with PCV13. The gender ratio was 121 males (53.3%) and 106 females (46.7%). Species identification was based on routine methods and PCR detection of lytA gene. To determine the serotypes of the strains, a combination of conventional serology (Pneumotest Latex Kit and Quellung test) and PCR was used. MICs were determined with agar dilution or Etest, according to the EUCAST guidelines. Out of the 227 children, 94 were carriers (=41.4%). The prevalence of serotypes in ranking order was the following: 11A/D/F (n=18), 15B/C (n=15), 35F (n=15), 23B (n=14), 23A (n=13), 10A (n=6), 6C (n=5), 19A (n=2), 24 (n=2), 8 (n=1), 19F (n=1), 16F (n=1) and 21 (n=1). Based on these figures, the serotype coverage would be only 1.1% for PCV7 and 3.2% for PCV13. The macrolide resistance of the isolates was 21.7%, 20.7% were intermediately resistant to penicillin and 17.4% resistant to tetracycline. The strains were sensitive to cefotaxime, levofloxacin, moxifloxacin and vancomycin. The carriage rate was higher than observed earlier among children aged 3-6 years (32.9%). The serotype distribution changed further compared to the pre- and post-PCV7 era. Before 2010 the old ‘paediatric’ serotypes (6B, 23F, 19F, 14) represented 40.4% of all strains and serotype 3 had 6.0%. After PCV7 was used, these old types decreased strongly, and previously rare or absent serotypes emerged. Among these, serotype 19A became the leading type, followed by 23A, 15B/C, 11A/D/F 24A/B/F and 35F; serotype 3 had 6.8%. In the current post-PCV13 population, only two 19A isolates (=2.1%) were found and none of serotype 3. The resistance rate of these isolates was stable compared to previous years, but different serotypes were responsible for it: first the ‘paediatric’ serotypes contributed, later serotype 19A dominated alone, meanwhile now serotype 23A was mostly resistant. It seems that PCV13 is very successful indeed in the elimination of the PCV13-PCV7 serotypes.

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## THE USE OF GOLD-STANDARD GENOMIC INFORMATION IN THE DEVELOPMENT OF AN INDUSTRIAL PLATFORM FOR PROTEINS AND METABOLITES PRODUCTION

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The filamentous fungus *Aspergillus niger* is a versatile cell factory that is used widely for the production of starch-degrading enzymes, recombinant proteins and organic acids. The genomes of two strains of *A. niger* used industrially for the production of enzymes (strain CBS 513.88) and citric acid (strain ATCC 1015) have been sequenced. The research community however works primarily with strain NRRL3 and its derivatives. We have sequenced and assembled the NRRL3 genome to a quality that is higher that than the reported CBS 513.88 and ATCC 1015 genomes. The NRRL3 genome has been assembled to eight chromosomes with all 16 telomeres. Missing are parts of the seven centromere sequences. Genome comparison showed that the NRRL3 genome is near identical to the citrate producer strain ATCC 1015, suggesting that they were likely derived from the same isolate. We used a recently developed SnowyOwl gene prediction pipeline to generate a set of accurate gene models. Moreover, we have manually curated, and corrected when needed, all potential gene models as well as manually annotated the function of the gene models.
With the resulting "gold-standard" information, we show here the ease and precision of manipulating the genome to improve protein production and to identify the genes involved in the production of secondary metabolites.

## DISTRIBUTION AND ECOLOGY OF PHOTOHETEROTROPHIC BACTERIA IN HUNGARIAN SHALLOW LAKES

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It has been discovered only during the last decade, that bacteriochlorophyll a-producing bacteria, the so-called aerobic anoxygenic phototrophs (AAP) – which use near-infrared radiation to generate metabolic energy – are quite abundant in the plankton of the upper oceans and thought to be important players in oceanic carbon cycling. These microorganisms are barely studied in freshwaters, notwithstanding their presence in the oxic layer has been justified during the last years in Central European mountain and North European humic lakes. Our aim was, therefore to study the occurrence and ecology of AAP in Hungarian shallow lakes. As a result, AAP was found in high abundance in the studied lakes: their presence was verified in Lake Balaton, in soda lakes of Seewinkel, in fishponds of Somogy County and shallow turbid or humic soda pans of the Danube-Tisza Interfluve. Highest abundances (~ 100 million cells per milliliter) were detected in more productive waters. A positive correlation was found between AAP abundance and phytoplankton biomass (chlorophyll a) as well as between AAP abundance and organic carbon content. The contribution of AAP to total bacterial abundance ranged between 4 and 40% which indicated the important role of these microroganisms in carbon and energy flow. A pronounced seasonal dynamics was observed in Lake Balaton with high AAP abundances in summer and lower values in winter.
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## BACTERIAL COMMUNITY STRUCTURE CHANGES DURING COLONIZATION AND FRUITING BODY PRODUCTION OF OYSTER MUSHROOM USING A COMPOSTED NATURAL SUBSTRATE

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Oyster mushroom (*Pleurotus ostreatus*) is a saprotrophic, edible, white rot fungi. In Europe it is grown on composted and pasteurized wheat straw (substrate) with Actinobacteria, Firmicutes and *Thermus* spp. as the dominant bacteria identified recently using Sequence-aided T-RFLP (Terminal Restriction Fragment Length Polymorphism). Bacteria in the mature substrate hinder the colonization of competing and pathogenic microbes of *Pleurotus* sp. However their interactions with oyster mushroom during its substrate colonization are not yet clarified. As a first step, we wanted to monitor the changes of bacterial community during oyster mushroom production. The oyster mushroom cultivation, carried out at a local mushroom farm consisted of two cycles of substrate colonization and FB production. Bacterial community changes were monitored with 16S rRNA gene based T-RFLP and selected samples were subjected to NGS (Next Generation Sequencing) pyrosequencing analysis. Bacterial community showed a clear parallel succession in the different substrate blocks forming 4 groups according to the four consecutive phases of mushroom production. Bacterial community was predominated from the beginning by the Firmicutes phylum (*Bacillus* spp. and related species) with continuous increase. Also the ratio of the halotolerant *Halomonas* spp. increased. Parallel members of Actinobacteria and *Thermus* spp. disappeared from the community. In the near future the absolute amount and activity of Firmicutes bacteria should be analysed to reveal whether they just survive or they have an active role in *Pleurotus* production.

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## PREVALENCE OF BINARY TOXIN POSITIVE *CLOSTRIDIUM DIFFICILE* STRAINS AT THE CLINICS OF THE UNIVERSITY OF DEBRECEN, HUNGARY

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The number of infections caused by *Clostridium difficile* (CDI) is increasing worldwide and in Hungary as well. The bacterium is an anaerobic, spore- forming, Gram-positive rod, which is able to produce binary toxin besides the A and B Large Clostridial cytotoxins. The binary toxin is an actin-specific ADP-ribosyltransferase. It consists of two parts: an enzymatic (CDTa) and a binding (CDTb) component. The binary toxin positive strains are resistant to ciprofloxacin, therefore the fluoroquinolone treatment may select them. The purpose of our study was to measure the prevalence of binary toxin positive strains at the Clinics of the University of Debrecen, and to analyse the toxin production in the context of age and mortality. Between 08.01.2014. -10.31.2014. 37 samples from 32 patients proved to be positive for GDH antigen and A/B toxins using an immunochromatographic

rapid test. We searched for the presence of binary toxin with polymerase chain reaction (PCR) using primers targeting the binding subunit (*cdt*B). As an internal control we used primers specific for the *cdd*3 gene which is present in all *C. difficile* strains. Finally, we compered the results with clinical data. Based on our studies, out of 32 strains 24 (75%) produced both A/B and binary toxin. 8 strains (25%) produced only A/B toxin. The mortality of binary toxin positive strains was 41.6%, while mortality of the negative strains was 37.5 %. The median age of the patients was 67 years. All patients recieved antibiotics previously. 19 patients (59.3%) were treated with fluoroquinolones. The median age of the fatal cases was 72 years. Two-third of the strains isolated at our Clinics produced both A/B and binary toxins. This result is concordant with the Hungarian data.
In most of the cases patients get colonized with binary toxin positive strains during prior hospitalization. These patients receiving fluoroquinolone therapy have a high risk for CDI. The mortality of binary toxin positive strains is higher, primarily among the older patients. Considering these data, the use of fluoroquinolones should be restricted, these types of antiobiotics should be administered only in critical circumstances.

## DETECTION OF *CHLAMYDIA TRACHOMATIS* ATTACHMENT TO EPITHELIAL CELLS BY THE CHLAMYCOUNT SOFTWARE USING IMAGES PRODUCED BY A DNA-CHIP SCANNER

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Chlamydiae are obligate intracellular bacteria that propagate in the inclusion, a specific niche in the host cell cytoplasm. The infection starts with the attachment of the infectious form of *Chlamydia*, the elementary body (EB) to the plasma membrane of the host cell. Characterizing and quantitation of the attachment process is principally performed by fluorescent microscopy or radioactively labeled elementary bodies. Both methods are labor-intensive and not user friendly. We have already applied a regular DNA-chip scanner to detect and quantitate chlamydial inculsions. We further developed this method to follow and quantitate the attachment process. HeLa and McCoy human and murine epithelial cells cultivated on chamber slides were infected with various MOIs of *Chlamydia trachomatis*. Immediately after infection HeLa cells were washed to remove unbound elementary bodies, fixed and stained for the attached EBs with an Alexa647-labeled anti-chlamydia LPS antibody. The attached EBs were scanned with an Axon GenePix 4100A DNA chip scanner and were counted automatically with the custom ChlamyCount software using the scanned image. The attachment process was also quantitated in the presence of the already described attachment inhibitor heparin, and novel attachment inhibitor compounds. Measurement of the MOI dependent EB attachment revealed that the ChlamyCount software was able to measure the attached EBs on a close to 1-log dynamic range with a high correlation to the theoretical counts. Moreover, our method was able to detect the attachment inhibitory effect of heparin, with a good correlation to the previously described concentration dependence of the inhibition. Our method was also used to identify novel compounds that could inhibit the attachment of C. trachomatis. Besides the measurement of chlamydial growth, the ChlamyCount method was able to measure quantitatively the attachment process and the effect of known and novel inhibitors on *C. trachomatis* attachment.

## ISOLATION AND IDENTIFICATION OF BACTERIA FOUND IN HONEY SAMPLES

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Honey is considered as traditional medicine providing several health benefits. With some extent, its antibacterial effects attributed to metabolic products of different microorganisms living in the gastrointestinal track of the honeybee which can be isolated from honey as well. We have collected 10 different honey samples, some of them were still in honeycomb at that time. Primarily, we were interested in isolating lactic acid bacteria, so our isolation conditions preferably supported their growth. PCR, specific for *Lactobacillus*, provided a DNA fragment and its sequence indicated that Lactobacillus plantarum existed in the samples tested but we were not able to culture them. However, a bacterium was isolated from a honey sample obtained directly from the honey stomach of a honeybee. Sequence of its 16S rRNA gene identified it as *Lactobacillus helsingborgensis*. We have also isolated some culturable bacteria and their identities were defined by determination of the sequences of their 16S rRNA gene. Antibacterial effects of the isolates and their plasmid content are currently under investigation. Some of the isolates deserve to be subjected to genome sequencing.

## ANTIBACTERIAL EFFECT OF ESSENTIAL OIL COMBINATIONS

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Many essential oils (EOs) have antibacterial, antifungal and antiviral effects. Their use as natural antimicrobials gained attention in the recent years. The use of EOs in the food industry is limited due to their strong aroma which can cause off-flavors and off-odors. One solution is the combination of EOs that can lead to reduction of the necessary concentration. Our aim was to determine the antibacterial effect of marjoram, thyme and juniper EOs alone and in combination (marjoram-thyme, marjoram-cinnamon, cinnamon- thyme and cinnamon-juniper EOs) on food spoilage bacteria: *Escherichia coli, Pseudomonas putida* and pathogens: *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus* and *Listeria monocytogenes*. The minimum inhibitory and bactericide concentrations were evaluated by microdilution method and plating. To the assessment of interaction between the EOs checkerboard method was used.

The fractional inhibitory concentration index (FICI) got the type of interaction: synergy, addition, indifference or antagonism. In most cases the MBC was below 6.3 mg/ml and cinnamon EO proved to be the best antimicrobial with the lowest MIC and MBC. Gram-negative bacteria were less sensitive to the EOs than the Gram-positives. The interaction of EOs was additive in most combination with reduced MIC values correlated to the MIC of single EOs. These results could give basic information for the development of natural disinfectants.

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## NOVEL DEVELOPMENTS IN PLANT BIOMASS DEGRADATION BY FUNGI

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With the current push towards a biobased economy the efficient conversion of plant biomass into valuable building blocks or products is high on the research agenda. Fungi play a major role in this as they in nature are the main organisms responsible for decomposition of plant biomass. The use of fungal enzymes for modification of plant biomass has a long history as they are involved in a wide range of biotechnological applications such as the production of paper & pulp, food & feed, beverages, whine and textiles. However, these applications do not require complete conversion of the plant biomass to monomers, while this is desirable for the recent development of plant biomass based fuels and chemicals. Development of more efficient fungal enzyme cocktails is needed for this. In nature fungi do not work as individual organisms in the decomposition process, but as consortia of many species, so understanding the role and abilities of different fungi is crucial to be able to develop more efficient mixtures for a range of applications. In this presentation novel insights in the diversity of plant biomass degradation by fungi will be addressed. Finally, the possible role of extrachromosomal elements in this process will be touched upon. These elements are known in fungi, but have so far not been addressed in the context of plant biomass degradation.

## *DE NOVO* GENOME PROJECT OF THE POLYSACCHARIDE DEGRADER BACTERIUM *XYLANOBACILLUS XYLANOSILYTICUS* SP. NOV, GEN. NOV

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The termotolerant *Xylanobacillus xylanolyticus* sp. nov, gen. nov was originally isolated from overheated compost pile on xylan-containing agar plate. Its taxonomic position is near the Gram-positive *Paenibacillus* group and the closest relative is *Paenibacillus montaniterrae* with 93.3% 16S rDNA sequence homology. *X. xylanolyticus* strain K13 cells cultivated at 50 $^{o}$C, 200rpm for two days in xylan containing liquid medium were used for total DNA isolation. Mate-paired libraries were prepared from total DNA and genome sequencing of the strain K13 was performed using sequencing-by-synthesis on MiSeq system (Illumina) resulting in ~2 million 2x250bp paired-end sequences, with ~200X coverage of the whole genome . According to the whole genome comparisons the most similar genomes are from the representatives of the *Paenibacillus* genus: *Paenibacillus sabinae* T27, *Paenibacillus terrae* HPL-003, *Paenibacillus mucilaginosus* 3016, *Paenibacillus mucilaginosus* KNP414, *Paenibacillus polymyxa* SQR-21. The analysis of the annotated genome of *X. xylanolyticus* revealed the existence of numerous putative glycoside-hydrolases belonging to different GH-families. GH5 is the largest glycosyl hydrolase family in strain K13 (7 title). Interestingly at least one of the different cellulase, xylanase and mannanase enzymes

harbor 3 consecutive S-layer domains at the C- or the N-terminus. Genome data suggest that strain K13 uses a full set of cell surface bonded polysaccharide-degrading enzymes. More interestingly, dozen long proteins decorated with cadhesin, IG, fibronectin and different cellulose-binding domains contain also S-layer triplet domain motive. These structures may act as arms for anchoring the polysaccharide substrates to the cell surface.

## COUPLED HYDROGEN AND BIOGAS PRODUCTION FROM ALGAL-BACTERIAL BIOMASS

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Microalga biomass offers a promising alternative to the commonly used maize silage in biogas technologies. It can be cultivated on areas not suitable for other agricultural activities, algae do not compete with food production and may yield more biomass per unit land than any terrestrial plant. Alga biomass utilization can be part of a complex biorefinery strategy to supply high value chemicals, e.g. carotenes, enzymes, or other energy carriers such as hydrogen before the spent biomass is used for biogas generation. A two-stage energetic utilization, using a natural mixed population of algae (*Chlamydomonas* sp. and *Scenedesmus* sp.) and mutualistic bacteria (primarily *Rhizobium* sp.), was tested for coupled biohydrogen and biogas production. The microalgal-bacterial biomass generated hydrogen without sulfur deprivation. Algal hydrogen production in the mixed population started earlier but lasted for a shorter period relative to the benchmark approach. The residual biomass after hydrogen production was used for biogas generation and was compared with the biogas production from maize silage. The gas evolved from the microbial biomass was enriched in methane, but the specific gas production was lower than that of maize silage. Sustainable biogas production from the microbial biomass proceeded without noticeable difficulties in continuously stirred fed-batch laboratory-size reactors for an extended period of time. Co-fermentation of the microbial biomass and maize silage improved the biogas production: The metagenomic results indicated that pronounced changes took place in the domain Bacteria, primarily due to the introduction of a considerable bacterial biomass into the system with the substrate; this effect was partially compensated in the case of co-fermentation.

The bacteria living in syntrophy with the algae apparently persisted in the anaerobic reactor and predominated in the bacterial population. The Archaea community remained virtually unaffected by the changes in the substrate biomass composition.

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