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INTERGENIC REGIONS AS DETERMINANTS OF GENE ORDER CONSERVATION IN THE FISSION YEASTS

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Genomes are changing continuously both at the nucleotide level (substitutions and indels) and the chromosome level via genomic rearrangements (translocations, inversions, fusions and fissions). Despite these changes, recent results suggest that locations of the genes, their order and even the sites of the chromosomal rearrangements are entirely not random in the genomes. Many genome shaping forces have been described so far, such as DNA double strand break (DSB) hotspots, co-expression- and co-regulation of genes. Besides, the intergenic regions (IGRs) (nucleotide sequences between coding regions of genes) could be major determinants of gene order conservation, since DNA is more prone to breaking in large IGRs. Moreover, sequence motifs that induce DSBs are mostly localized in IGRs. As one of the basal groups of Taphrinomycotina, the fission yeast clade has a distinct life history from other yeasts. More importantly, they share common biological features like chromosome structure and metabolism, spliceosome components, G2/M cell cycle control and cytokinesis with metazoans. The genus comprises four species: *Schizosaccharomyces pombe* which is a well-known model organism; *S. octosporus*, the recently described *S. cryophilus* and the most divergent branch of the clade, the dimorphic *S. japonicus*. It is reported that gene content and order are substantially conserved among them despite their evolutionary breadth. Based on the above features, we suppose that fission yeasts can be good models for genome evolution studies. Thus, in this study comparative genomic analyses of the fission yeasts were carried out and features of the IGRs were investigated, after previous identification of the locally collinear blocks (LCBs), which consisted of at least five orthologous genes in the same order and orientation among the four fission yeast species. The aims of the study were to examine the IGRs' properties and to reveal the IGRs' contribution to gene order conservation in the fission yeast genomes. In order to do this, we compared the intergenic distances (IGDs); the intergenic guanine-cytosine percentages (IGGC%); the strandedness (orientation) of the adjacent genes and the overlaps of untranslated regions (UTRs) between LCBs and their edges. We compared the IGDs within LCBs (n = 1,790) with the IGDs of the LCBs' edges (n = 532) and we found that the values of the latter were significantly higher (Mann-Whitney U test, $P < 0.0001$) in all the different species. We observed the same tendencies in the examination of the IGGC% (Mann-Whitney U test, $P < 0.05$). In general, adjacent genes in convergent orientation (→←) have lower IGDs and motif densities in their IGRs, while divergent pairs (←→) have higher IGDs and motif densities. To test which determinant has a higher impact, we examined the strandedness of the adjacent genes at the edges of the LCBs and the properties of their IGRs. As we had expected, the most frequent orientations were tandem (→→ / ←←). The convergent orientations proved to be the less frequent types and their IGDs were the smallest, too. Thereafter we counted the overlaps of the UTRs in the genomes and we calculated the percentages of the overlaps from an estimation of maximum possible UTR overlaps. We found the smallest number of overlaps at the edges of the LCBs. Taken together, our results suggest that, like other species, small IGD is one of the strongest determinants of gene order conservation in the genomes of the fission yeasts.

MICROBIAL COMMUNITIES IN AQUATIC ENVIRONMENTS

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Algae and cyanobacteria are the primary producers of aquatic environments. They also have an important role in the industry as bio-fuel producers and in the remediation of waste-waters. Nevertheless, bacteria and fungi co-existing with algae and cyanobacteria are often considered as contaminants. However, there is emerging evidence which shows that algal growth could be enhanced by different compounds produced by bacteria. It has been also reported that certain cyanobacteria and molds can be in symbiotic relationships. On the other hand, evolution and interaction (whether symbiotic or parasitic) of these microbial consortia cannot be understood through individual examination. To learn more about aquatic microbial communities, we started to examine different xenic algal and cyanobacterial strains and their concomitant organisms obtained from different culture collections (UTEX and SAG). The algal and cyanobacterial species were the following: *Picochlorum oklahomensis* (UTEX 26); *Dunaliella salina* (UTEX 200 and 1644); *Arthrospira maxima* (SAG 84.79); *Synechococcus elongatus* (SAG 89.79) and *Synechococcus cedrorum* (SAG 88.79). As a first step, we isolated bacteria and fungi from the algal and cyanobacterial culture broths. We managed to separate almost 30 isolates on agar plates. Thereafter, we extracted genomic DNA from them and amplified their certain sequences with PCR (16S rDNA for bacteria and ITS for fungi). The PCR fragments were sequenced and submitted to BLASTn searches.

Our preliminary analyses suggested the following species: *Thalassospira* sp. and *Bacillus* sp. from culture broth of *P. oklahomensis*; *Pseudosphingobacterium* sp., *Olivibacter* sp., *Mesorhizobium* sp., *Limnobacter* sp. and *Orchrobactrum* sp. as concomitants of *D. salina* strains. We identified a *Halomonas* sp. from culture broth of *A. maxima*. Moreover, we isolated two mold-like fungi from cultures of *S. cedrorum* and *S. elongatus*, which turned out to be a *Phialophora* sp. and a *Simplicillium* sp., respectively. Further analyses to identify the exact taxonomic positions of these isolates and reveal the interactions with their "hosts" are underway.

DEVELOPMENT OF A BIOFILM BASED PETROLEUM HYDROCARBON DEGRADING LABORATORY-SCALE MODEL SYSTEM

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Recently, beside traditional Pump & Treat systems Permeable Reactive Barriers (PRBs) are more and more used in groundwater remediation. Already in 2010 the United States Environmental Protection Agency referred to PRBs as "green remediation" technologies. PRBs based on biological processes (mainly on metabolic capabilities of certain bacteria), known as BioBarriers (BBs), are relatively new types of remediation technologies and are considered to be the most cost effective and sustainable groundwater decontamination approaches. For a more efficient and "greener" in situ remediation of polluted groundwater the scientific literature concerning the use of BBs urges laboratory-scale tests

by using model systems. Accordingly, our research aimed at developing a lab-scale biobarrier model system for subsequent use to determine interactions between metabolically active bacteria - carrier material - organic/inorganic pollutants - volumetric flow rates - physico-chemical parameters etc. By using the model system the obtained results can be implemented in situ for BB based groundwater decontamination. After the establishment of the model system a bench scale test has already been conducted by using (i) a prolific biofilm forming and simple aromatic hydrocarbon degrading bacterium, (ii) an easily biodegradable aromatic compound benzoic acid (30 mg/L) used as model contaminant and (iii) shredded waste tyre as filling material and carrier for biofilm. By the end of the test (48 hours) under the applied conditions the concentration of the model contaminant decreased to almost zero. The developed model system is consistent with global trends, environmental and economic aspects of remediation and is suitable for testing BB based elimination of more recalcitrant compounds as well (e.g. BTEX-compounds).

IDENTIFICATION OF A [D5,6] DONOR-DISRUPTED STWINTRON IN THE TRANSCRIPT OF A RETICULON-LIKE PROTEIN CONSERVED ACROSS THE PEZIZOMYCOTINA SUBPHYLUM

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In the primary transcript of nuclear genes, coding “exons” usually alternate with non-coding “introns”. These latter sequences have to be excised with precision by a catalyzed splicing reaction to form the functional mRNA species, in the large majority of cases by the major U2 spliceosome. Spliceosomal twin introns (“stwintrons”) are complex intervening sequences where a standard “internal” U2 intron interrupts one of the canonical splicing motifs of an “external” U2 intron (viz. 5’ donor; 3’ acceptor; motif around lariat branch point), i.e., the external intron can only be spliced out after prior excision of the internal intron. We discovered stwintrons by chance while pursuing other objectives [1, 2]). To study spliceosomal introns, their excision, evolution and intrinsic characteristics, we can use fungal stwintrons as model systems. Thereto, we created a simple bio-informatics tool to aid the search for stwintrons in *Aspergillus nidulans*, an amenable model organism with well-developed molecular and classical genetic toolboxes, including a genome sequence assembled into 8 super scaffolds. The search strategy is based on the short consensus sequences for the three sequence elements necessary for U2 splicing: the donor at 5’ (6 nt), the acceptor at 3’ (3 nt) and the element containing the lariat branchpoint (6 nt). Relaxed variants of their consensus sequences reported by Kupfer et al. [3] were used and generally applicable limits to the length of introns and minimal distance requisites for the donor, lariat branchpoint- and acceptor elements, were taken into account. Here, we illustrate the utility of our stwintron search tool as well as its imperfection with a new type of stwintron we have identified. We found a 151-nt long [D5,6] donor-disrupted stwintron in *A. nidulans* in the gene at locus AN5404, where a 75-nt long internal intron is situated within the donor element of a 76-nt long external intron, between the fifth and the sixth nt [5’-GUAAG|U]. We collected orthologue genes for the reticulon-like protein from the NCBI DNA databases and found that this stwintron is present in all 9 classes of Pezizomycotina in which species have been genome-sequenced, in each case, as the first intervening sequence within the coding region. In certain lineages, the second intervening sequence in the CDS is also a [D5,6] stwintron. Loss of the stwintron was apparent in only a handful of species. We have experimentally confirmed the [D5,6] stwintron(s) in species representing the Eurotiomycetes, Dothideomycetes, Leotiomycetes and Sordariomycetes. Extant EST data provided evidence of its existence in *Tuber melanosporum*

(early divergent class of the Pezizomycetes). However, due to the allowed variation at most positions of the (6-nt) donor- and lariat branchpoint elements, the [D5,6] stwintron would not have been identified with our search criterion in all species where it is present. We continue to improve the performance of our search tool to reduce the importance of extensive manual curation of candidate stwintrons before experimental verification.

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FUNCTIONAL CHARACTERIZATION OF HMBA, A CHROMATIN ASSOCIATED PROTEIN IN THE MODEL ORGANISM *ASPERGILLUS NIDULANS*

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“High Mobility Group-box" (HMGB) proteins are non-histone architectural components of the chromatin of eukaryotic organisms that modulate most of the structural changes in chromatin. This large and varied family plays an important role in tumor genesis, in developmental and differentiation processes, has an effect on transcription, replication and recombination as well as in the repair of damaged DNA. Extremely, they can also escape into the extracellular space and participate in the formation of immune responses as cytokine. Each member of the HMGB family has one or more copy of a specific DNA binding domain of 70-80 amino acids (HMG-box), that interacts with DNA. The HMGB proteins also interact with wide range of chromatin associated proteins, chromatin modulating factors and thereby promote the stable binding of chromatin remodeling complexes, other activators or repressors to chromatin. Earlier, we identified 3 HMGB proteins in the eukaryotic model organism *Aspergillus nidulans*: AN2885 (HmbA), AN1267 (HmbB) and AN10103 (HmbC). The physiological function of HmbB has been investigated so far, while the role of HmbA and HmbC remained undiscovered. In our present project we aimed the detailed functional characterization of the HmbA protein to explore its physiological role. We obtained *hmbA* deletion, reconstituted *hmbA* and *hmbA*-GFP fusion strains and investigated their micromorphology, C- and N-source utilization ability, stress tolerance (osmotic, temperature, oxidative), secondary metabolite production and cell wall composition in comparison to *hmbA+* control. We found that HmbA affects the chitin content of the cell wall, the morphology and growth rate of the vegetative hyphae, oxidative stress tolerance as well as the production of the secondary metabolite sterigmatocystine and penicillin.

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EXPERIMENTAL STUDY OF THE KARST BIOFILM FORMATION ASSOCIATED WITH THE DISCHARGING THERMAL WATER UNDER CONTROLLED CONDITIONS

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The Buda Thermal Karst System (BTKS) is one of the modern analogues of hypogenic karsts where active dissolution and precipitation can be observed. Recently, several microbiological studies have been performed with respect to the BTKS, a part of the Hungary's largest karst reservoir. However, the phases of biofilm formation under controlled conditions (at a stable volume discharge) and the factors influencing have not been clarified so far. Therefore, the aim of this study was to monitor the biofilm formation by establishment an *in-situ* model system in an artificial tunnel inside the Gellért Hill. under. Biofilm formation was studied by scanning electron microscopy and next generation amplicon sequencing to observe the changes of bacterial community structure and composition. During the twelve-week experiment, detailed water chemistry measurements were performed with parallel to the microbiological investigations. Samples were taken at different distances (1, 8, 20, 40, 60, 80, 100 and 120 m) from the spring of continuously flowing thermal water. For the evaluation of biofilm formation and adhesion of bacteria, glass sides were placed at all sampling points in the tunnel. In the case of the physicochemical parameters rapid changes were detected after the outflow (at the first 50 - 100 m), while after the 100 m stabilization were found. The appearance of the precipitates formed between 8 and 120 m showed also significant differences. On the glass slides placed in the tunnel, reddish spherulitic biofilm was detected between 1 and 8 m while crystalline white biogeochemical precipitate was observed between 20-120 m. According to the SEM images, loose attached precipitates formed by filamentous and rod-shaped bacteria and crystal structures of calcium carbonate served as a surface for colonization of bacterial cells.

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MONITORING THE PREVALENCE OF TRANSMITTED DRUG RESISTANCE AGAINST HIV-1 PROTEASE, REVERSE TRANSCRIPTASE AND INTEGRASE INHIBITORS IN HUNGARY

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Widespread accessibility of antiretroviral therapy has caused emergence of transmitted drug resistance (TDR) among HIV-positive, treatment-naive individuals. The aim of this study was to monitor the presence of transmitted drug resistant HIV-1 strains in newly diagnosed, treatment-naive patients in Hungary. 127 HIV-infected individuals diagnosed between 2013 and 2017 were included into the study. Antiretroviral drug resistance was determined by sequencing the HIV protease and partial reverse transcriptase coding regions. Extensive implementation of integrase inhibitors into the

medical care initiated to investigate transmitted drug resistance of this drug class as well. The majority of patients belongs to the MSM (men who have sex with men) group. The sequences were analyzed using the Stanford HIV Drug Resistance Database algorithm to determine surveillance drug resistance mutations and susceptibility to antiretroviral inhibitors. Phylogenetic analysis was used to confirm the detected subtypes of HIV-1 and to identify transmission clusters among patients. Genotyping of pol sequences revealed that the majority of patients carried subtype B of HIV-1 strains (95.28%), while subtype F, A, G and CRF02_AG was also detected. The overall prevalence of transmitted drug resistance was 11.02% (14/127; 95% CI: 6.68 – 17.65). Nucleoside reverse ranscriptase inhibitor (NRTI) associated resistance mutations were the most frequent indicators of TDR (12/127; 9.,45%; 95% CI: 5.49 – 15.79), followed by resistance mutations associated with non-nucleoside reverse transcriptase inhibitors (NNRTIs) (2/127; 1.57%; 95% CI: 0.43 – 5.56) and protease inhibitors (PIs) (1/127, 0.79%; 95% CI: 0.14 – 4.33). Transmitted drug resistance of integrase inhibitors were tested in 70 samples.
Major resistance mutation (T66A) conferring high-level resistance to elvitegravir and low-level resistance to raltegravir was detected in 1 case (1/70; 1.43%; 95% CI: 0.25 – 7.66). The prevalence of TDR in Hungary is comparable to the data reported in other studies on subtype B HIV-1- infected MSM groups in Europe. Forward spread of drug resistant HIV-1 strains may account for the somewhat higher occurrence of NRTI associated resistance observed in our study.

DIFFERENCES IN CARBAPENEM RESISTANCE LEVELS IN *ACINETOBACTER BAUMANNII* ISOLATES MAY HAVE PLAYED ROLE IN RECEDING OF THE FORMERLY DOMINANT STRAIN

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New resistance genotype of carbapenem resistant *Acinetobacter baumannii* (CRAb) carrying *blaOXA*-24/40-like carbapenemase replaced the formerly dominant *blaOXA*-23-like carriers in a tertiary-care center. We hypothesize that alterations in antibiotic consumption may have contributed to this switch. To identify drugs possibly involved, changes in CRAb incidence and in drug use (monthly DDDs/100 bed-days between 2010 and 2016) were examined by changepoint-analysis (pruned exact linear time method). Whole genome of 22 isolates from 2010 (n = 8, each *blaOXA*-23-like carrier) and 2017 (four *blaOXA*-23-like and ten *blaOXA*-24/40-like carriers) was sequenced. Representative sequenced isolates were chosen for time-kill assays with meropenem (16 and 128-1,024 mg/L; 14 isolates) and colistin (2-32 mg/L; 14 isolates), the drug of choice against CRAb. Killing rates (k) were calculated by nonlinear regression. Changepoint-analysis identified December 2014 as the most recent changepoint in CRAb incidence, which corresponded only to changepoints in carbapenem and cephalosporin consumption (October 2014) both as well as the appearance of *blaOXA*-24/40-like. Whole genome sequencing showed that among *blaOXA*-23-like carriers in 2010 ST218 was the most common sequence type, which was also found in 2017. The *blaOXA*-24/40-like carriers became dominant in 2017, ST502, which was absent from the 2010 strains, was the most prevalent. Meropenem was bactericidal at 256-1,024 mg/L (k = 0.346 - 0.859) against *blaOXA*-23-like carriers, while only at 512 – 1,024 mg/L (k = 0.156 - 0.421) against *blaOXA*-24/40-like carriers; *blaOXA*-24/40-like carriers were not killed at 256 mg/L (k = -0.174 - 0.097). Colistin time-kill curves

showed moderate differences, these were evenly distributed among *blaOXA*-23-like and *blaOXA*-24/40-like carrier isolates; k values always indicated bacteriostatic activity.
Meropenem was clinically ineffective against both *blaOXA*-23-like and blaoxa-24/40-like carriers, but the resistance level of *blaOXA*-24/40-like positive isolates were significantly higher, which may explain their emergence and the strain switch.

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EFFECT OF STERIGMATOCYSTIN AND AFLATOXIN CONTAMINATED FEED ON GLUTATHIONE REDOX AND LIPID PEROXIDATION PARAMETERS OF BROILER CHICKEN

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Authors studied the effect of naturally, on ground corn substrate, produced sterigmatocystin (STG), ultraclean (99%) sterigmatocystin (USTG), and aflatoxin (AFB1) contaminated feeds on lipid peroxidation and glutathione redox parameters in some tissues of broiler chickens. A total of 144 Cobb 540 broiler chickens were divided randomly into four experimental groups (control, STG, USTG, and AFB1) consisting of 36 animals, and each of these groups was divided into 2 sub-groups (n = 16). The basal diet was a commercial broiler grower complete feed (Vitafort Ltd., Dabas). For the treated groups the feed was experimentally contaminated with naturally, on ground corn substrate, produced STG by *Aspergillus versicolor*, ultraclean (99%) STG (Romer Labs, Tulln, Austria) in concentration of 2,000 µg/kg, and with 200 µg/kg AFB1, respectively. Mycotoxin exposure begun at 3rd week of age. Six randomly selected birds from each group were sampled at 1st, 2nd, 3rd, 7th, and 14th day of mycotoxin exposure. Blood (plasma and red blood cell haemolysate) and post mortem liver samples were taken, in which the amounts of conjugated diens (CD) and - triens (CT) and MDA concentration was measured as initial and terminal phase markers of lipid peroxidation processes. As parameters of glutathione redox system, concentration of reduced glutathione (GSH) and glutathione peroxidase (GPx) activity were also determined.
Both applied doses of STG/USTG and AFB1 had rapid pro-oxidant effect and caused marked but not significant increase in CT and CD levels in liver. The increase of free radical formation in liver activated the glutathione redox system, as slightly elevated GSH concentrations were found at 1st and 14th day of AFB1 exposure compared to control. At 2nd day AFB1 treatment caused significantly higher GSH concentration in liver than the USTG treatment. GPx activity of liver showed similar changes as its co-substrate (GSH), resulting higher values at the start and at the end of AFB1 treatment compared to control. At 2nd day of mycotoxin exposure, USTG treatment caused significantly lower GPx activity than in control and in AFB1 treated group.

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LACTOBACILLI AS POTENTIAL PSYCHOBIOTICS – TESTS ON MICE

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Gut microbiome has significant impact on bodily functions such as growth, metabolism, immune functions, and on the central nervous system. Probiotics, which act through the gut-brain axis on the brain functions, are recently referred to as psychobiotics. The aim of these studies was to investigate possible psychobiotic function of some recently isolated lactobacilli. *Lactobacillus plantarum* AT51, *Lactobacillus salivarius* SK45, and *Lactobacillus pentosus* TV45 strains were given to C57Bl6 mice at $2x10^8$ CFU/ml in the drinking water for 14 days. Control animals received drinking water only. Food intake and body weight gain were measured and body composition was checked by EchoMRI. The relative weights of the adrenals, thymus, colon, and testis were also calculated. The social behavior of the mice was assessed in the resident-intruder test. Animals that received the TV45 strain gained more weight than the controls and those treated with SK45 or AT51. These mice had the highest lean (muscle) weight and the lowest score for body fat following TV45 treatment. TV45 treated mice displayed higher relative adrenal and testis weights and were significantly more social than controls or those received other lactobacilli. Furthermore, TV45 reduced the aggressive and defensive behavioral markers. These data reveal some beneficial effects of *Lactobacillus pentosus* TV45 strain, which might be tested as a potential psychobiotic in preclinical/clinical trials.

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TARGETED GENOME EDITING IN *COPRINOPSIS CINEREA* USING THE CRISPR/CAS9 SYSTEM

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Basidiomycota fungi comprise important model systems for several biological questions, yet routinely applicable genome editing tools are hardly available. We set out to implement the CRISPR/Cas9 system in *Coprinopsis cinerea*, one of the most widely used Basidiomycota model organism. The CRISPR/Cas9 system is a genome editing tool with versatile applications. It enables the precise editing of the nucleotide sequence in the target DNA sequence by causing double-stranded DNA break(s) with mutation(s) incorporated during the repair process. The repair process can generate indels (insertion/deletion mutations) during the non-homologous end joining (NHEJ) or even precise alteration during the homology directed repair mechanism if a donor DNA is used as a repair template. Our approach includes the transformation of *C. cinerea* protoplasts with Cas9 and gRNA co-expressing vectors, followed by selection marker and DNA sequencing-based screening of potential mutants before phenotyping. For our experiments we use AmutBmut strains: trp1-1,1-6 (tryptophan auxotroph) and pab1-1 (a para-amino-benzoic acid auxotroph). These strains also carry mutations in their mating type loci A and B, which makes them self-compatible and capable of fruiting body formation in the absence of a compatible mating partner. For transformation, we use

protoplasts of the asexual spores (oidia); These uninucleate haploid oidia have a big technical advance since even the recessive mutations can be expressed. We plan to complement the above mentioned strains with a Cas9 and gRNA co-expressing vector containing the appropriate selectable marker gene. We produced a codon optimized *cas9* gene, while for our gRNA constructs we use overlap extension PCR to be able to replace the target specific sequence or any part of the gRNA. We amplified the U6 RNA polymerase III promoter of *C. cinerea* strain #326 to drive the gRNA construct. Since the optimal length of the promoter is not known, we amplified different lengths of the above mentioned promoter and assembled with the gRNA sequence. We are using the target RNA sequence in different variations to account for the nucleotide preference of the RNA polymerase III and to possibly avoid off-target effects. Our aim ultimately is to speed up the generation of gene knockouts in *C. cinerea* by a routinely applicable CRISPR/Cas9 implementation, to serve large-scale knockout experiments of developmentally relevant genes.

CLASSICAL AND 'OMICS' APPROACHES TOWARDS THE BIOLOGICAL CONTROL OF DEVASTATING FOREST PATHOGENS FROM THE GENUS *ARMILLARIA*

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Certain members of the basidiomycete genus *Armillaria* are devastating soil-borne forest pathogens causing root rot in fruit and timber trees as well as nut crops all over the world. Native forest ecosystems are affected by their vegetative diploids as regular decay drivers. Their subterranean colonies can be characterized with long life span, some of them being among the largest known terrestrial organisms of our planet. The abundant spread by soil-borne, root-like structures known as rhizomorphs enable *Armillaria* species to form extensive ecological networks and reach multiple hosts. The genus *Armillaria* entered the genomic era: the number of sequenced and published genomes is rapidly growing. Recent genomic studies revealed that *Armillaria* species possess a unique repertoire of cell-wall-degrading enzymes and numerous lineage-specific genes expressed in developing fruiting bodies and rhizomorphs. Investigation of damaged and healthy forests in both Austria and Hungary resulted in the identification of oak- and conifer-specific species, including A. gallica, a member of the genus showing a remarkable genetic diversity.

Metagenomic studies are in progress to study the *Armillaria*-associated microbiota and search for potential bacterial endosymbionts. *Bacillus* and *Pseudomonas* species producing antifungal metabolites, as well as *Trichoderma* species exerting antagonistic activities against plant pathogenic fungi based on competition, antibiosis and/or mycoparasitism may be promising for the biological control of *Armillaria* root rot. Screening large numbers of *Bacillus*, *Pseudomonas* and *Trichoderma* isolates derived from forest soils infested with Armillaria in antibiosis and *in vitro* antagonism experiments revealed the potential of certain *Pseudomonas* and *Trichoderma* strains to control *Armillaria* species. Certain selected biocontrol candidates were found to be able to produce indole-3-acetic acid and siderophores as well as to efficiently mobilize phosphorous, which are plant growth

promoting traits that may also be exploited in environment-friendly, ecological strategies for the management of *Armillaria* root rot.

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ENDOPHYTIC FUNGI AND SECONDARY METABOLITES OF MUSHROOM COMPOST AND ITS RAW PLANT MATERIALS

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Plants are colonized by - among others - endophytic microbes (such as bacteria and fungi, colonizing plants internally and asymptomatically) and therefore, contain both plant- and endophyte-derived secondary metabolites. These endophytic microbes and secondary metabolites might have influence on the subsequent use of plant materials in different industrial and agricultural activities. Such an example is the compost production for mushroom cultivation, where the compost, which serves as substrate for the cultivated mushroom, is produced mainly from plant materials. Our aims were to isolate and identify endophytic fungi and to determine secondary metabolites of plant materials, used in compost production of white button mushroom cultivation at the Bio-Fungi Ltd. (Áporka, Hungary). Raw plant materials of the compost (wheat-, barley-, rapeseed straw and sunflower seed hulk), as well as compost samples from different stages of the process were screened in both winter and summer. To isolate endophytic fungi, plant materials were surface-sterilized with different methods. The isolated fungi were identified based on the sequence of their nrDNA ITS region, the DNA barcode region of fungi. Different staining techniques were carried out to visualize fungi, colonizing the raw plant materials. To determine secondary metabolites of the samples, methanolic extracts were made, whose components were separated by RP HPLC, and detected by DAD UV/Vis and MS detectors. As a result, we got almost 300 fungal isolates, majority isolated from the raw plant materials, especially from wheat and barley. Both the number and diversity of isolates decreased during composting. The isolates, identified till now, belong to genera that typically occur in the plants used, and during the different stages of composting.

By using different staining techniques on the raw plant materials, we detected extensive fungal colonization. In terms of metabolic composition of the samples, we could reproducibly detect three metabolites in the extracts of wheat and barley. The identification of these metabolites - whose amount decreased notably during the first steps of composting - is in progress; however, based on their UV spectra, they might be flavonoids. We plan further experiments to determine secondary metabolites of the fungal isolates and to test, whether these metabolites or the fungi themselves can influence the efficiency of compost production and mushroom cultivation.

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CHARACTERISTIC STRUCTURAL AND FOLDING PROPERTIES OF ASPERELINE PEPTIDES

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Asperelines are the representative members of the group of short-sequence peptaibols, and these peptides composed of 10 amino acid residues were isolated from the fungus *Trichoderma asperellum*. Like the majority of peptaibol molecules, these peptides are also synthetized as a microheterogeneous mixture. In the course of our theoretical study, for various asperelines, a comprehensive structural investigation was carried out by means of molecular dynamics methods. In the case of aspereline peptides, the backbone conformations were studied, and the appearance of different secondary structural elements (i.e. beta-turns and helical conformations) was examined along the entire sequence of molecules. Nevertheless, the occurrence of intramolecular H-bonds was investigated, which play a role in the structural stabilization of conformational states and secondary structures. Based on the results derived from the simulated annealing calculations, it was concluded that for the aspereline peptides, the types I, III and III' beta-turns, as well as their stabilizing i←i+3 H-bonds appeared. However, beside the various beta-turn structures, shorter and longer helical conformations could be also found regarding the conformational states of asperelines. On the basis of molecular dynamics simulations, it was studied how the typical structural properties of aspereline peptides alter as a function of time. The results led to the observation that the asperelines could be characterized by a sort of helical structure (i.e. 310- and alpha-helix). Moreover, based on the analysis of molecular dynamics trajectories, it was observed that the aspereline peptides could adopt not only the right-handed, but also the left-handed helical conformation. On the basis of our detailed structural investigation, the conformational properties of asperelines were determined, as well as the characteristic folding features of these short-sequence peptaibols were identified.

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EFFECT OF INOCULUM SIZE ON THE EX SITU BIOREMEDIATION OF A USED LUBRICATING OIL-POLLUTED SOIL

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Excessive consumption of petroleum products carries the risk that these chemicals can enter natural environments during transportation, usage or storage. In this regard, soils are extremely compromised because pollution can persist in soils for a long time, thus, several toxic components can accumulate leading to serious environmental or health issues. Used lubricating oils (ULOs) are complex chemicals consisting of a hydrocarbon mixture with varied carbon counts and diverse structures, additives and a considerable level of heavy metals. They can strongly bind to soil particles, so physico-chemical remediation procedures are preferable in many cases but the application of more environmentally friendly and often cost-effective bioremediation techniques are also worth considering. Bioaugmentation involves the inoculation of degrader microorganisms introduced into the polluted site, while biostimulation is performed via nutrient addition exclusevily so native microflora can be stimulated and biodegradation of contaminants can be achieved. Aiming to model the ex situ bioremediation of a ULO-polluted railway station area for 40 days, we found that applying a smaller inoculum size for bioaugmentation, *Rhodococcus* sp. C, *Rhodococcus erythropolis* PR4, *Rhodococcus* sp. PAE1 and *Rhodococcus* sp. PAE8 strains were able to utilize used MK8 engine oil in small scale soil experiments with a bioconversion of 23.58, 29.24, 28.04 and 28.59%, respectively. Biostimulation was responsible for 22.39% of ULO-removal. Increased inoculum size enhanced

bioconversion to 41.39, 31.83, 37.97 and 39.87% in augmented samples, respectively. In all cases, augmented CFUs decreased to the level of stimulated samples in 20 days possibly due to the transition to viable but non-culturable (VBNC) state of newly introduced degrader strains.

APPLICATION OF THE CRISPR/CAS9 HIS-FLP SYSTEM IN *CANDIDA PARAPSILOSIS*

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The prevalence of fungal pathogens is increasing worldwide. Among these *Candida albicans* is still the most commonly isolated from patients, however infections caused by non-albicans species has increased in the past decades. Along with this shift from *C. albicans*, *Candida parapsilosis* become the third or in some cases the second most common cause of invasive candidiasis, infecting mainly low birth weight infants. There are several methods available for the investigation of fungal genes which presumably have an effect on the pathogenicity of fungal pathogens. As *C. parapsilosis* is a diploid organism without a known mating cycle, classical genetical analyses are not applicable in this case. With molecular biological methods null mutant strains can be generated, however these processes have disadvantages, as they rely on the use of pre-established auxotrophic strains and are less efficient regarding the generation of homozygous reintegrated mutants. Because of these disadvantages, the recently described CRISPR/Cas system has a potential to improve the investigation of the fungal genes in *C. parapsilosis*. Currently there are several CRISPR/Cas9 based genome editing systems available in *C. albicans*. In *C. parapsilosis* however, only one such strategy has been developed. This previously applied method depended on transformation with unstable plasmids that are lost after the editing event. In order to test a novel HIS-FLP CRISPR/Cas9 system in *C. parapsilosis*, we targeted a fatty acid desaturase gene named FAD3 to generate knock-out and reintegrated mutants in three different clinical isolates, later used as wild type strains (CLIB214, GA1, CDC317). For mutant generation, the CRIPSR components were amplified via PCR and transformed into the cells in one step along with the proper donor DNA (dDNA). After a homologous recombination event, the Cas9-gRNA cassette integrated into the HIS1 locus. The single guide RNA (sgRNA) encoding oligonucleotide contained a 20 nucleotide sequence specific to the targeted gene. After the activation of the integrated cassette, the Cas9/sgRNA complex initiated a double strand break (DSB) in the FAD3 gene. The formation of the DSB together with the presence of the dDNA induced homology-directed repair (HDR) over non-homologous ends joining (NHEJ) repair. Since, HDR uses dDNA as a template for repair, a specific tag sequence can be introduced, as well as an additional PAM target motif which can be later used for the reintegration process. After the genome editing event, the CRISPR components were eliminated using maltose induced FLP recombinase activity. With the CRISPR/Cas9 HIS-FLP method, we were able to generate null mutant strains in all three backgrounds. Overall, the method is more efficient in the CDC317 background, where reintegrated mutants were also obtained. In the case of the reintegrated strains, they contained the *fad3* gene in a homologous form in the native locus.

Since with the previously used methods the reintegration was only partially achieved, the addback system of the CRISPR/Cas9 is most beneficial for gene characterization in the future.

INVESTIGATION OF THE ROLE OF CHROMATIN-ASSOCIATED HIGH-MOBILITY GROUP-BOX DOMAIN PROTEINS (HMGB) IN THE SEXUAL DIFFERENTIATION IN *ASPERGILLUS NIDULANS*

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Although the Ascomycota haploid filamentous fungus *Aspergillus nidulans* is one of the most studied model organism, we have limited knowledge about the function of the architectural HMGB proteins, which function through interacting with both DNA and protein components of chromatin. Our research group previously identified three HMGB proteins (HmbA, HmbB and HmbC) in *A. nidulans* and studied one of them (HmbB) in details. The function of the other two is completely unknown, yet. Previously we obtained gene deletion mutants for each HMGB gene (*hmbAΔ, hmbBΔ* and *hmbCΔ*) and observed that selfed cleistothecia did not ensure the survival of the *hmbA* deletion mutants and hmbC cleistothecia contained reduced amount of ascospores in comparison to wild type. As we reported earlier, the *hmbBΔ* strain produces less ascospores than *hmb+* with three orders of magnitude and only 0.4% of them are viable. Study of the 12 HMG-box domain proteins of *Podospora anserina* unravelled sexual development governing role of architectural HMGB proteins through the regulation of HMG-box domain containing transcription factors that plays role in the sexual development. Based on these results and our observations we presume that all the three architectural HMGB protein have an important master regulatory role in the sexual development. Beside we conduct a thorough morphological analysis on sexual structures of wild type and *hmbAΔ*-, *hmbBΔ*- and *hmbCΔ* strains of *A. nidulans* in our work, we also monitor the expression of other HMG-box domain protein coding genes in them, and additionally, all the 36 known transcriptional factors that are involved in the sexual development.
We also investigate possible functional interaction between HmbA, HmbB, HmbC and VeA, the master regulator of sexual development through comparative analyses of *hmbAΔ*, *hmbBΔ* and *hmbCΔ* deletions in *veA+* and *veA1* genetic background.

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CHANGES IN ENTEROVIRUS SEROTYPES DETECTED IN HUNGARY 2010 - 2017

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In the 2010 - 2017 period the Hungarian National Enterovirus (EV) Reference Laboratory received 3,424 clinical samples for diagnostic testing of which 331 were found positive for EV nucleic acid using the standard WHO recommended method of 5'UTR amplification. These corresponded to 210 cases of EV infection with a median age of patients of 5.0. A gradual shift was observed among the most frequently identified serotypes/year. In 2010 EV-A71 was found to cause the majority of diagnosed EV infections and 82% of these cases had neurological manifestations. In 2011 and from 2014 - 2017, CV-A6 was genotyped most often. Initially CV-A6 was detected exclusively in patients with skin related symptoms including Hand-foot-and-mouth disease (HFMD), but in 2016 and 2017 solely neurological symptoms were recorded in an increasing ratio of cases with at 11.4% and 19%

respectively. Since 2,014 samples from infections affecting skin on average accounted for 62% of all yearly cases, apart from year 2015, when it was 33.3% and total sample number well below average. Each year either Genogroup A or B viruses dominated the landscape with Enterovirus C and D only sporadically detected. EV-D68 was identified only in 2010 and 2013 years, when samples from patients with neurological symptoms still counted for over two-thirds of total processed samples.

CHLAMYDIA RESEARCH - FROM MOLECULAR MECHANISMS TO CLINICAL CHARACTERISTIC OF INFECTION

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Members of the *Chlamydia* genus are Gram-negative bacteria with special biphasic life cycle. *Chlamydia pneumoniae* is responsible for 10% of the community acquired pneumonia cases and is a causative agent of upper respiratory infections. Further role in the pathomechanism of chronic diseases like atherosclerosis is based on seroepidemiological, *in vitro* and *in vivo* studies. The other important human pathogen in the genus is *Chlamydia trachomatis*, the most common sexually transmitted bacterium. Acute infection with the pathogen can be followed by severe consequences like chronic pelvic inflammatory disease, ectopic pregnancy and infertility. In the last two decades our research group made efforts to investigate the immunological mechanism of chlamydial diseases to identify the biological substances which can influence the replication of the pathogen in the human beings and to find the immunogenic proteins of the pathogens eliciting the effector mechanism of the immune response serving as vaccine subunit candidates. To evaluate the impact of IFN-γ on the transcriptome of murine epithelial cells infected with the human *C. trachomatis* L2 serovar and the murine strain *C. muridarum* DNA microarray was used for infection . We proved that IFN-γ is a key cytokine that primes epithelial cells to activate adaptive and innate immune response and to express anti-chlamydial effector genes acting both intracellularly and extracellularly. Furthermore, the IFN-γ inducible MIG/CXCL-9 was found to have direct anti chlamydial activity in *in vitro* and *in vivo* conditions. The effect of IL-17 on *C. pneumoniae* infection was investigated in mice. The *in vivo* neutralization of IL-17A resulted in a higher *C. pneumoniae* burden in the mouse lungs, a decreased cell influx, and diminished chemokine levels. To find a vaccine subunit candidate, the chlamydial plasmid antigen pGP3 and pGP4 were investigated. Immunization of mice with recombinant pGP3 or pGP4 proteins caused a significantly lower chlamydial burden in the lungs of the infected mice; the lower IFN-γ level indicated a reduced extent of inflammation. We proved that adoptive transfer of the CD4+ spleen cells isolated from the immunized mice resulted in a significantly reduced bacterial burden. Our data indicate that the CD4+ cells that are the cell type responsible for the protective effect of the immune response to plasmid proteins. The immunogenicity and protective effect of the immunization with recombinant LcrE protein of *C. pneumoniae* combined either with Freund's or Alum adjuvant was investigated in mice. The results confirm that LcrE induces protective immunity in mice and show that Alum is able to activate the CD4+ cell-based cellular immunity. Thus, it can be regarded as an alternative adjuvant during vaccine screening and as a useful adjuvant in a potential protein vaccine against *C. pneumoniae* infection. In a recent study we demonstrated that the treatment with the commonly used mucolytic, N-acetyl-cysteine resulted in approximately a six-fold enhanced *C. pneumoniae* growth in tissue culture, and this effect was shown to be based on the increased binding of the bacterium to the host cells. NAC-treated mice infected with *C. pneumoniae* had prolonged and more severe infections than the control mice. Hydroxyethyl cellulose (HEC) is a

commonly used gelling agent which can be found in vaginal gels. We showed the concentration-dependent growth-enhancing effect of HEC on *C. trachomatis* serovar D and E *in vitro* and *in vivo*.

EFFECTS OF KYNURENIC ACID AND KYNURENIC ACID ANALOGUES ON TUMOUR NECROSIS FACTOR-α AND TUMOUR NECROSIS FACTOR STIMULATED GENE-6 PRODUCTION INDUCED BY *CHLAMYDIA PNEUMONIAE* AND *STAPHYLOCOCCUS AUREUS*

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Kynurenic acid (KYNA) is a product of one branch of the kynurenine pathway of tryptophan metabolism following indoleamine 2,3-dioxygenase (IDO) activation. In recent years, the anti-inflammatory and immunosuppressive functions of KYNA have been of particular interest. Previously we observed that KYNA attenuated the TNF-α production of human mononuclear cells activated by heat-inactivated *Staphylococcus aureus* (SA1). The aim of the present study was to investigate the effects of KYNA and novel synthetic KYNA analogs on the production of human TNF-α by U-937 monocytic cells infected with *Chlamydia pneumoniae* or treated with SA1. Additionally, the potential role of TSG-6 (TNF-α-stimulated gene-6), a considerable anti-inflammatory mediator, in this process was investigated. U-937 monocytic cells were stimulated for 24 h with heat-killed SA1 or infected with *C. pneumoniae*. Direct quantitative PCR was used for the detection of *C. pneumoniae* DNA in the infected cells. TNF-α concentrations were determined in the supernatants of the cells by ELISA, TSG-6 mRNA expression was quantified by reverse transcription PCR. KYNA attenuated the TNF-α production of U-937 cells stimulated by heat-inactivated SA1. The suppressive effect of some KYNA analogs was more potent than that of an equimolar concentration (250 - 500 μM) of KYNA. *C. pneumoniae* induced also a considerable level of TNF-α (125 ± 25 pg/ml) in U-937 cells, but in contrast, it was not suppressed by KYNA, moreover, KYNA and one of the KYNA analogues increased the TNF-α level (350 ± 120 pg/ml and 425 ± 50 pg/ml, respectively). These substances, however, inhibited the *C. pneumoniae* mediated TSG-6 transcription (which in turn inhibits TNF-α production). Further KYNA analogs with different molecular structure inhibited *C. pneumoniae*-induced TNF-α synthesis, and at the same time augmented TSG-6 mRNA expression. There was a negative correlation between TNF-α production and TSG-6 expression. Both *C. pneumoniae* and *S. aureus* are able to induce TNF-α and TSG-6 in monocytic cells. Further studies are necessary to elucidate the mechanism of the different action of KYNA on TNF-α production induced by intracellular and extracellular bacteria and its role in antimicrobial host defense. The induction of TSG-6 following bacterial infections is an essential feedback loop, and its modulation by KYNA derivates indicates a novel approach toward therapy of inflammatory diseases.

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DEVELOPING A SOIL INOCULANT VALIDATION METHOD

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The Hungarian legislation requires the documentation of soil inoculants on which utilized microbes must be indicated. This allows making the TRFLP analysis of the product, according to the data of the type strains. This research aims the development of a method to analyze the effects of soil inoculants on different soil types in laboratory circumstances (using conventional and molecular microbiology methods). Moreover, to map the TRFLP fingerprints of the 2 most common soil types of Hungary (brown earth, chernozem). Before validation of a soil inoculant product, the ingredients strains restriction places has to be identified properly, after that the DNA content of the soil itself has to be investigated by specific restriction enzymes, thirdly soil samples were treated by soil inoculants and the two TRFLP data has to be compared. During the conventional analysis of the chernozem and the brown earth soil four medium were used (TGE, CMC, Cetrimid, Mannitol) for growing microbes, the 16S rDNA sequencing is continued. 35 strain was isolated from the chernozem soil (6 strain is nitrogen fixing), 32 strain was isolated from the brown earth (5 strain is nitrogen fixing). Two differently utilized fields (abandon and intensive) were sampled and treated by the 2 most common soil inoculant products on the Hungarian market.
Because the bacteria strains used for the products are common soil bacteria, the presents of them was expected, the ratio was question after the treatment. A 25 day long pot experiment was carried out, the treatment was 10 kg/ha dose for each product, pots were sampled at the end, 4-4 pots for each treating, and 2-2 for each control, with altogether 40 samples total DNA were extracted and treated by restriction enzymes (RsaI, TasI, Hin6I) specific to the 2 products for interpreting TRFLP data. The bacteria's of the two products where analyzed in silica, and also were compared by the type strains restriction results, the restriction places were identified, on the TRFLPs of the soils. The expected common occurrence of the bacteria was not proved by the data's of the control soils in every case. Both products ingredients strains occur in the treated soil samples, compared to the control soils after 25 days at least in the case of one restriction enzyme.

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POLYMORPHISMS IDENTIFICATION OF *SLC30A* (ZNTS) AND *SLC39A* (ZIPS) FAMILY GENES IN HUNGARY

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Zinc is the second most common trace element and catalytic cofactor in the human body. Zinc plays an important role in wound healing, biosynthesis and homeostasis of various connective tissues. A large number of proteins are known to regulate cellular Zn homeostasis and the most significant proteins belong to two protein families of Zn transporters. The solute carrier family 30 (SLC30A, also called ZnTs) and solute carrier family 39 (SLC39A, also called ZIPs) regulate Zn influx and efflux in the cells as well as intracellular compartments. Over the past few years, new knowledge accumulated in the international literature concerning Zn transporters. Fourteen ZIP and 10 ZnT proteins have been identified. The Hungarian population has not been investigated so far. Our research aims to eliminate this shortage. The present study was designed to test single nucleotide polymorphisms (SNPs) of the *slc30A* and *slc39A* genes. Samples of human blood were collected from 32 Hungarian women before their surgery for benign gynecological reasons. 24 markers were designed for the genetic variability of Hungarian isolates. Zinc transport loci could be amplified successfully in all patient samples included in the analysis. Thus more, than seven hundred samples will be applied in this work. Our results suggest that the 24 primers tested were found to be

appropriate tools to detect the genetic variability of zinc transporters in the human blood samples. These primers will be applied in further studies to reveal the structure of Zinc transporters of the Hungarian populations.

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HUMAN CYTOMEGALOVIRUS INFECTION – EXPERIENCE OF LABORATORY DIAGNOSTICS

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Human cytomegalovirus (HCMV or Human herpesvirus 5) was classified as belonging to the *Cytomegalovirus* genus within the subfamily Betaherpesvirinae of the Herpesviridae family. HCMV is composed of a large double-stranded DNS genome, its linear genome is the largest among human herpesviruses. HCMV can infect various cell types, including epithelial cells, fibroblasts, monocytes, macrophages, and lymphocytes. The virus is transmitted between individuals via all body fluids (e.g. saliva, blood, breast milk, semen, vaginal secretions, urine) as well as through bone marrow and solid organ transplants and vertically from the mother to the fetus. After the primary infection, HCMV is capable of latency and reactivation. The propensity of the virus for reactivation following immunosuppression or immunodeficiency is an important factor leading to HCMV-associated diseases. The virus usually infects the population in early childhood and later at the time of sexual activity. The primary infection is often asymptomatic in the healthy host, or it can cause self-limiting diseases, such as certain cases of infectious mononucleosis. In contrast, serious disease frequently occurs in the immune suppressed individuals. Diagnoses of life-threatening HCMV pneumonitis and retinitis are increasing among transplant recipients and people living with AIDS. HCMV infection is a major infectious complication of transplant recipients, causing significant morbidity and mortality. The primary HCMV infections of seronegative pregnant women's transplacental transmission during pregnancy leading to fetal damage may cause severe congenital damage in the neonates. The most common worldwide congenital viral infection is the HCMV infection. Congenital HCMV infection is the leading viral cause of hearing impairment, sight impairment and mental retardation in newborn infants. Congenitally infected newborns are symptomatic at birth (they often display hepatosplenomegaly, jaundice, microcephaly, intracranial calcification, chorioretinitis, skin manifestations, including petechiae and purpura), but infected infants appear normal at birth, clinically silent congenital infection leads to neurological sequelae which may progress throughout early childhood. Several techniques exist allowing rapid diagnosis of HCMV with high sensitivity. Serologic tests for antibody to HCMV are useful for determining whether a patient had HCMV infection in the past, a determination of great clinical importance for organ and blood donors, and in the pretransplant evaluation of prospective transplant recipients. Maternal HCMV infection is typically diagnosed by serology. The avidity of IgG antibody increases with time after initial infection and demonstration of low HCMV-IgG avidity can improve the accuracy of identification of recent primary infection. Antibody tests are not useful in the diagnosis of HCMV disease in the immunocompromised host. Serology determination of either IgG or IgM has no place in the diagnosis of HCMV infection or disease but is useful to determinate risk of subsequent HCMV infection. In the immunocompromised host, newer rapid diagnosis assays such as antigenaemia and real-time HCMV PCR of blood or plasma have made preemptive treatment feasible to reduce morbidity and mortality.

The most commonly used tests for diagnosis of HCMV infection are the detection of DNA or mRNA. Use of a quantitative assay (Q-PCR) gives additional information valuable for patient management.

ARE TONSILS AND/OR ADENOIDS SITES FOR HUMAN POLYOMAVIRUS INFECTIONS?

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Based on DNA prevalence data and the high rate of the seropositivities in human population, respiratory transmission of human polyomavirus 3, 4 and 9 is suggested. Nevertheless, the sites of the virus replications have not been clarified. These viruses might replicate in the respiratory tract, and secondary lymphoid tissues might have rule in human polyomavirus infections. In our study tonsil and adenoid tissue samples were collected from altogether 146 patients. Upper respiratory sample was also collected using throat swab from each patient just before the operation, and middle ear fluid sample was obtained if serous otitis media was suspected. The aim of our study was to collected DNA prevalence data about human polyomavirus 3, 4 and 9 data in respiratory and tissue samples, to analyze the sequence variability of the non-coding control region (NCCR) and complete genomes. Viral DNA was detected by quantitative, real-time PCR. NCCRs were amplified, sequenced and analyzed. Complete genomes of HPyV4 were amplified by inverse PCRs, sequenced, and then phylogenetic analysis was performed. Complete HPyV9 genome was obtained by primer walking and sequencing. HPyV9 was detected in one tonsil tissue sample. Comparing the genome to the reference sequence, point mutations were detected. HPyV3 was detected in adenoids (the first report of viral DNA positivity), tonsils and throat swab samples.
The non-coding control regions of the viral DNA did not show variability. HPyV4 was detected in all sample types collected, even in middle ear fluid specimen. Each of the NCCR showed mutations compared to the reference sequence. Analysis of the putative transcription factor binding sites revealed that these mutations may alter the binding of these factors. Separated clades of HPyV4 were determined by phylogenetic analysis of the complete genomes.

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CYCLOMALTODEXTRIN GLUCANOTRANSFERASE PRODUCTION BY DIFFERENT *BACILLUS* FERMENTATION

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Cyclomaltodextrin glucanotransferase (CGTase, EC 2.4.1.19) is a multifunctional enzyme, which catalyzes reversible intermolecular and intramolecular α-l,4-transglycosylation and hydrolytic

reaction for starch and related compounds. Production of cyclodextrins is a great alternative for starch utilization. CGTase creates from starch cyclodextrins, which are circular oligosaccharides of 6, 7 or 8 (α-, β- and γ-cyclodextrin) glucose monomers. They have special characteristics, because the outer surface of the cyclic oligosaccharide is hydrophilic while the ring inside has rather hydrophobic character. They can be used for molecular wrapping, which may improve for example the water solubility of steroids. Packing into cyclodextrins gives a protection against microbial degradation of substances. In our experiments we investigated three *Bacillus* species, which may produces the CGTase enzyme. These three bacilli were *Bacillus licheniformis* DSM-13, *Bacillus coagulans* DSM-1 and *Geobacillus stearothermophilus* DSM-2313. For the first screening we used impedimetric BacTrac system. This is a high-throughput, scaled down and online measuring flexible system, providing the opportunity of measurements via direct/indirect, aerobe/anaerobe, media/electrode impedance measuring technics. In order to compare the above bacilli we selected the proper method in BacTrac. Further investigations were done to compare an inorganic media for Bacilli with the generally used CGTase production media (Horikoshi). Since the enzyme is extracellular, cell free supernatants are used for activity determinations. The better media and the best enzyme producer strain is then scaled up 10-fold to shaking flask, in which two different temperatures are also compared. Finally, all 3 bacilli were sued for enzyme fermentation in 1L scale, and crude enzyme solutions were examined in term of temperature optimum and pH, and stability. The enzyme preparation are compared end statistically evaluated with two samples t-tests.

GRAPE BERRY AND *BOTRYTIS CINEREA* PARAMETERS DURING NOBLE ROT OF TOKAJ'S GRAPE SAMPLES

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Botrytized („aszú") wines are produced from ripe grape berries subjected to a unique interaction with the filamentous fungus *Botrytis cinerea*. This special interaction between the berries and *Botrytis* is called noble rot. During noble rot the berries gradually dehydrate and shrivel and the increasing concentration of cellular constituents results in an inhibition of fungal growth. The process of noble rot takes several days and the berries go through four distinct phenotypic stages. We have developed a high-throughput semi-automatic technique to assess the ratio of living and dead plant cells in the berries and some crucial physical parameters for each phenotypic stage. The level of *Botrytis cinerea* biomass was simultaneously evaluated by ELISA and the results were also validated by qPCR. Furthermore, the biomass of *B. cinerea* was quantified in the peel, pulp and stem separately in the four stages of noble rot, thus the pattern of the fungal colonization was estimated. Thirty-nine botrytized grape berry samples were sequenced by RNA-seq enabling us to gain insight into the complex relationship between the berries and the fungus during noble rot.

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DNA BARCODING DATABASE OF MACROFUNGI IN HUNGARY

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Species identification using DNA sequences (barcoding) is essential in the taxonomic and diversity studies of fungi, particularly in genera where distinguishing morphological characteristics are cryptic and/or the number of species is high. True evolutionary relationships of species and other lineages can also be detected with DNA barcoding. The recently proposed nuclear ribosomal DNA ITS region as the official DNA barcode of fungi was successfully applied in numerous studies and national projects (e.g. ABOL, FinBOL, NorBOL). Previously in Hungary, occurrence of ca. 3,000 macro fungi species was estimated solely based on morphological species concept. This number is hardly reliable, because verification or revision of this data using modern methods were generally lacking. No systematic study of macro fungi in Hungary using DNA barcoding techniques has been done to date. Our aim is to create a DNA barcode database of the Hungarian macro fungi in order to significantly improve our knowledge on their taxonomy, diversity and distribution. We performed a deep taxon sampling among the morphologically well-documented fungal specimens from various personal herbaria. We focused on species rich and taxonomically challenging ascomycetous and basidiomycetous genera such as *Agaricus*, *Amanita*, *Clitocybe* s.l., *Cortinarius*, *Entoloma*, *Geastrum*, *Gyromitra* s.l., *Hebeloma*, *Helvella* s.l., *Hygrophorus*, *Inocybe*, *Lactarius*, *Lepiota*, *Mycena*, *Peziza* s.l., *Polyporus* s.l., *Russula*, and *Tricholoma* s.l., as well as species of other important higher taxa like Bankeraceae, Boletales, Cantharellales, and Morchellaceae. For DNA extraction and PCR procedures we applied standard methods. Based on our preliminary results, beside the increasing dataset, taxonomic revision of several genera (e.g. *Cortinarius*, *Entoloma*, *Geastrum*, *Gyroporus*, *Hortiboletus*, *Morchella*) has also been started.

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THE GLUTATHIONE DEGRADING PATHWAY OF *ASPERGILLUS NIDULANS* AND ITS SIGNIFICANCE UNDER CARBON STRESS

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Glutathione (GSH; gamma-L-glutamyl-L-cysteinyl-glycine) is an abundant and widespread multifunctional stress metabolite in fungi. It functions not only as antioxidant, but it contributes in several detoxification processes and also serves as N, S, or C/energy store. Previously we found that GSH content of *Aspergillus nidulans* decreased to the 20 - 25% of the original values in carbon stressed (carbon starved or carbon limited) cultures. This process was not followed by the accumulation of oxidized glutathione (GSSG) but was accompanied by elevated gamma-glutamyl transpeptidase (gammaGT) activities. Deletion of the *ggtA* gene eliminated all the gammaGT activities but interestingly did not influence the GSH content of the cultures. Here we demonstrate that instead of GgtA the putative glutamine amidotransferases DugB and DugC were responsible for GSH degradation in *A. nidulans*. These proteins are orthologues of *Saccharomyces cerevisiae* Dug2p and Dug3p, respectively, which are the key enzymes of the DUG (cytosolic GSH degradation) pathway. Using a Δ*dugB* x Δ*dugC* double mutant we show that the intensive GSH degradation was responsible for the elevated ROS (reactive oxygen species) content of starving cultures and enhanced autolytic cell wall degradation, melanization and extracellular proteinase secretion.
The evaluation of transcriptome data of carbon stressed and growing Δ*dugB* x Δ*dugC* and control cultures will also be summarized in this presentation.

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CALCIUM-CARBONATE PRECIPITATING BACTERIA FROM BARADLA CAVE

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The Baradla Cave as part of Aggtelek Karst (NE Hungary) has a length of 20.5 km and constitutes of diverse carbonate speleothem formations in its various connecting side branches developing by epigenic speleogenesis. Bacteria are presumed to contribute to speleothem formation (calcium-carbonate precipitation) through the extracellular polymeric substances in biofilms and bacterial metabolism. The aim of this study was to reveal the cultivable bacterial community in Baradla Cave and observe the bacterial involvement in $CaCO_3$ precipitation based on *in vitro* experiment. The surface of speleothems is covered with biofilms containing bacterial cells with different morphology examined by electron microscopy. The surface of stalagmites, stalactites, soda straws and the dripping water were sampled and serially diluted to different subsurface-environment specialized culture media containing low amount of nutrients, minerals and extract of soda straws. Following 4 weeks of incubation at 21°C, altogether 361 bacterial strains were isolated. The 16S rRNA genes of the strains were amplified and compared by ARDRA method. Most of the 117 ARDRA representatives were identified as members of phyla Actinobacteria and Proteobacteria (classes Alpha-, Beta- and Gammaproteobacteria), but representatives of Firmicutes and Bacteriodetes were also detected. Due to the specific cultivation, strains belonging to species *Bacillus*, *Streptomyces*, *Pseudomonas*, *Micrococcus*, *Rhodococcus* and *Brevundimonas* spp. were found in the highest number among the 50 genera detected. To test the calcium-carbonate precipitating capacity, the selected strains were incubated in parallels on calcium-acetate, glucose and yeast extract containing agar medium (B4) for weeks at 21°C and observed by light and electron microscopes.
Majority of strains identified as *Arthrobacter*, *Bacillus*, *Micrococcus*, *Rhodococcus*, *Brevibacterium*, *Microbacterium*, *Pseudarthrobacter*, *Pseudomonas*, *Acinetobacter*, *Aminobacter*, *Herbiconiux*, *Kitasatospora*, *Paeniglutmicibacter*, *Stenotrophomonas* and *Streptomyces* induced calcium-carbonate precipitation on media from the second weeks. These bacteria had various cell wall components (lipopolysaccharides, teichoic acids, mycolic acids) and produced extracellular polymeric substances, suggesting that the precipitation mechanism generally based on the bacterial metabolism (e.g. protein and amino acid degrading activities).

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ANTIMICROBIAL ACTIVITY OF NCR PLANT PEPTIDES STRONGLY DEPENDS ON THE TEST ASSAYS

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The symbiosis specific NCR247 and NCR335 cationic plant peptides of *Medicago truncatula* have been shown to exert antimicrobial activity against a wide range of microbes. However, their antimicrobial efficiency is clearly limited by divalent cations. Here, the antibacterial and antifungal activities of NCR247 and NCR335 peptides were compared to those of the well-characterized peptide antibiotics polymyxin B and the aminoglycoside streptomycin on three model microbes, *Escherichia coli*, *Bacillus subtilis* and *Saccharomyces cerevisiae* as representatives of Gram-negative and Gram-positive bacteria as well as eukaryotic fungi. The aim of the study was to assess how the killing efficiency of these peptides depends on various, widely used antimicrobial susceptibility assays. Validated resazurin microdilution assay was used to determine minimal growth inhibitory concentrations in three general test media (MHB, MHBII and low-salt medium LSM). Bactericidal/fungicidal activities were determined by the commonly used drop plate assay. The natural plant peptides showed distinct characteristics, NCR247 had a generally high sensitivity for Ca^{2+} and Mg^{2+} in the medium, while NCR335 proved to be a robust and strong antimicrobial agent with comparable efficiency values to polymyxin B.
Activity data were confirmed visually, both NCR247 and NCR335 treatments at minimal bactericidal concentrations induced complete disruption of the membranes and provoked cell lysis on all tested microorganisms as observed by scanning electron microscopy.

MICROBIAL AND ECOTOXICOLOGICAL MONITORING OF LAKE BALATON AND ITS WATERSHED

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Within the framework of GINOP-2.3.2-15-2016-00004 project our department has an opportunity for the comprehensive ecotoxicological and microbial monitoring of Lake Balaton. In this study we would like to shortly delineate the results of last summer. To unfold the impact of main streams flow into the lake 37 sampling points were selected on the lake and its catchment area. Both water and sediment samples were analyzed by molecular (T-RFLP) and ecotoxicological (*Aliivibrio fischeri*, BLYES, BLYAS) methods. Isolation of aerobically cultivable bacteria from water samples were carried out on 4 different media (TGY-1, Endo, Leeds *Acinetobacter*, *Aeromonas*). Multi-pesticide residues (451 chemical agents) were also screened from the water samples of Balaton. The results of ecotoxicological assays demonstrated that neither water nor sediment samples had observable estrogenic, androgenic or cytotoxic effect. It was also positive that only one pesticide from the hundreds of chemical agents reached the detection limit of the analytical methods. The colony forming unit values decreased with the distance from the Zala river since this is the main inflow stream which brings organic matter to the lake. By this time more than 200 bacterial strains were isolated and identified. According to the results it seems to be obvious that the diversity of cultivable heterotrophic bacteria of samples taken from the lake and the water flows are markedly different. T-

RFLP fingerprinting results also revealed notable differences between the microbial communities of the lake and inflow streams, both of sediment and water samples. The 16S rDNA amplicon sequencing of the selected samples is in progress by the time of writing.

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ANALYSIS OF A MANGANESE TRANSPORTER IN *ASPERGILLUS NIGER*

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Citric acid (2-hydroxi-propane-1,2,3-tricarboxilyc-acid) is an industrially important organic acid with an annual global production of over 2 million tons. Citric acid can form chelates with several metal ions, thus is used as a metal cleaning agent. Furthermore, it is widely applied in food industry as additive. Production of citric acid is based on submerged fermentation by the filamentous fungus *Aspergillus niger*. To achieve high yields, it is critical to adjust several parameters such as dissolved oxygen levels and the pH of the medium. Adjusting correct levels of the trace elements iron, zinc, copper, and manganese in the medium is a further crucial precondition for high yield citrate production, and citric acid production will only occur when these ions are present in very low concentrations which are suboptimal for growth. The effect of manganese (II) ions is particularly striking, as concentrations >2 ppb already reduce acid accumulation by about 20%. Such concentrations are easily introduced into the medium by other nutrients, such as the carbon source, and therefore manganese must be removed from the feedstock. We identified a putative manganese transporter (locus NRRL3_07789) in *A. niger*. To investigate the effect of this carrier on the citric acid production, the gene was both deleted and overexpressed (OE). NRRL 2270 was used as reference strain. Under producing conditions both the reference and the deletant strains displayed molar yields over 80%, but the growth rate of the mutant significantly decreased. In contrast, in OE cultures, molar yield dropped to 17% of that of the reference strain, and biomass formation doubled. Importantly, OE cultures shifted from the tight pellet morphology to branched mycelial clumps, while the reference and deletant strains retained their pellet-like morphology. Our results imply that the studied gene is probably involved in Mn^{2+} transport in *A. niger*.

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CO-CULTIVATION OF HYDROCARBONOCLASTIC AND BIOFILM FORMING BACTERIA

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Our study aimed at developing a bacterial consortium, made of prolific biofilm forming and hydrocarbon degrading bacteria, which can be used for subsequent development of innovative,

biofilm based in situ bioremediation systems (biobarriers). Biobarriers thus obtained can be used in the future for containment and decontamination of simple aromatic hydrocarbon polluted groundwaters. During our analyses previously characterized prolific biofilm forming (isolate BFHA4_7) and simple aromatic hydrocarbon (BTEX-benzene, toluene, ethyl-benzene, o-, m-, p-xylene) degrading (isolates BUG14 and BFB1_13) bacteria were investigated. First and foremost, the optimal cultivation conditions of the selected bacteria were determined (optimal culture medium, pH-, temperature, NaCl concentrations). In the second phase of the study, under optimal conditions the co-cultivation of the isolates was assessed. By using a cultivation independent approach, the terminal restriction fragment length polymorphism (T-RFLP), in function of the incubation time the relative abundance of the three bacteria was determined within the same culture medium. For developing an effective bacterial consortium bacteria with outstanding and complementary metabolic properties should be able to proliferate in high number in the presence of other members of the consortium. Results indicated that for cultivation of isolates Nutrient medium, pH 7, 30ºC and 1% NaCl concentration is the most suitable. T-RFLP analyses indicated that isolate BFHA4_7, irrespective of the co-cultivation experiment, within one week of incubation over proliferated the other two members of the consortium. Further optimizations are needed.

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GENOME-BASED TAXONOMIC COMPARISON OF TYPE STRAINS OF *PUSILLIMONAS*-RELATED SPECIES AND STRAINS REPRESENTING POTENTIALLY NEW BACTERIAL SPECIES (FAMILY ALCALIGENACEAE)

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Some members of the family Alcaligenaceae were isolated from various contaminated environments. In our previous study, where unconventional methods were used to cultivate the widest possible range of bacteria from a landfill leachate-treating bioreactor, a surprisingly high diversity of Pusillimonas-related strains were isolated from a single sample. Two novel genera within the same phylogenetic cluster have been already described by our research group from the same site, *Caenimicrobium hargitense* gen. nov., sp. nov. and *Quisquiliibacterium transsilvanicum* gen. nov., sp. nov. This study aimed the detailed taxonomic characterization of the other strains which represent eight potentially novel species. New species candidate strains were subjected to comparative polyphasic taxonomic analyses and in addition to these, the genome-based comparison with closely related type strains was also performed. In this study, the de novo genome sequence of 12 type or newly isolated strains was determined. The genome size of the studied *Pusillimonas*-related strains ranged from 2.8 to 6.5 Mb (average 4.3 Mb), while the G+C content of the genomic DNA was between 50.5% and 64.6% (average 58.5%). Genome analyses and classical polyphasic studies both supported that new isolates represent novel species and that taxonomic revisions are needed (reclassification of some *Pusillimonas* and *Candidimonas* species).
The significance of individual taxa within the studied group in bioreactors treating different wastes was revealed by a meta-analysis based on 16S rRNA gene sequences.

ACTINOBACILLUS PLEUROPNEUMONIAE – AN AGENT OF THE PORCINE RESPIRATORY DISEASE COMPLEX

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Actinobacillus pleuropneumoniae can cause hemorrhagic, necrotic pneumonia and fibrinous pleuritis in grower and finishing pigs alone or together with viruses and other bacteria. The bacterium has two biotypes; biotype 1 strains need nicotinamide adenine dinucleotide to their growth, while biotype 2 strains can be cultured without it. There are virulence variants influenced by the presence or the absence of virulence factors (toxins, fimbriae, outer membrane proteins, enzymes, biofilm production etc.). No major difference could be detected in the carbon source utilization of the two biotypes when it was examined using the Biolog system. All strains could utilize 20 carbon sources and a part of them could use further 27 ones. There was no correlation between the biotype, serotype and carbon source utilization of the strains. On the basis of our results extension of the data base of the Biolog system would be necessary to apply it to the identification of *A. pleuropneumoniae* strains. On the basis of surface polysaccharide antigens *A. pleuropneumoniae* strains can be assigned into 18 serotypes, their frequency shows geographical differences. Serotypes 2 (39.5%) and 13 (15.4%) were dominant in Hungary when 255 field isolates were typed. In addition to serotype 13 we described three new serotypes, serotype 16, 17 and 18. Serotype 16 (8.8%) was detected only in Hungary till now, and its pathogenicity was confirmed in a challenge experiment. Serotype 2 strains proved to be diverse when they were examined with pulse field electrophoresis, they could be assigned into 9 clusters, while only each two ones could de differentiated among strains of serotype 13 and 16 isolated in several herds without epidemiological connection. Presence of toxin gene *apxIV* that is typical for *A. pleuropneumoniae* was confirmed in all strains, while serotype 2 strains harboured *apxII* and *apxIII* toxin genes. Serotype 9 and 16 strains could produce ApxI and ApxII toxins, this pattern is connected with increased virulence. The frequency of serotype 16 strains can be explained with their higher clonality and virulence. Serotype 13 strains carried only *apxII* and *apxIB* genes so they could produce only ApxII toxin but not ApxI and ApxIII that is why they have lower virulence. The epidemiology of the *A. pleuropneumoniae* strains can only be partially explained by the differences in the virulence so further characterization of the strains is necessary.

SEROLOGIC SURVEY OF CRIMEAN-CONGO HAEMORRHAGIC FEVER VIRUS INFECTION AMONG RODENTS IN HUNGARY

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Crimean-Congo Hemorrhagic Fever Virus (CCHFV) is one of those viruses that is considered "high-risk pathogen" by the WHO, as it can potentially cause epidemic in the future. CCHFV is one of the most widely spread virus, as it has been detected from China to Europe and in African countries. CCHFV is a tick-borne virus, most often spread by species from the Hyalomma genus. Due to climate change, the geographical range of the species is expanding continuously, and it has been

detected in Hungary too. The rodent populations are highly exposure group of tick bites. In Hungarian forests, the dominant species of rodents are yellow-necked mouse (*Apodemus flavicollis*), striped field mouse (*Apodemus agrarius*), wood mouse (*Apodemus sylvaticus*) and bank vole (*Myodes glareolus*). The CCHFV circulates among ticks, rodents, wild and domestic animals and causes high lethality hemorrhagic fever among humans. The aim of the study was to survey the prevalence of CCHFV among rodent populations. Further aim was to examine the potential correlation between population densities, seasonality and seroprevalence. Rodents were trapped at 13 sampling plots on a 11 ha area in the Mecsek Mountains, Hungary from March to October between 2011 and 2013 using a capture - mark - recapture method. Rodent sera were tested for antibodies against CCHFV by dot-blot and immunofluorescence assay. Among the 2,078 tested sera samples 205 were positive for CCHFV by dot-blot method. Compared to 2011, the rodent sample number doubled for 2012, but both seroprevalence remained around 10%. While from 2012 to 2013 the number of samples fell to fifth, the value of seroprevalence stayed around 10%. In conclusion, the seroprevalence was about 10% every year despite of the variable number of rodents in years. The seroprevalence was the highest for the bank vole (*Myodes glareolus*) in every year. Summarizing the above research of CCHFV infections, monitoring the reservoirs and predict the emerging hot-spots enable us to perform large-scale surveillance that should help us to gain a clearer picture of the epidemiology and the ecology of CCHFV infections and reduce the risk of the virus transmission in Hungary.

CHARACTERISATION OF VANCOMYCIN-RESISTANT *ENTEROCOCCUS FAECIUM* CLINICAL ISOLATES AT A TEACHING HOSPITAL IN DEBRECEN

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Vancomycin-resistant enterococci (VRE) have emerged as important global nosocomial pathogens and this trend has been associated with the dissemination of a genetic lineage designated clonal complex 17 (CC17). The aim of this study was to phenotypically and genotypically characterize VRE isolated from different clinical specimens between 2012 and 2015 at ateaching hospital in Debrecen, Hungary. We used MALDI-TOF mass spectrometry for species identification. Antibiotic susceptibility patterns were determined by either disc diffusion or E-test. This study involved PCR amplification of the vancomycin resistance genes (*vanA*, *vanB*, *vanC*) and three virulence genes (*ace*, *agg*, and *esp*). We also investigated the relationship between these virulence factors and biofilm production. Clonal relatedness was determined by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Among 7,799 enterococci 73 grew on the VRE screening plates originating from 43 independent patients. All isolates were identified as *Enterococcus faecium* with a high score value and confirmed by PCR. The strains were 100% resistant to ampicillin and ciprofloxacin while 81.4% were resistant to gentamicin and all of them were susceptible to newer antibiotics such as linezolid, tedizolid, tigecyclin and daptomycin. PCR analysis revealed the presence of *vanB* in 40 (93%) and *vanA* in 3 (7%) isolates. Among the virulence genes only *esp* was found in 7 cases (16.3%). Modified microtiter-plate test showed 13 (76.5%) weak and 4 (23.5%) moderate biofilm producer isolates. Based on the chi-squared test there was no statistically significant correlation between the presence of *esp* and biofilm production ($p = 0.059$). Among selected 23

isolates PFGE analyses revealed 9 pulsotypes based on a similarity cutoff value of ± 85%. 74% of the strains could be grouped into three main clusters indicating monoclonality. Selected 14 VRE isolates could be assigned to six different sequence types (ST17, ST117, ST203, ST78, ST412, ST364) all belonging to CC17. Four of them (ST17, ST117, ST78, and ST203) represented major hospital-derived ampicillin-resistant clones. We found an alarming emergence of multidrug-resistant VREfm belonging to CC17 at the University hospitals of Debrecen. We also found VREfm isolates belonging to ST412 and ST364 the first time in Central Europe. Fortunately, we detected no outbreak but still the increasing prevalence of VREfm is of concern and dissemination must be prevented with proper infection control measures and regular VRE screening.

MEASUREMENT OF BRASSINOSTEROID CONTENT OF *KLEBSORMIDIUM* SP. BEAE IDA_0061B AND THE OPTIMIZATION OF THE RICE LAMINA INCLINATION BIOASSAY

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The auxin-, and cytokinine-like phytohormone producing ability of various algal strains is well-known. Gibberellin activity has been demonstrated in brown algae. The presence of steroid plant hormones like brassinosteroids (BR) are essential for plant growth and development. Among lower organisms, algae are the primary producers of these molecules. 24-epi-castasterone and 28-homo-castasterone were detected from *Hydrodictyon reticulatum* green algae. Brassinosteroids promote cell elongation and proliferation, increase ethylene production, stimulate root growth, accelerate plant growth and increase yield and plant resistance against environmental impacts. Interaction with gibberellins and abscisic acid stimulates the germination of seeds. *Klebsormidium* species are considered as one of the most ubiquitous group of biological soil crust (BSC) organisms with high ecological value. These filamentous green algae can grow in the upper 5 - 10 cm layer of soil of both meadows and forests of Central Europe, between 5 - 30 °C. The strain *Klebsormidium* sp. BEAE IDA_0061B is able to accumulate brassinosteroids that can be detected in algal cultures by extraction and HPLC analytical methods, and presumed with seedling bioassay methods. High performance liquid chromatographic separation with fluorimetric detection was used to detect the brassinosteroid concentration of microalgae cultures. During the evaluation of the chromatograms it was found that brassinolides can be detected in *Klebsormidium* sp. BEAE IDA_0061B algal cultures, however, the method needs further development. In addition to instrumental analytical procedures, we also tried to detect the presence of brassinolides by rice leaf lamina inclination bioassay.
Several varieties of rice were tested to investigate the relationship between brassinosteroid concentration and leaf lamina inclination using an analytical grade brassinolide standard. On the basis of results, we are developing a method of detecting brassinosteroids from lyophilized and wet algal cultures of *Klebsormidium* sp. BEAE IDA_0061B.

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PREVALENCE OF ANAEROBIC BACTERAEMIA IN A TERTIARY CARE TEACHING HOSPITAL: A 5-YEAR RETROSPECTIVE STUDY

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Anaerobes are important human pathogens, however, the specific requirements of their isolation from clinical samples (e.g., strict anaerobic environment, long incubation time, laborious workup) makes the identification of these pathogens time-consuming. According to literature, anaerobes are causative agents of bacteraemias in 0.5 - 2% of cases, however, this field lacks recent data from our region. Matrix Assisted Laser Desorption Ionization Time-of-flight Mass Spectrometry (MALDI-TOF MS) has revolutionized all aspects of clinical microbiology. Thanks to the use of this technology, a vast variety of human pathogenic anaerobic bacteria can be identified on the species level, allowing for precise reporting of pathogens to clinicians. The aims of this study were to determine the prevalence of bacteremias due to anaerobes at the Albert Szent-Györgyi Clinical Center located in Szeged and to compare epidemiological trends with a previous study performed in a similar timeframe (2005–2009), where classical biochemical identification methods were used. Episodes of anaerobic bacteremia were identified retrospectively by reviewing the computerized microbiology records of the Department of Clinical Microbiology, University of Szeged. The data screening included blood cultures submitted to the Department of Bacteriology between 1st of January 2013 and 31st of December 2017. The anaerobic blood culture bottles were incubated in a BacT/ALERT 3D blood culture system (bioMérieux). The identification of isolates was carried out using MALDI-TOF MS (Bruker Daltonics). From the 116,371 blood cultures 422 (0.36%) strict anaerobic isolates were detected. The percentage of anaerobes in positive blood cultures remained constant throughout the study period (3.45 ± 0.12%). The mean age of patients was 66.21 years (range: 18 - 96), 55.45% were male. The number of different anaerobic species increased substantially (48 vs. 26), compared with the previous study. *Propionibacterium* sp. accounted for 53.32% of isolates, followed by *Clostridium* sp. (13.98%), *Bacteroides fragilis* group (13.03%) and Gram positive anaerobic cocci (GPAC; 4.98%). Compared to the results of the previous 5-year study, the number of strict anaerobic isolates increased by 38.36%, whereas their ratio decreased when compared to the overall number of blood cultures received. The introduction of MALDI-TOF in diagnostics allows for the verification of anaerobes as significant pathogens in bacteremia and the identification of novel species, not yet described from blood cultures (e.g., *Collinsella*, *Flavonifractor*, *Solobacterium*, *Tissierella*) in this region.

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STENOTROPHOMONAS MALTOPHILIA BLOODSTREAM INFECTIONS: PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY

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Stenotrophomonas maltophilia is a non-fermenting, aerobic Gram-negative bacillus, found in various aquatic environments, which has been recognized as an emerging pathogen in both community and nosocomial settings. This microorganism is intrinsically resistant to several classes of antibiotics, the therapy of choice is trimethoprim-sulfamethoxazole (TMP-SMX) in these infections, and resistance

to this drug is between 2 - 10% in the Western hemisphere. The aim of this study was the detailed epidemiological characterization of *S. maltophilia* bacteremia at the tertiary care teaching hospital located in Szeged and to assess the resistance trends associated with this pathogen. Episodes of *S. maltophilia* bacteremia were identified retrospectively by reviewing the computerized microbiology records of the Department of Clinical Microbiology, University of Szeged. The data screening included patients admitted to the Albert Szent-Györgyi Clinical Center between 1st of January 2013 and 31st of December 2017 who had at least one positive aerobic blood culture for *S. maltophilia*, associated with clinical signs or symptoms of infection. The blood culture bottles were incubated in a BacT/ALERT 3D blood culture system (bioMérieux). The identification of isolates was carried out using MALDI-TOF MS (Bruker Daltonics). A total of 134 episodes of *S. maltophilia* bacteremia were identified (26.8 ± 8.04/year) during the study period. 64.18% of patients were over 50 years of age and they presented with male dominance (62.69%). Most of the isolates originated from the Department of Internal Medicine (39.13%), the Intensive Care Unit (33.70%) and the Department of Surgery (13.04%). Malignancies (21.64%), cardiovascular illnesses (17.16%), respiratory failure/pneumonia (17.16%) and recent trauma (15.67%) were characterized as underlying diseases present in patients with *S. maltophilia* bacteremia. 17.16% of isolates were resistant to TMP-SMX and 5.97% of isolates were also resistant to fluoroquinolones. This study represents a large series of *S. maltophilia* bacteremia, representing a broad patient population, reported from a general tertiary care hospital in Hungary and highlights several features of *S. maltophilia* bloodstream infections. Although infrequent, the importance of this pathogen increased in recent years as *S. maltophilia* bacteremia is associated with a high mortality rate (25 - 35%).

QUORUM SENSING INHIBITION BY SULFUR AND SELENIUM-CONTAINING ORGANOCHALCOGENS: A COMPARATIVE *IN VITRO* STUDY

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The emergence and spread of antimicrobial resistance, together with the lack of newly developed antibiotics is a serious public health issue worldwide. The modulation of the redox-homeostasis and of oxidative stress has been described as an attractive target in the field of antimicrobial research. Novel antibacterial compounds containing chalcogens (elements in the VI.A group of the periodic table) have attracted substantial attention in the field of experimental chemotherapy. Bacterial quorum (QS) sensing inhibitors are considered as attractive alternatives for the control of infectious pathogens. The aim of our study was to determine the potency of sulfur and selenium-containing organochalcogens as QS inhibitors using bacterial model systems *in vitro*. Twenty-one compounds (eleven selenoesters and ten thiourea derivatives) were evaluated in our study. The antibacterial activity of the tested compounds was investigated by broth microdilution method according to CLSI standards. The effects of the tested compounds on QS were evaluated using the sensor strain *Chromobacterium violaceum* CV026, N-acyl homoserine lactone producer *Enterobacter cloaceae* 31298 and *Serratia marcescens* AS-1. The toxicity of sulfur and selenium compounds was assessed

on murine (NIH/3T3) and human (MRC-5) fibroblast cell lines by MTT method. The predicted physicochemical and pharmacokinetic properties of the compounds were determined *in silico*, using OSIRIS Molecular Property Explorer and PreADMET software. The tested compounds did not show relevant antibacterial activity against the strains tested in our study, while two thioureas and five selenoester derivatives exhibited strong QS-inhibitory properties. Apart from ketone-containing selenoesters, the compounds investigated did not have any cytotoxic effects on the cell lines tested. All tested compounds complied with Lipinsky's Rule of Five (RO5) and the predicted percentages of intestinal absorption are excellent (≥95%) for all respective compounds. Some of the selenoesters and thioureas showed promising quorum quenching (QQ) activity and their predicted ADME properties are of the highest standards. Derivatization and the synthesis of novel chalcogen-based organic compounds could introduce alternative antibacterial drugs with selective activity.

PHARMACEUTICAL COMPOUNDS AS QUORUM SENSING (QS) INHIBITORS

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Many studies report the high prevalence of multidrug resistant (MDR) bacterial pathogens. Because infections caused by MDR bacteria are more difficult and more expensive to treat, they represent a growing public health threat, as they are associated with increased mortality and decreased quality of life (QoL). Bacteria can communicate with surrounding bacterial cells using diffuse signaling molecules (autoinducers) forming multicellular communities. This cell-cell communication is called quorum sensing (QS), which changes the expression of various bacterial genes, resulting in phenotypic changes (e.g., antibiotic resistance, biofilm production, virulence factors, competence) depending on the density of the bacterial cell population. QS-inhibiting compounds represent a potential therapeutic alternative to antibiotics, as they do not exert selection pressure on microorganisms. Despite the scientific interest in such therapeutic vehicles, none of these compounds were included in a clinical trial thus far. The aim of the study was to determine the ideal experimental conditions for the disc diffusion QS inhibition assay (e.g., incubation time and temperature, culture media) as well as the *in vitro* QS inhibitory activity of various compounds currently used in human medicine. In the present study, the QS inhibitory activity of 30 pharmaceutical compounds (NSAIDs, antineoplastic agents, antipsychotic drugs, antiviral agents, mucolytics, antihistamines, vitamins) were evaluated using the disc diffusion method. For effective compounds, the concentration dependence of QS inhibitory activity was also determined. *Chromobacterium violaceum* wt85 (wild type, violacein pigment producer) and CV026 (Tn5 mutant, AHL-indicator), *Sphingomonas* sp. Ezf 10-17 (AHL-producer), *Enterobacter cloacae* 31298 (AHL-producer) and *Serratia marcescens* AS-1 (prodigiosin pigment producer) strains were used. The antibacterial activity of the compounds was investigated by broth microdilution method according to CLSI standards. The experiments were performed on Luria-Bertani agar supplemented with 2 g/L glucose and a microelement stock solution. The diameters of QS-inhibition were measured after 48h incubation at room temperature. Metamizole sodium, 5-fluorouracil, cisplatin, methotrexate, leomycin, promethazine, chlorpromazine and thioridazine had concentration-dependent QS-inhibitory effect on the bacterial strains examined. Nowadays, thousands of drug compounds are marketed for human therapeutic purposes and they can be considered as potential sources of QS inhibitory agents with different chemical structures and

mechanisms of action, since the pharmacokinetic parameters and tolerability of these compounds have already been verified *in vivo*. In our experiments, we have demonstrated the QS inhibitory effect of various drug molecules. Further experiments involving additional pharmaceutical compounds are warranted.

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INVESTIGATION OF THE MYCOBIOTA OF GRAPEVINE TRUNKS AFFECTED BY TRUNK DISEASES

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Grapevine trunk diseases (GTDs) are fungal infections of grapevine. This group of diseases consist of several different syndromes with various causal agents: Esca (*Phaeoacremonium minimum*, *Phaeomoniella chlamydospora*); Black dead arm (*Botryosphaeria* spp.); Eutypa dieback (*Eutypa lata*, *Eutypella* spp.); Phomopsis disease (*Diaporthe* spp.). The common characteristics of GTDs are the follows: The related pathogenic fungi colonize the woody tissues and develop black necrotic regions in the trunks. Some diffusible effector molecules (proteins, polysaccharides, secondary metabolites) of the pathogens can reach the leaves and berries and develop symptoms (chlorotic and necrotic regions) on these parts of the plant. GTDs often lead to the death of the affected plants, therefore are responsible for great losses for winegrowers. The appearance of the above symptoms are irregular, which is due to the complex nature of these diseases. The pathology of GTDs believed to depend on the weather conditions, canopy management techniques and also microbial interactions. Our aim was to compare the mycobiota of GTD-affected grapevine and healthy plants revealing the changes due to the different stages of the infections. Cabernet sauvignon trunks were monitored for GTD symptoms (impaired shoots, dead plants) in the spring of 2018 at the vineyard of Eszterházy Károly University. A total of 36 trunks were sampled alongside with 6 healthy plants. Three disks were cut from the trunks and used for the isolation of fungi after surface sterilization. A total of 108 different filamentous fungal strains were isolated and subjected to identification. The diversity of the isolated fungi was the lowest in healthy plants (1.8 strain/sample), it was much more higher in plants with impaired shoots (3.75 strain/sample) and it showed a moderate value in dead plants (2.8 isolate/sample) The isolation rate of three substantial GTD pathogens (*Botryosphaeria* spp., *P. minium*, *P. chlamydospora*) was also analyzed. In dead plants *Botryosphaeria* spp. were the most abundant (90%), followed by *P. minimum* (21%) and *P. chlamydospora* (11%). In grapevines with impaired shoots, the occurrence of *Botryosphaeria* spp. was decreased (50%) while these plants showed increased incidence for *P. chlamydospora* (40%) and a similar rate for *P. minimum* (17%). The healthy trunks showed the lowest incidence for *Botryosphaeria* spp. (40%), relatively high rate for *P. chlamydospora* (60%) and there was no isolate belonging to *P. minimum*. Our results pointed out the importance of external factors in the development of GTDs, since both *Botryosphaeria* spp. and *P. chlamydospora* occurred in significant portion of asymptomatic plants. Grapevines with impaired shoots showed an increased isolation rate for *P. minimum*, while dead plants showed the highest rate for *Botryosphaeria* spp. compared to healthy plants.

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SIMPLE METHODS FOR SELECTION OF *SACCHAROMYCES* STARTER YEAST FROM LOCAL MICROBIOME

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Controlled wine making is used in the majority of wineries to avoid the risk of slow or incomplete fermentation. They apply commercial available starter yeasts which were selected according to beneficial oenological properties. Most of starter cultures contain *Saccharomyces cerevisiae* or other *Saccharomyces* species, which can ensure suitable ethanol level (10-15%) in the final product. However, the use of commercial yeast strains can result in uniform character of wines independently from the wine region. In spontaneous fermentation different yeast species can produce different secondary metabolites and as a consequence influence the character of wine. These indigenous strains were adapted better to local conditions highlighting the specific character of the wine. In the present study we applied a selection method in order to develop new *Saccharomyces* starter strains for wine fermentation. The strains investigated were isolated previously from RIVE-NARIC vineyard, Badacsonytomaj. Selection of *Saccharomyces* strains were carried out in two main steps. First, we used molecular biological technology for identification of *Saccharomyces* strains and afterwards beneficial properties of studied strains were analyzed with microbiological methods. Strains belonging to genus *Saccharomyces* was identified with ITS4 and ITS5 primers and different strains were separated from each other based on polymorphism of interdelta region. The interdelta pattern of strains were compared to 35 commercial starter strains to avoid re-isolation and from 238 local strains only 10 showed the same pattern to commercial yeasts investigated. 21 strains with different interdelta pattern were chosen for microbiological tests and the following oenological features were analyzed: ethanol tolerance, fermentation capacity, hydrogen sulfide production and killer activity. Our results show that all selected isolates can grow in the presence of 15% ethanol and 15 of them start fermentation of glucose at the third day at 16°C. 18 strains show killer activity, a beneficial feature of starter yeasts. Hydrogen sulfide production is an insufficient character of starter yeasts because it causes disagreeable smell and taste of wine. Among the investigated strains only 8 isolates produced low level of hydrogen sulfide and one strain produced higher level, other strains were non-producer. We tested the selected strains in microvinification procedure and analyzed ethanol concentration, pH, volatile acid, total acid, reducing sugar parameters of final product.
Taken together it was turned out that 12 strains from the 21 isolates can be applied as a "terroir" starter culture. In the future we will investigate these strains in mezovinification and analyze profile of aroma compounds of "organic" wine with gas chromatography.

COMPARISON OF CARBON STARVATION AND CARBON LIMITATION STRESS RESPONSES IN *ASPERGILLUS NIDULANS*

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The filamentous fungus *Aspergillus nidulans* - like any other microorganisms - has to cope with carbon stress frequently in its natural habitats, coming from either carbon starvation or carbon limited environmental conditions. The quality and availability of carbon sources influence many aspects of its life including growth, asexual and sexual developments or even secondary metabolite production. Carbon stress commonly induces the production of versatile extracellular hydrolases such as carbohydrate-active enzymes (Cazymes) and different peptidases as well. Genome-wild transcriptional changes caused by carbon stress in *Aspergillus nidulans* cultures were analyzed by RNA sequencing. Carbon starvation and carbon limitation stresses were induced by transferring exponentially phase hyphae, pre-grown on glucose, into carbon source free and lactose containing media, respectively. A significant overlap between the carbon starvation and carbon limitation stress responses were detected at the level of both stress responsive genes and processes. The detected transcriptional changes included the followings: i) Up-regulation of autophagy genes, extracellular proteinase genes and genes involved in chitin and ß-1,3-glucan utilization in carbon starving cultures. ii) Up-regulation of genes involved in lactose utilization including *lacD* and *lacpA* (encoding respectively the main ß-galactosidase and lactose permease of *A. nidulans*) on lactose. iii) Up-regulation of the D-galactose oxidoreductive pathway genes and several Cazyme genes on both carbon starving and lactose utilizing cultures. More than 50% of stress inducible Cazyme genes (all together 127 genes) were up-regulated in both carbon starvation and carbon limitation stresses. Up-regulation of *galX*, *galR*, *lacpA* and *lacD* as well as elements of the D-galactose oxidoreductive pathway was also recorded on arabinogalactan by RT-qPCR.
Our results support the view that production of plant cell wall degrading enzymes is an important element of carbon stress response of *A. nidulans*: Although carbon starving cultures can utilize nutrients liberated by macroautophagy and autolytic cell wall degradation, seeking for plant cell wall materials in their environment can be an important strategy to survive this stress. Production of a wide range of enzymes and transporters involved in hemicellulose utilization makes fungi capable to grow on unique disaccharides like lactose.

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ISOLATION OF GREEN ALGAL STRAINS, ESTABLISHMENT OF A CULTURE COLLECTION AND DESCRIPTION OF TWO NEW *CHLOROCOCCUM* SPECIES

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Nowadays algae are widely used as foodstuffs or for industrial and agricultural purposes. Among them, the biofuel research is a major focus. Lack of adequate raw materials is currently a limiting factor in biofuel production. Halotolerant microalgae can grow in brackish or saline waters that are unsuitable for traditional agricultural production. As algae are not seasonal, they are 10 to 30 times more productive than land-based crops. Halotolerant algae can play a key role in the successful development of biofuels and can effectively contribute to the recycling of atmospheric carbon dioxide. During the creation of our culture collection we aimed to isolate thermotolerant and halotolerant algae. The isolation was carried out from 2 sites (Lake Kőhegyi and Lake Velence, Hungary). Four water samples were collected from both sites during the summer. Lake Kőhegyi is an

astatic lake with low pH and low conductivity, while Lake Velence is a salt (soda) lake having alkaline pH and relatively high conductivity. Water samples were processed using classical microbiological methods: sequential plating on agar plates after enrichment for 1 week in liquid medium. The culturing was carried out with continuous illumination at 25°C for 7 days. The culture collection of Albitech Ltd. currently contains 45 identified and 15 unidentified algal isolates. According to partial 18S rRNA gene sequence-based identification, it was found that some algal strains could be described as new species. During the characterization of the two new *Chlorococcum* species, phylogenetic trees were constructed based on 18S rRNA gene, ITS (internal transcribed spacer between rRNA genes) and RuBisCO (ribulose-1,5-bisphosphate carboxylase oxygenase) gene sequences. Morphological studies were carried out by light microscopy. After lipid staining with nile red, the intracellular lipid droplets were visualized in 2-week cultures. Pictures were also made by transmission electron microscopy to visualize other cellular structures. Native algal cultures were deposited in the SAG (Culture Collection of Algae at Goettingen University) and lyophilized algae were deposited in the Algal Collection of the Hungarian Natural History Museum.

INVESTIGATING THE INTERACTION BETWEEN *AZOSPIRILLUM BRASILENSE* SOIL BACTERIA AND *SCENEDESMUS RUBESCENS* ALGAL STRAIN BY USING MICROFLUIDIC METHODS

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Nowadays the use of algae for industrial or agricultural purposes, including their use as foodstuffs, is spreading globally. *Scenedesmus* species are important for both the food industry and agriculture due to its nutritional properties. Previous research and development on a *Scenedesmus*-based foliar fertilizer raised many questions on effectiveness of large-scale algal culturing and the potential effects of algae on soil bacteria. Co-cultivation with *Azospirillum* species is a way of optimization of *Scenedesmus* culturing, however, the operation of co-cultivation system is not well-known. *Azospirillum brasilense* can produce indole-3-acetic acid, which being a plant hormone, could promote the propagation of algae. However, the effects of algae on *Azospirillum* cultures are not well known. *Azospirillum* species are associative nitrogen fixing rhizobacteria, one of the most studied plant growth promoting bacteria in the practice of agricultural microbiology. They can fix nitrogen under microaerophilic conditions. Under anaerobic or microaerophilic conditions they can denitrify, and they are capable to assimilate ammonia, nitrate and nitrite. Replacing plants with microalgae is a good method for modeling bacteria-root interactions. According to recent literature motile bacteria may move in the direction of algae due to the presence of chemoeffectors – in a similar way they use to move towards root exudates (sugars, amino acids). According to our previous experiments, the *Scenedesmus rubescens* BEA D01_12 can produce certain secondary metabolites that can be used by *A. brasilense* as a carbon source. In order to reveal what kind of interactions are present between these two strains we used a microfluidic set up. This device consists of two large reservoirs and an observation channel separated by a membrane. Chemical gradient forms in the channel within minutes, and lasts for several hours. We studied the behavior of gfp- labeled *Azospirillum brasilense* CdS strain in the microfluidic gradient generator under different chemical conditions (e.g. the presence of a neighboring *Scenedesmus rubescens* BEA D01_12 strain). We followed the spatial distribution of bacteria by fluorescent time-lapse microscopy. With this method we are able to detect

specific motility patterns, such as bacterial chemotaxis and the growth rate of bacteria in the channel can be analyzed as well. We performed qualitative and quantitative analyses of the images. Based on our results, the algal culture is likely to contain chemoattractant compounds.

MICROBIAL COMPOSITION OF BOTRYTISED GRAPE BERRIES FROM TOKAJ

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One of the most renowned dessert wines of the world is produced in the Tokaj wine region from botrytised berries. The phenomenon, which leads to raisined ‘‘aszú,, berries, is called noble rot and is different from the well-known grey mold and bunch rot diseases caused by the same fungus. Despite of many articles which pay attention to the determination of the suitable microclimatic conditions of botrytisation, the process has not been investigated in detail. The aim of our study was to characterize the botrytisation process concentrating on the different stages of the development of infection and determine the microbial diversity. We investigated one specific vineyard in Tokaj region in Mád. For characterization of botrytised grape berries two local varieties have been chosen that rot in a different way according to organoleptic observations. Berries were collected during ripening/rotting, distinguishing four infection phases (I. healthy berries, II. botrytised, not rotten berries, III. botrytised and rotten berries, IV. rotten berries with latent mycelia).
Bacteria, yeast and filamentous fungi composition of all phases and cultivars were analyzed by molecular biological methods. In order to get an overview about the microbiome populations the following sequences were analyzed: 16S V4/V5 region was examined in the case of bacteria, for the yeast composition the 18S D1/D2 region was used and for the filamentous fungi the fungal ITS1/ITS2 region was analyzed. In conclusion, the composition of the populations of identified microorganisms showed variances in the different phases.

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TESTING THE EFFECTIVENESS OF FUNGICIDE ACTIVE AGENTS AGAINST *BOTRYTIS CINEREA* BY GRADIENT PLATE METHOD

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Three different active fungicide agents were tested against Botrytis cinerea such as fenhexamid, tebuconazole and fluopyram. Fenhexamid is a narrow spectrum fungicide belonging to the chemical family of hydroxyanilides inhibiting sterol biosynthesis (SBI). Fenhexamid blocks the demethylation at C4 of sterols. Tebuconazole is a triazole fungicide relies on inhibiting fungal CYP51 (lanosterol-14α-demethylase) and sterol biosynthesis. Fluopyram is a succinate dehydrogenase inhibitor (SDHI) which blocks cellular respiration and inhibiting ATP production. In the present study the effectiveness of the active agents were tested by optimizing the gradient plate technique. The

technique allows a more cost-effective and time-saving assay of EC50 values comparing to the traditional dilution method. Nine different *B. cinerea* strains were used in the research. Seven *B. cinerea* isolates were collected from different wine regions of Hungary and two *B. cinerea* strains were used as reference strains. To prepare the gradient plates, the active agents were mixed with PDA medium in the required concentration and poured into slant placed petri dish. After the first layer is solidified, a fungicide-free PDA medium was poured horizontally onto the previous layer. This results in a linear concentration gradient of the fungicide agent in the agar medium. The strains used for the test were grown on sterile toothpicks, which were then placed on the fungicide-amended medium along the gradient line. As a control, a fungicide-free PDA medium was inoculated with the same strains. The strains were incubated at 21°C then the mycelial growth was measured after 96 hours. The concentration of the fungicide agents related to the 50% reduction of growth rate (EC50 values) were determined. According to our results the gradient plate technique works well to determine the EC50 values, and stands for a faster and more cost-effective method than the agar dilution method.

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INTERKINGDOM INTERACTIONS BETWEEN COMMON COLONIZERS OF THE CYSTIC FIBROSIS AIRWAYS *IN VITRO*

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Cystic fibrosis (CF) is one of the most common genetic disorder resulted by the mutation of the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The impaired function of CFTR leads to a nutrient rich airway surface liquid with high viscosity, which cannot be cleared effectively by the muco-ciliary transport system. This supports the colonization of the airways by opportunistic bacterial and fungal species. The CF lung microbiota is a complex community, where *Pseudomonas aeruginosa* is the most prevalent bacteria, while members of the *Scedosporium apiospermum* species complex are the second most common fungal pathogens after *Aspergillus fumigatus*. It was previously reported that a bacterial infection may promote the subsequent colonization of *A. fumigatus*, but the background and the main risk factors for the airway colonization of scedosporia has been less investigated. Thus, we aimed to study how *P. aeruginosa* might influence the appearance of *Scedosporium* species in the CF lung using *in vitro* models. Direct interactions between fungal and bacterial strains were examined using the disk inhibition method. In these tests bacterial strains inhibited fungal growth, but the degree of inhibition varied between culture media and bacterial strains. Indirect interactions via volatile organic compounds (VOCs) were investigated by the plate - in - plate method on various media. Although the two pathogens were cultured physically separated from each other in these tests, the presence of bacteria stimulated the growth of several fungal strains. In order to identify the group of VOCs responsible for these effects, fungal isolates were cultured on various nutrient-depleted minimal media. Our results suggest, that volatile nitrogen- and sulphur-containing compounds might be in the background of the growth-stimulating effect. Indirect interactions via media-soluble molecules of *P. aeruginosa* were also investigated by a cellophane assay on solid media and by culturing fungi in liquid RPMI-1640 pre-treated with *P. aeruginosa*. On solidified RPMI-1640 media mainly complete inhibition zones, while on synthetic CF media only partial inhibition zones were observed. Furthermore, the growth of scedosporia was significantly higher in non-treated RPMI-1640, than in those media pre-treated with

P. aeruginosa. According to these results, a prior infection with *P. aeruginosa* might increase the chance of *Scedosporium* colonization in a cystic fibrosis lung via volatile molecules, but after direct physical contact their relationship most probably becomes antagonistic.

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A COMPARATIVE STUDY ON THE VIRULENCE OF ENVIRONMENTAL AND CLINICAL ISOLATES OF THE *FUSARIUM SOLANI* SPECIES COMPLEX

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Fusarium species are widely distributed in soil as harmless, saprophytic organisms. Besides, they are also able to infect plants, animals and humans. The members of the *Fusarium solani* species complex (FSSC) and among them *F. falciforme* are the most frequently isolated causative agents of the sight-threatening disease, fungal keratitis in South India. The aim of this study was to investigate the background of the dominance of *F. falciforme* in South-Indian *Fusarium* keratitis cases. To this end, FSSC strains from South-Indian keratomycosis patients and environmental samples were isolated and identified at the species-level and the species diversity of the two groups were compared. Furthermore, Oregon R (wild type) and MyD88c03881 (MyD88-mutant) *Drosophila melanogaster* flies were infected with *F. keratoplasticum*, *F. falciforme* and *F. solani* s. str. strains isolated from clinical and environmental sources in order to compare their virulence. According to our results, the majority of isolates - both clinical and environmental - were confirmed as *F. falciforme*. Virulence seemed to be independent of the source of the isolates, since no significant differences were observed in the survival of flies infected with either clinical or environmental strains. Higher mortality rates were found in case of the MyD88-mutants than the wild-type flies, which reconfirmed that MyD88 was essential in *D. melanogaster* to eliminate fusaria. In wild-type flies, clinical *F. falciforme* and an environmental *F. solani sensu stricto* strains proved to be avirulent, while another *F. falciforme* and two *F. keratoplasticum* strains reduced the survival significantly. In conclusion, these intraspecies differences in the virulence of *F. falciforme* isolates suggest that virulence is more like a strain-specific, than a species-specific feature and the dominance of *F. falciforme* in fungal keratitis could be attributed to its higher environmental frequency compared to other FSSC species.

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RESISTANCE TO QOI FUNGICIDES IN THE GRAPE BLACK ROT PATHOGEN, *GUIGNARDIA BIDWELLII*, AND RELATED SPECIES, IN THE LIGHT OF THE CYTB GENE STRUCTURE

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Strobilurins, belonging to the group of Quinone outside Inhibitors (QoIs), are considered as single-site of action fungicides which inhibit the electron transfer in mitochondria by binding to the cytochrome bc1 enzyme complex. It has repeatedly been shown that a single point mutation in codon 143 of the mitochondrial gene CYTB, which encodes cytochrome b, confers complete resistance to QoI fungicides in many plant pathogenic fungi. However, in some species, such as *Puccinia* spp., neither QoI resistance nor this mutation, designated as G143A, have been detected so far. This was explained by the presence of an intron in the CYTB gene right after codon 143 in these plant pathogens: it was predicted that a G143A mutation would prevent the splicing of this intron and, thus, the production of functional cytochrome b proteins. Consequently, in these intron-containing species the G143A mutation is considered to be lethal and the risk for QoI resistance is predicted to be low. *Guignardia bidwellii* (anamorph: *Phyllosticta ampelicida*), the causal agent of grape black rot, is considered as a CYTB intron-containing species with low risk for the development of QoI resistance in the field. We amplified and cloned CYTB fragments in several *G. bidwellii* strains, and also in some other *Guignardia* spp., including authentic strains of *G. citricarpa*, the causal agent of citrus black spot, and also *G. gaultheriae*, *G. mangiferae* and *G. aesculi* obtained from CBS, to sequence the intron located after codon 143. Surprisingly, no intron was detected in the predicted position in several *G. bidwellii* strains isolated from different grape varieties in Hungarian vineyards. Also, the intron was not found in either an authentic *G. bidwellii* strain obtained from LGC ATCC, or the *G. aesculi* and a *G. gaultheriae* strains included in this study, while the intron was identified, and sequenced, in all other *Guignardia* spp. strains examined by us. *In vitro* fungicide resistance tests did not show a clear correlation between the presence/absence of the intron in *Guignardia* spp. strains and their sensibility to QoI compounds.

This might suggest that other mechanisms may also be involved in their QoI resistance. So far, our results indicate that at least some *G. bidwellii* strains causing grape black rot could contain the G143A mutation and might be able to develop QoI resistance in this way in the field.

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ROLE OF EXTERNAL CONDITIONS IN THE ANTIFUNGAL ACTIVITY OF PULCHERRIMIN PRODUCING *METSCHNIKOWIA* YEASTS

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In order to be suitable for biological control, a potential microbial antagonist should have important inherited features. It is desirable for these microbes to show, among other things, wide range of antifungal activity, to be non-pathogenic for humans, to be resistant to pesticides, to be genetically stable and to be able to survive under variable environmental conditions. Since yeasts often have special features, such as tolerance to extreme environmental conditions (low and high temperatures, drought, high relative humidity, low oxygen level, extreme pH, UV radiation) a relatively high number of yeasts was reported among the antagonistic microorganisms which could be used as biocontrol agents against postharvest pathogens. One of the most potent antagonistic yeasts is the pulcherrimin producing *Metschnikowia* genus, which can inhibit growth of other microbes by immobilizing iron in the medium. Correlation between their pulcherrimin pigment production and their antifungal activity has only recently proved. Earlier results suggested that besides the internal inherited features, the external environmental factors can also seriously influence pigment production. Hence it is very important to learn which external factors can and how they can influence the pulcherrimin production. Earlier data suggested that iron content of the medium or oxygen level or agar concentration could affect it. To expand our knowledge about these influencing external factors, further experiments have been carried out. Our results revealed that concentration and type of the carbon sources significantly influence production of the pulcherrimin pigment. Furthermore, pigment production seemed to be pH and temperature dependent.
The highest amount of pulcherrimin was produced at low pH and high temperature. These data can contribute to finding the optimal conditions of antagonism and they can be useful in the improvement of biocontrol capacity, which is a potential alternative to synthetic fungicides.

METSCHNIKOWIA ANDAUENSIS CAN BE A MORE EFFECTIVE BIOCONTROL AGENT THAN OTHER PULCHERRIMIN PRODUCING *METSCHNIKOWIA* STRAINS

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Problems of pest control and postharvest fruit protection include declining effectiveness of fungicides, formation of resistant microbes and environmental pollution. Thus, over the last decades, a number of research programs have evolved around the world to discover new biocontrol agents. Antagonistic yeasts which can be suitable for biological control have received particular attention. The pulcherrimin producing *Metschnikowia* genus is very effective against several pre- and postharvest pathogens. Consequently, they can be used to protect a different kind of fruits and vegetables against pathogens during ripening or storage periods. Several studies have been directed at using them against those microbes, such as *Cryptococcus laurentii* or *Hanseniaspora uvarum*. *Metschnikowia pulcherrima* and *M. fructicola* produce a red pigment called pulcherrimin, which can inhibit even the mycelial growth of several postharvest molds. As long as effectiveness of *M. pulcherrima* and *M. fructicola* has been reported by several researches, whereas the antagonistic effect of *M. andauensis*, which is also able to produce pulcherrimin is less studied. Thus, our aim was to study antagonistic capacity of the *M. andauensis*. Our results show that *M. andauensis* is more effective against the *B. cinerea* and *P. expansum* than the type-strains of *M. pulcherrima* and *M. fructicola in vitro*. These experiments also revealed that presence of the *M. andauensis* negatively influenced growth of several other yeasts, such as *Candida stigmatis*, *Candida orthopsilopsis*, *Cryptococcus albidus*, *Issatchenkia terricola*, *Pichia anomala*, *Pichia kudriavzevii*,

Wickerhamomyces orientalis and *Aureobasidium pullulans*. These findings can be very important in designing a possible antagonistic mixture which contains different yeast species.

PLEOSPORALEAN DARK SEPTATE ENDOPHYTES COLONIZING ROOTS OF *STIPA KRYLOVII* IN MONGOLIAN GRASSLANDS

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Plants form symbioses with various microorganisms through their roots. Endophytic fungi are important members of these communities, comprising the wide spread group of dark septate endophytes (DSE). These endophytes are common in harsh environments such as arid regions and however their function in ecosystems is still unclear. Our information is more limited on the DSE community of Asian grasslands, and only few studies focused on the root endophytes of Mongolian steppe. In this study, we examined the root colonizing fungal endophytes of *Stipa krylovii*, a common gramineous plant species of semiarid grasslands in Mongolia. Our aims were to (i) carry out molecular identification of pleosporalean isolates previously gained collected from the roots of *S. krylovii* from a natural Mongolian Steppe and to (ii) test the symbiotic feature of the fungi in resynthesis experiments. For molecular identification of the isolates, total DNA was extracted and the internal transcribed spacer (ITS) region of the nrDNA was amplified for all the isolates investigated. Besides the ITS region, which is accepted as the barcoding region of fungi, the partial translation elongation factor 1-α (TEF) gene and the large subunit (LSU) region of rDNA was also amplified and sequenced in case of isolates representing different lineages. Multilocus molecular phylogeny was carried out using our sequences and representative sequences from GenBank. *In vitro* tests were performed using leek (*Allium porrum*) inoculated with the representatives of each lineages resulted by the analyses of ITS sequences. We considered a fungus as true DSE if it colonized the roots without causing a negative effect on the plant. Altogether, ITS region of more than 80 pleosporalean isolates were sequenced representing numerous lineages. Further DNA regions of 41 representative isolates were also sequenced. Most of the isolates could be identified on species or genus level and novel taxa are surely present among these endophytes. Majority of the isolates caused no negative effect the leek. We have found numerous lineages not only from Mongolia, but from prairies of the United States and sandy grasslands of Europe, too. Common and dominant pleosporalean lineages of grasslands, such as *Periconia macrospinosa* and *Darksidea* species were also found here.

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MICROBIOLOGICAL CHARACTERISATION OF COLIFORM BACTERIA ISOLATED FROM THE FAECES OF HERBAL EXTRACTS AND ZINC OXIDE TREATED PIGS

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Members of the Enterobacteriaceae are widespread in nature, and many genera of this family belong to the normal intestinal microbiota of humans and animals. Some of them are harmless or beneficial symbionts, but many of them known as pathogens (*Salmonella*, *Yersinia*, *Shigella*) or disease causing bacteria (*Escherichia*, *Klebsiella*, *Proteus*, *Enterobacter*, etc.). The type strain of this family is *E. coli*, and they are commonly called "enteric bacteria" or "coliforms". The occurrence of *E. coli* in water and food can be used as the indicator of fecal contamination and the potential occurrence of pathogenic bacteria. Due to their clinical importance there are many semi-selective and differential media moreover biochemical tests to separate and identify the different coliform genera. Coliforms caused diseases plays important role in livestock breeding, especially in pigs. The European Union banned the use of antibiotics as feed additives in 2006 and other chemicals, like zinc oxide, frequently used as feed additive in pig to improve health. Zinc oxide is very efficient against post-weaning diarrhea, but the increased use of zinc can cause the emergence of multi resistant *E. coli* strains and can mask the use of other antibiotics, therefore the use of zinc will be banned too. The heavy metal contamination of soil may pose risks to humans and the ecosystem therefore, the livestock industry is searching for environmentally friendly supplements, like pre/probiotics or herbal extracts. In our experiment three different herbal extracts and zinc oxide (as a control) were used as feed additives during a 23-week period. The composition of fecal microbiota was monitored every two weeks and more than 300 coliform strains were isolated and characterized by microbiological (colony morphology in Eosin Methylene Blue and Chromogenic Coliform Agar) and biochemical (indole, methyl red, citrate, H_2S production tests) methods. The changes of composition and the total number of coliforms were determined in the case of treated and control animals. The biochemical tests were confirmed molecular biological methods (PCR amplification of *lacZB*, *lacZ3*, *uidA* and *yaiO* genes) and the 16S rDNA of some representative strains of each biochemical group were sequenced. More detailed analyses of the strains are in progress.

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THE POSSIBLE ROLE OF *AUREOBASIDIUM PULLULANS* IN THE DEVELOPMENT OF ESCA DISEASE OF GRAPEVINE THROUGH THE INTERACTION WITH AN ASSOCIATED FUNGAL PATHOGEN

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The Esca disease belongs to the group of grapevine trunk diseases. These syndromes are caused by several fungal species and are responsible for great losses in vineyards. Esca disease is caused by two ascomycetous fungal species, *Phaeomoniella chlamydospora* and *Togninia minima*. These pathogens colonize the woody parts of the plants and develop necrosis at the site of the infection and on the leaves. The expression of the symptoms shows irregular distribution in time, which is the result of the complex nature of the disease. Environmental conditions and microbial interactions believed to affect the development of the symptoms. In the present study the interaction between the nonpathogenic fungus *Aureobasidium pullulans* and *P. chlamydospora* and the possible effects of this interaction on the development of the Esca disease were investigated. In confrontation tests, both *A. pullulans* and *P. chlamydospora* showed decreased growth rate toward the confrontation zone, however no signs of cell death can be observed on the mycelia. Tests with the culture filtrates of the two fungi suggested that both *A. pullulans* and *P. chlamydospora* secrete substances which affect the development of the other fungus without damaging it. On plates amended with the culture filtrate of a fungus, the other

fungus showed decreased growth rate and enhanced sporulation. Germination tests verified that the culture filtrates are not toxic for the confrontation partners even at 50% v/v concentration. To study the in planta interaction of the two fungi, single and co-inoculations were done on detached green shoots of Cabernet sauvignon. 15 days after the inoculations the plants were examined. Mock inoculated shoots did not show foliar symptoms, neither necrotic lesions at the wounds. Plants inoculated with *A. pullulans* did not show necrosis on the canes, but 30% of the leaves showed discoloration. All plants which inoculated with *P. chlamydospora* developed necrosis at the inoculation point, but the leaves were asymptomatic. The co-inoculations were resulted in the severe discoloration or necrosis of the leaves and the frequent necrosis of the canes even at parts apart from the inoculation points. Our results suggest that *A. pullulans* can promote the development of the foliar symptoms of Esca disease of grapevine. *In vitro* interaction tests suggest that this phenomenon may due to the interaction between *A. pullulans* and *P. chlamydospora*, which may alters the spreading and the metabolism of the pathogenic fungus. *A. pullulans* can directly damage the leaves of grapevines, but with a low efficacy, according to the in planta tests. This latter result can be explained by the pullulan production of *A. pullulans*, which is a polysaccharide produced also by *P. chlamydospora* and believed to take part in disease development.

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COMPARISON OF DRAFT GENOMES OF COMMENSAL AND PATHOGENIC *HISTOPHILUS SOMNI* ISOLATES

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Histophilus somni is an opportunistic pathogen of sheep and cattle, may be present as commensal on the genital and upper airway mucosa of animals, while at the same time, it is a major pathogen capable of causing epizootics of pneumonia and disseminated disease. Despite it is a major economic problem, its virulence properties and differences between commensal and pathogenic strains or between strains from different host species are not well understood. Earlier a cluster of *H. somni* isolates from pneumonia of calves present in multiple epidemiologically distinct herds was identified by pulsed-field gel electrophoresis in a 100-isolate collection from Hungary. Aim of this work is to compare the whole genomes of two isolates of this cluster to genomes i) of two reference strains sharing the same pulsotype with members of the pathogenic cluster; ii) of an isolate from calf pneumonia showing a different pulsotype; iii) of three isolates from genital samples of sheep and cattle. Two complete genomes from the GenBank (CP000436, from a genital commensal isolate and CP000947, from a pneumonia isolate) were used as references. As lipooligosaccharide (LOS) phase variation is a known factor determining virulence, we concentrated on genes putatively associated with LOS biosynthesis and glycosylation. Genomes were sequenced using Illumina NextSeq500, de novo draft genomes were assembled with SPAdes Genom Assembler 3.11. and auto-annotated using the RAST server. Genomes were searched for genes coding for antibiotic resistance, putative LOS biosynthesis and glycosylation. Antibiotic resistance genes were not found in any of the genomes. Of the 18 genes putatively involved in LOS biosynthesis, only four were present in all ten genomes compared; genes for a putative alpha-1,3-galactosyltransferase, ADP-heptose-LOS-heptosyltransferase, LOS-heptosyltransferase I and the gene *wadA*; all involved in formation of the

core oligosaccharide directly linked to lipidA. Another six genes were ubiquitous among the isolates sequences and were absent only from one or two isolates. No genes were characteristic to host species or to pathogenicity; though a glycosyl transferase with an exactly unknown function was only found in our isolates originating from genital tissues, however, this gene was also present in the genome of a reference strain (CP000436) assigned as a genital commensal strain. Highest variation was found in the gene cluster responsible for phase variation, where variously truncated copies of the gene cluster were frequently found. Isolates representing the putatively virulent cluster were highly, but not totally uniform, and there were no genes differentiating it from non-cluster isolates. Analysis of the biosynthetic apparatus of the LOS did not yield unequivocal differences between commensal and pathogenic isolates or between isolates from different host species.

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QUANTIFYING ASSOCIATION BETWEEN DRUG USE AND CARBAPENEM RESISTANCE IN *ACINETOBACTER BAUMANNII* USING NON-LINEAR TIME-SERIES ANALYSIS

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Restriction of antibiotic use is an effective and frequently used method to contain antibiotic resistance. However, over-restriction may hinder access to effective treatment and lead to substitute prescribing which creates new selection pressures or resistance problems. A method is needed to balance access to effective treatment with control of resistance. As a development of linear dynamic regression approaches, non-linear time-series analysis can identify the most important antibiotic groups for resistance problems, but in addition identify levels of use above which resistance shows abrupt increases. We utilized nonlinear time-series analysis, i.e. generalized additive models (GAMs) and multivariate adaptive regression splines (MARS) to find threshold levels of drug use in tertiary hospital between Oct 2004 and Aug 2016 (n = 143 months). Explanatory variables were monthly hospital use of carbapenems, fluoroquinolones (FQ), piperacillin - tazobactam (PipT), third generation cephalosporins (3GC), and aminoglycosides measured in defined daily doses/1,000 OBDs (DoD); outcome variable was monthly incidence density of carbapenem resistant *Acinetobacter baumannii* (CRAb) (non-duplicate isolates/per 1,000 occupied bed days, OBDs). Explanatory variables (operating at lags of 1 - 5 months), autoregressive and seasonal autoregressive terms were entered into a GAM to identify significant linear or non-linear associations with CRAb. We then applied a MARS procedure to obtain an efficient model with easily interpretable thresholds. Drug use thresholds identified were: carbapenem use (lag 3 months) above a threshold of 14.9 DoD; PipT use (lag 3 months) above 6.2 DoD; and FQ use (lag 1 month) above 85.2 DoD. In a slightly less efficient model, FQ use was replaced by 3GC use (lag 2 months) above 36.2 DoD. Previous CRAb incidence was always a significant predictor (lag 1 and 2 months). When applied to the total hospital population, these thresholds suggest limiting use to 84 carbapenem, 35 PipT, 478 FQ and 203 3GC 7-day regimens/month. Non-linear time-series analysis identified critical thresholds in use-resistance

relationships. These indicate quantitative targets for antibiotic stewardship which balance use of key agents with the need to contain carbapenem resistance in *A. baumannii*.

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ISOLATION AND MOLECULAR DETERMINATION OF DSRNA VIRUSES IN MUCOROMYCOTA FUNGI

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Detection of double-stranded RNA (dsRNA) elements in fungal isolates may suggest the presence of mycoviruses. Mycoviruses can be transmitted only intracellularly by horizontal transmission via hyphal anastomoses or protoplast fusion or by vertical routes through sexual or asexual spores. Although, mycoviruses are common in fungi, they usually remain latent and their presence is typically symptomless. However, some of them can cause smaller or greater phenotypic changes in the host. The most important effect is that they may enhance or reduce the virulence of the host causing hypervirulence or hypovirulence, respectively. Although majority of the fungal phyla contain virus harboring species, we have only limited information about fungal viruses compared to those of plants and animals. Mycovirus harboring is even less explored in the Mucoromycota than in other fungal groups. Therefore, the aim of this research was the isolation and characterization of the detected dsRNA fragments in *Umbelopsis* and *Mucor* isolates, as well as to determine the sequence of the detected dsRNA fragments. We would also like to detect virus-like particles purified from the virus harboring strains. We have detected dsRNA fragments in some *Umbelopsis* and *Mucor* isolates and we have started the molecular analysis and the identification of the detected dsRNA fragments of *Umbelopsis ramanniana* SZMC 11078 and *Mucor mucedo* SZMC 12034 strains. In *U. ramanniana* SZMC 11078, four discrete fragments of approximately 5.3, 5.0, 3.0 and 2.8 kb, respectively could be observed while *M. mucedo* SZMC 12034 contained one clear fragment of approximately 6.1 kb. The sequence analysis revealed that the detected viruses from both isolates may have at least two open reading frames (ORFs). One of the ORFs encodes a putative RNA-dependent RNA polymerase (RdRp) and the other ORF encodes a putative capsid protein (CP). Results of the sequence analysis suggest that the detected mycoviruses of the *Umbelopsis* and *Mucor* isolates belong to the Totiviridae family. This is also supported by the observation of conserved domains characteristic to the Totividae family in the putative amino acid sequences as well.

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ENVIRONMENTALLY TRANSMITTED OPPORTUNISTIC BACTERIA DURING PRODUCTION AND COLD STORAGE OF FISH

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Aquaculture is a dynamically growing agro-sector in Hungary with priorities defined by the National Aquaculture Strategy. The primary goals determined by this Strategy can be reached when the productivity and safety of fish farming, processing and storage are ensured. The microbial quality of fish is greatly affected by the indigenous microbiota of the aquaculture and presumably have an influence on the quality and the shelf life of fish product. Therefore, the aim of this research was to examine the microbial community of a chosen fish farm and to follow the changes in the bacterial condition of fish during processing and storage. Traditional microbial methods were used for enumeration and cultivation of environmental microorganisms using selective and differential media. Common carp (*Cyprinus carpio*), one of the key species of the Hungarian fish production, was chosen for 4 days long cold storage (on 2 - 4°C and 6 - 8°C) and was periodically examined by standardized microbial and molecular methods. Bacterial strains isolated from different (environmental and fish) samples were identified on the basis of 16S rDNA sequencing, community profiles of the stored fish samples were determined by T-RFLP analysis. Based on our results, the examined aquatic environment and the mucus of farmed carp (with or without ulcer) were dominated by the *Aeromonas* genus. Fish arriving from fish pounds had a diverse microbial community of *Chryseobacterium*, *Aeromonas*, *Citrobacter* and *Pseudomonas* species. Cell counts dramatically decreased by the end of processing. During cold storage, temperature greatly affects the cell count and shelf life of fish filet, but T-RFLP patterns suggest that the diversity of microbial community does not change under different storage conditions. As key bacterial species of stored carp filet, the following bacteria were identified: *Pseudomonas psychrophila*, *P. fragi*, *Shewanella xiamenensis* and *S. putrefaciens*. With additional selective/differential cultivation, *Hafnia alvei* and *Kurthia zopfii* were isolated and identified as potential contributors in spoilage of carp filet. Our results clarified that several uncommon, presumably environmentally transmitted bacteria are detectable during storage of common carp filet with a possible role in spoilage. The future investigations on these bacterial strains enable to find an effective solution for the prolonged storage of freshwater fish.

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FIRST INSTANCE OF A NEW CLASS OF SPLICEOSOMAL TWIN INTRON (STWINTRON) FROM *ASPERGILLUS NIDULANS*

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In the primary transcript of nuclear genes, coding sequences – exons - usually alternate with non-coding sequences - introns. The latter must be excised by the spliceosome, to join exons and thus create the mature mRNA ORF that translates into the functional peptide product. Ubiquitous intron splicing provides a means of post-transcriptional regulation by coupling alternative splicing with nonsense-mediated mRNA decay, a quite common feature in higher eukaryotes but broadly ignored in fungi. To study spliceosomal introns and their excision, we use fungal spliceosomal twin introns ("stwintrons") as model systems. Stwintrons are unconventional intervening sequences where a standard "internal" intron interrupts one of the canonical splicing motifs of an "external" intron, and that consequently, can only be removed by consecutive splicing reactions. Previously, we have characterized occasions where the internal intron interrupts either the donor- or the acceptor sequence of the external intron (i.e., the first and second classes of stwintrons, respectively). We have demonstrated that stwintrons can emerge by the appearance of a new intron within a pre-extant intron. Here we present a new class of stwintron in which the internal intron is nested within the

conserved sequence element around the lariat branch point A of the external intron (consensus sequence: 5'-DYURAY), more specifically, between the 4th and 5th nucleotides separating the branch point A5 from the almost absolutely conserved U3. This lariat branch point motif-interrupted stwintron ([L4,5]: 5'-GCUA|AU) occurs in the gene coding for a variant form of heat shock protein 12 in *Aspergillus nidulans*, but lacks from the orthologue gene in the related species *A. sydowii* and *A. calidoustus*, which instead carry a standard intron at the very same position as the internal intron of the *A. nidulans* stwintron. This new stwintron thus emerged recently by a different mechanism which involves "intronisation" of exonic sequences on either side of a pre-extant standard intron. Interestingly, excision of the external intron removes the only possible translational start codon from the transcript, implying that the RNA requires to retain the external intron to deliver a protein – a form of alternative splicing known as "intron retention". We show that the steady state levels of the RNA species from which only the internal intron is excised, increase considerably when *A. nidulans* biomass is transferred from a minimal growth medium without extra salt to one including 1 M NaCl, and graduately decreases back to its initial levels when the biomass is subsequently transferred back to a medium without added salt. In an example of genetic flexibility provided by intron sequences, the chance formation of the external intron has offered the fungus an opportunity to develop extra means of regulation of the variant HSP12 protein inextant in *A. sydowii* and *A. calidoustus*.

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IMPACT OF GRAPHENE-OXIDE NANOPARTICLES ON AEROBIC GRANULAR SLUDGE UNDER SHOCK LOADING

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Graphene-oxide nanoparticles (GO NPs) are one of the most important graphene based nanomaterials which are widely used in the field of electronics, optics, energy conservation and storage technology due to its excellent electrochemical, optical, thermal properties both in pure and nanocomposite form. Effects of GO NPs on environment have been investigated extensively, because the special properties that make nanomaterials useful may also cause some nanomaterials to pose hazards to humans and the environment, under certain conditions. Widespread application of GO NPs results large volume wastewater production with relatively high GO NPs content. The effective wastewater treatment particularly depends on the viability of the various microbial communities presented in the wastewater. The aerobic granular sludge (AGS) wastewater treatment is a new technology where bacteria and fungi form aggregates that have many advantages over conventional activated sludge treatment methods. The granules provide protection and more efficient nutrient uptake for the microorganisms. To date, the effect of the shock loading of GO NPs on the reactor performance and microbial activities of AGS still remain unknown. Therefore, the major purpose of the present study is to assess the impact of the shock loading of GO NPs on the biological treatment processes in an AGS bioreactor, microbial activities and extracellular polymeric substances (EPS) production. During our experiment, synthetic wastewater was applied, where the carbon, nitrogen and phosphorus ratio were set to 100:10:1. The chemical oxygen demand (COD), ammonia (NH_3), nitrite (NO_2^-), nitrate (NO_3^-) and phosphate (PO_4^{3-}) were measured daily. The EPS and the mixed liquor suspended solids (MLSS) were measured weekly. Structure and development changes of granules were followed by

scanning electron microscope (SEM). After the granulation occurred, GO NPs was added to the system in different concentrations (5, 15, 25, 35, 45, 55, 65, 75, 85 and 95 mg/L). Based on our results, the formation of the granules caused a fivefold increase of sedimentation velocity compared to the initial value. Treatment efficiency of the system was 96% in case of 3.12 kg/m^{3}day COD loading. The exposure of 65 mg/L GO NPs resulted significant inhibition of ammonia oxidation: the ammonia concentration increased from 0.2 mg/L to 0.8 mg/L, while the nitrite and nitrate were remained stable (0.09 and 0.3 mg/L). At 95 mg/L GO NPs concentration the ammonia concentration was 4.7 mg/L and the sedimentation ability decreased. The content of polysaccharides (PS) remained stable (5.1 - 5.3 mg/g MLSS) at varying levels of GO NPs exposure. However, the GO NPs stimulated the secretion of protein (PN) 10 - 20.2 mg/g MLSS, respectively, in contrast with the control of 4.1 mg/g MLSS. Our observations suggest that the AGS treatment was achieved successfully with a high treatment efficiency rate. GO NPs stimulated the secretion of EPS, especially the proteins, which play a vital role in protecting from the inhibition of GO NPs. In addition, we demonstrated that the GO NPs over 65 mg/L concentrations have a negative impact on the efficiency of wastewater treatment.

FILOVIRUS IN HUNGARY – FIRST IDENTIFICATION AND CHARACTERIZATION OF LLOVIU VIRUS FROM BATS WITH HAEMORRHAGIC MANIFESTATIONS

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The discovery of Lloviu virus (LLOV) in *Miniopterus schreibersii* bats in Spain 2002 dramatically changed our understanding of the genetic diversity, geographic distribution, and host preference of filoviruses. However, the ecology of LLOV is largely unclear mainly due to the lack of reports following the first report of LLOV. In our study, we try to solve the open questions regarding LLOV genetics and ecology, based on a continuous screening of a selected *M. schreibersii* colony, proved to be positive for LLOV during a mortality event in 2016. We established a countrywide surveillance system in Hungary for the early detection of *M. schreibersii* dye-offs in 2012. Multiple events were examined during the past few years with viral metagenomic analyses and LLOV-specific TaqMan-based real-time PCR screening. In 2016, we detected LLOV virus in tissue samples of a *M. schreibersii* individual. Partial sequences of the nucleoprotein and the RNA-dependent RNA-polymerase gene suggests a close genetic relatedness with the original isolate in Spain. Following this event, several additional mortalities were registered to date with the same gross pathology of hemorrhagic symptoms, but no other positives were verified with PCR method, possibly because of the bad conditioned carcasses. In 2018, we started a monthly sampling activity, after the maternity period, in order to examine the seroprevalence, and other related factors of the virus in this cave. Here we present the current results of our survey programme, presenting the relation of the Hungarian isolate to the original Spanish virus. A major goal of our presentation is to call attention to this pathogen, possibly affecting the stability of *M. schreibersii* colonies across Europe, representing a

paramount concern for conservation biology. We discuss the possible factors leading to the dispersal of the virus in Europe and the possible transmission routes between bats to be examined in future studies and we also summarize current knowledge about the virus.

HEPATITIS E VIRUS IN COMMON VOLES (*MICROTUS ARVALIS*) FROM AN URBAN ENVIRONMENT, HUNGARY: DISCOVERY OF A CRICETIDAE-SPECIFIC GENOTYPE OF ORTHOHEPEVIRUS C

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Hepatitis E virus is a major causative agent of acute hepatitis worldwide. Despite its zoonotic potential, there is limited information about the natural chain of hepevirus infection in wildlife, and the potential reservoir species. In this study, we performed a HEV survey in an urban environment and investigated the prevalence of the virus among native rodent species. A total of 338 rodent samples were collected, belonging to five species (*Apodemus agrarius*, *Apodemus flavicollis*, *Apodemus sylvaticus*, *Microtus arvalis* and *Myodes glareolus*), in the city of Pécs (Hungary). Collected animals were screened for HEV by heminested RT-PCR, using gene-specific primer sets. HEV was detected in 3.2% (n = 11) of all investigated animals. Out of the examined five species, interestingly only Common voles (*M. arvalis*) found to be positive for the virus. Based on the phylogenetic analysis, our strain showed the closest homology with European strains detected in fecal samples of birds of prey and red fox, supporting the possibility of the dietary origin of these previously described strains. Furthermore, our samples showed close homology with a South American strain detected in *Necromys lasiurus* (Cricetidae), but separated clearly from other Muridae-associated strains, suggesting the presence of a Cricetidae-specific genotype in Europe and South-America. Furthermore, we hypothesize the reservoir role of *M. arvalis* rodents for the European Cricetidae-specific Orthohepevirus C genotype.

EFFECT OF BACTERIAL SIGNAL MOLECULES ON LIFE PROCESSES OF YEASTS AND FUNGI

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Microbial cross-kingdom interactions play a crucial role in food production. They are based on specific signal molecules like N-acyl homoserine lactones (AHL) for bacteria and farnesol for yeast species. This type of communication regulates virulence, resistance, attachment to surfaces and biofilm formation. It has been shown that these molecules can also be detected by eukaryotes. This might result in physiological and morphological adaptation of these communities and have a high impact on the quality and safety of foods. This work aimed to explore the effect of long-chain (C12) and short-chain (C4) AHL bacterial signal molecules on yeasts and fungi. Molecules were used in 1 μM concentration to investigate growth rates, morphological changes, AHL degrading capacity and biofilm formation of *Saccharomyces cerevisiae*, *Pichia membranifaciens* and *Kluyveromyces*

marxianus yeasts. Growth rate of *Penicillium brevicompactum* and *Penicillium expansum* strains was also tested. Morphological changes were detected by optical microscope and growth rates by measuring absorbance at different time intervals.
Biofilm biomass was stained with crystal violet and AHL degradation was tested with a biosensor-based system where β-galactosidase production was measured. Results showed growth inhibition with C4 treatment for *P. membranifaciens* and with C12 for *K. marxianus*. No changes were detected in the growth rate of *S. cerevisiae*. We detected inhibition of growth with both signal molecules only in *P. brevicompactum* after the 3rd day. *S. cerevisiae* showed potential C12-degrading capacities after 48 hours of incubation with the molecule. C12 inhibited biofilm formation of *K. marxianus* and enhancement of biofilm biomass was observed in case of *S. cerevisiae*. Our results indicate that bacterial signal molecules can effect life processes of yeasts and fungi tested. Therefore, ongoing investigations are needed to fully understand the mechanisms behind these changes.

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HYGIENIC STATUS OF HUNGARIAN IRRIGATION WATERS

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The aim of the NVKP_16-2016-0044 project is to follow the accumulation and utilization of trace elements trough water - soil, water - plant and plant - human nutriment connections. In 2017, irrigation waters were assessed by National Public Health Institute. One thousand Hungarian wells were investigated within the frameworks of the study. Among these 1,000 representatives were monitoring, agricultural-, drinking-water and other types of wells, too. The hygienic status of the well-waters was surveyed with the following bacterial parameters: heterotrophic plate counts at 22°C, coliforms, *E. coli*, *Enterococcus faecalis* and *Clostridium perfringens* based on standards MSZ EN ISO 6222:2000, MSZ EN ISO 9308-2:2014, MSZ EN ISO 7899-2:2000 and MSZ EN ISO 14189:2017, respectively. At the end of the study the complete data-series for 977 well water samples was available, although the processing of the data is not complete yet. Up to 200 samples have not shown any bacterial growth, and only the Petri dishes of the heterotrophic plate counts were positive in about half of the samples. Coliforms were found in approximately 25% of the wells; *E. coli* was present in 5%, *E. faecalis* in 15% and *C. perfringens* in 10% of the wells.
The bulk of the wells contained small amounts of pathogens (1 - 10 colony forming unit/100 ml), but in some cases the irrigation waters were like wastewater. In 24 cases 100 or more Enterococci were found and a minimum of 100 CFU *C. perfringens* were detected nine times from a 100 ml sample. More than 200 *E. coli* were revealed in 4 cases. As a general rule, drinking-water wells were hygienic, the agricultural and other wells proved to be the most contaminated. The next steps in the study are to analyze our data, compare them with the results of the water-chemical and microscopic biology investigations and discover the possible correlations.

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THE ROLE OF PH REGARDING ANTIBIOTIC SUSCEPTIBILITY OF URINARY TRACT PATHOGENS

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The severity of urinary tract infections (UTIs) depends on the virulence of the infecting bacteria and the antibacterial host defense. Antibiotics are applied during the treatment but there is a growing resistance to many drugs therefore the successful UTI therapy is complicated. Furthermore, it has been described previously that the pH of urine can have an impact on the activity of antibiotics. The main goal of our study was the investigation of the activity of antibiotics at pH 5, 6, 7 and 8 and the influence of gentamicin treatment on the relative expression levels of the antibiotic resistance and quorum-sensing activator genes at pH 5 - 8. The minimum inhibitory concentrations (MICs) of antibiotics (ampicillin, ciprofloxacin, gentamicin and erythromicin) were determined by two-fold broth microdilution method on reference and uropathogenic clinical bacterial strains (*Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Enterococcus faecalis*) over the range of pH 5 - 8. The relative gene expression levels of the multiple antibiotic resistance gene *marR* and the quorum-sensing activator gene *sdiA* were determined by real-time quantitative reverse transcriptase polymerase chain reaction (RT-qPCR). The gene expression levels were determined at pH 5 - 8 in the presence and absence of gentamicin on *E. coli* strains which were cultured at pH 5 - 8 and total RNA was isolated at 8 hour. The activity of erythromycin, ciprofloxacin and gentamicin were more effective at alkaline environment, furthermore ampicillin showed less activity at acidic pH on the tested strains. The pH 6 and gentamicin treatment initiated a significant stress response in the bacterium, for this reason the *marR* and *sdiA* genes were upregulated compared to the other pHs after 8 h of exposure. In contrast the *marR* and *sdiA* genes were downregulated at pH 8 and in the absence of gentamicin. These results suggested that the activity of antimicrobial drugs is pH dependent. The pH of urine can be influenced by nutritions in order to increase the efficacy of antibiotics. It can be concluded that *E. coli* at the pH 6 is more susceptible to antimicrobial agents leading to the over-expression of *marR* antibiotic resistance and *sdiA* quorum-sensing activator genes.

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SURVEY OF PHYTOHORMONE PRODUCTION OF *ARTHROBACTER* STRAINS IN ABIOTIC STRESS ENVIRONMENT

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Plant growth promoting rhizobacteria (PGPR) may improve quantity and quality of crops even under abiotic stress, like enhanced soil salinity, drought and heavy metal toxicity. Under harsh environmental conditions PGPR improve plant growth, for example by producing exopolysaccharides, phytohormones, 1-aminocyclopropane-1-carboxilate (ACC) deaminase, siderophores and osmolytes as reported by many studies. The *Arthrobacter* genus is described as halotolerant and psychrotolerant by several scientific reports. These bacteria can produce indole-3-acetic acid (IAA) plant hormone, ACC-deaminase and glycine betaine. Based on these features some *Arthrobacter* species may enhance crop plant growth on deteriorated soils. By producing IAA, these bacterial species enhance root elongation. ACC-deaminase reduces the stress ethylene level that inhibits growth of roots and shoots. Consequently, the presence of these bacterial species in the

rhizosphere can alleviate the effects of several abiotic stressors in plants. The aim of present study was to determine the ability of nine *Arthrobacter* strains for the production of potential plant growth promoting substances like IAA, ACC-deaminase, furthermore, to select the effective strain that preserve IAA production under abiotic stress conditions (osmotic, acidic, alcaline and cold stress). PGP profiles of the strains have been compiled through several methods. K, P and Zn uptake and siderophore production has been determined.
The ACC-deaminase activity was monitored by spectrophotometry. The phytohormone production was tested with thin layer chromatography. *A. crystallopoietes* (NCAIMB001424) was selected as the most effective strain and its IAA production was detected with thin layer chromatography at different temperatures (10°C; 20°C; 30°C), salinity (1%; 4%; 8%) and pH (4; 7; 10), respectively, on modified M9 medium with 200 µl/ml additional tryptophan (IAA precursor). Viability of the bacterial cultures was evaluated at each condition based on the optical density curve. From the obtained PGP profiles the *A. crystallopoietes* (NCAIMB001424) strain has shown outstanding results at phytohormone production under stress conditions. Tolerance of this bacterium for drought and long-term starvation has been verified. This strain is a component of the Biofil Normal® and Biofil Acidic® soil inoculants. This study supports that *A. crystallopoietes* (NCAIMB001424) strain is adequate for the inoculation of even droughty and saline soils.

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IDENTIFICATION AND VISUALIZATION OF ENDOPHYTIC FUNGI IN ABOVEGROUND TISSUES OF *VITIS VINIFERA* CV FURMINT ON DIFFERENT VINEYARDS IN HUNGARY

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In both managed and natural ecosystems, plants live together with a wide spectrum of endophytic fungi, which colonize plant tissues during some period of their life cycle yet cause no visible symptoms of tissue damage to their hosts. Varieties of grapevines (*Vitis vinifera*), the extensively grown, economically important crop, are also reservoirs of communities of fungal endophytes, which may have effect on its growing, resistance, health status and grape production. Although there is an increasing number of studies focusing on grapevine microbiome, our information is still limited on the communities of some cultivars, on their potential role. The visualization of the microbes in the plant tissues is also not a routine task. Our aims were to detect, identify and visualize fungal endophytes in above ground tissues of the *V. vinifera* cv. Furmint, a white grapevine variety on distinct vineyards. The Furmint is one of the most noted variety in the Tokaj, but also has plantations in other Hungarian wine regions as well. Samples were taken from four different vineyards from Tokaj and two sites from Eger wine region. Plants were sampled on different phonological stages, at the flowering and pea size berry stage, both young and matured leaves, flowers, and bunch of grapes comprising also pedicels and rachis were collected and set aside for investigation. For isolation of fungi, small parts of the different tissues were surface sterilized, placed on PDA media and hyphae growing out were transferred to separate plates. The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA was amplified and sequenced for identification of the isolates. For specific staining and visualization of fungal endophytes, fluorescence labelled lectin Wheat Germ Agglutinin

(WGA)-AlexaFluor488 conjugate was applied after clearing the plant tissues. Here we present the first information on fungal endophytes of Furmint grapevine in Hungary. More than 140 strains were isolated from different parts of Furmint vines. *Alternaria alternata*, *Aureobasidium pullulans* and *Botrytis cinerea* were found as frequent and dominant endophytes. We successfully applied the fluorescence visualization method of fungi and could prove the in planta fungal colonization.

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ANTIMICROBIAL EFFECT OF THE COMMONLY USED MUCOLYTIC AGENT, AMBROXOL

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Ambroxol (Ax) is a commonly used mucolytic agent in the health service. It is frequently prescribed by family doctors and recommended by pharmacists. In Hungary it was the second most frequently sold mucolytic with 603,779 packs in 2016. The mechanism of its action is to elevate the lysosomal function in the lungs and stimulate the synthesis and release of surfactant by type II pneumocytes, thus decreasing the viscosity of the mucus and facilitate expectoration. *Chlamydia pneumoniae* belonging to the Chlamydiaceae family is an obligate intracellular Gram-negative bacterium. It is a common cause of sinusitis, bronchitis, exacerbation of chronic bronchitis, pharyngitis and community-acquired pneumonia. *C. pneumoniae* is responsible approximately for 10% of the pneumonia cases. In an earlier study, we proved that Ax at 0.05 mg/ml concentration decreased the *C. pneumoniae* replication *in vitro* in association with elevated indoleamine 2,3 dioxygenase type 2(IDO) isoenzyme expression. We found that Ax at a dose equivalent to that used in humans does not alter the replication of *C. pneumoniae in vivo*. In this study our aim was to examine whether an increased concentration of Ax might influence the growth of *C. pneumoniae in vivo* and to investigate the effect of Ax on other human respiratory pathogens *in vitro*. To investigate the effect of Ax on chlamydial growth in *in vitro* conditions, permissive cells were infected with Ax-pretreated *C. pneumoniae*. Expression of genes with immunomodulating role was estimated by qPCR. In our *in vivo* experiment Balb/c mice were infected with *C. pneumoniae* and treated higher dose (4 times) of Ax than the commonly used human equivalent dose. The recoverable chlamydia inclusions from the mouse lungs were detected by an indirect immunofluorescent assay. The seroconversion of mice was tested with ELISA test. The antimicrobial effect of Ax against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* was measured in microdilution test. *In vitro*, the Ax treatment of the *C. pneumoniae*-infected cells besides IDO1 and IDO2 increased the expression of IL-6 and IFN-γ after 48-hour treatment. In the animal experiment the Ax treatment at a dose 4 times higher than the human equivalent one resulted in significantly lower (2.3-times) *Chlamydia* load in the mouse lungs, moreover the Ax accelerated the adaptive immune system response. In the control group 60% of mice whereas 100% of mice had chlamydia specific IgM in the Ax-treated mice had. The minimal inhibitory concentration of Ax was rather high, 0.8 mg/ml against *S. aureus* and *K. pneumoniae* but the Ax did not show any antimicrobial effect against *P. aeruginosa*. According to our results the higher dose of Ax treatment had beneficial effect during *C. pneumoniae* infection in mice but did not show significant direct antimicrobial activity against other extracellular pathogens.

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EFFECT OF CUII IONS ON ITACONIC ACID PRODUCTION BY *ASPERGILLUS TERREUS*

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Itaconic acid (IA), or 2-methylenesuccinic acid is commercially produced by the filamentous fungus, *Aspergillus terreus* using large-scale submerged fermentations. Polymerized esters of IA are frequently used during manufacturing of plastics and coatings. A few years ago, it has been described that, increased manganese(II) ion levels decrease IA yield, and simultaneously increase the fungal biomass. During citric acid production by *Aspergillus niger*; a process technologically and biochemically very similar process to IA fermentations, increasing the number Cu(II) ions up to a thousand times more than the number of the Mn(II) ions, antagonised their harmful effects. However it has been only described up to 14 µg/L Mn(II) ion concentration. The aim of this study was to analyze IA production of *A. terreus* at different manganese(II)- and copper(II) ion concentrations. Shake flasks cultivations were performed by employing *A. terreus* NRRL1960, a high IA producer strain. Cu(II) ion concentrations were accurately set between 400 mg/L and almost zero, Mn(II) concentrations were set between 300 µg/L and almost zero. The copper and manganese ions were monitored by ICP-QMS. The concentration of the D-glucose, and the itaconic acid were determined in the production medium by HPLC-RI. Increasing the Cu(II) ion concentration above 300 mg/L in the fermentation medium has suppressed the negative effects of Mn(II) ions, even as high concentration as 300 µg/L. At low manganese concentrations, like 10 - 25 µg/L, 50 mg/L copper concentration had satisfactory efficiency. The manganese limited cultures were barely viable at 250 mg/L and even higher copper concentrations, while cultures with additional manganese had shorter fermentation time, and average biomass. Increasing the Cu(II) ion concentration in the fermentation medium is a simple way to earn higher IA yields. This method could be useful in larger scale industrial fermentations, where leaching of manganese ions from metal parts of the vessel may be significant. The present study revealed that, not only the total limitation of manganese ions leads to high yield IA fermentation, but also the carefully balanced concentrations of Cu(II)- and Mn(II) ions.

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BUILDING UP A LAB STRAIN-COLLECTION FROM EXOTIC ANIMALS AND SCREENING IT FOR BIOTECHNOLOGICAL APPLICATIONS

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The exotic animals that live in zoo have a gut microflora consisting of bacteria received from their mother at birth and the other part obtained from their environment later. A part of the parental microbiota might be species specific. Since the microbiome of exotic animals has not yet been

thoroughly investigated, the aim of our research was the taxonomic study of the dominant lactic acid bacteria of the faces of selected exotic animals. We chose the lactic acid bacteria (LAB) as the focus of our study, as they are used in food preservation, as probiotics, and also some strains have special features like inhibition of mold's growth or mycotoxin binding abilities. LAB strains were isolated from the faces of exotic animals, preserved by deep freezing and a LAB-strain collection was formed. For the taxonomic identification of the almost thousand strains classical and molecular microbiological methods were done. For molecular taxonomy repetitive PCR method followed by agarose-gel electrophoresis was applied, and the strains were grouped based on their patterns. Representatives of each group were analyzed by 16S rDNA sequencing. In our screening process of the LAB-strain collection we focused on strains with pro- or synbiotic potential. In order to do this, known prebiotics (manno-, xylo- and fructo-oligosaccharides) utilization of LAB strains were studied. Several *Lactobacillus* species produce small molecular weight metabolites that inhibit the growth and toxin production of molds. Molds are major contaminants of forage plants like maize, wheat, or rice. Among them *Aspergillus flavus* is the most undesirable mold species, as many of their strains are able to produce aflatoxins, which is a very dangerous and harmful mycotoxin. One of the aims of our research was to study the antifungal activity of our LAB strains against *A. flavus* strains in order to select LAB for controlling molds during silage process. During this study, four *A. flavus* strains with high aflatoxin producing activity and near 100 LAB strains were used. In the experiments, modified agar-diffusion technique was applied for the co-cultivation of LAB and mold strains and the resulting inhibition zones were measured. When dealing with already contaminated substances, specific bacteria might be the solution with toxin binding abilities.
LAB strains from our collection were cultured in MRS broth in the presence of 1.0 ppm aflatoxin B1 (AFB1) or sterigmatocystin (ST). Both supernatant and biomass were analyzed separately by HPLC analytical method for the detection of AFB1 or ST concentrations. Based on our studies, the majority of our strains from our lactic acid bacteria collection can bind 30 - 50% of the ST and 5 - 20% of the AFB1 present in the sample. The most promising strains were *Lactobacillus plantarum* AT51, *Lactobacillus pentosus* TV45 and *Lactobacillus salivarius* SK45.

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IMMOBILIZATION OF *MUCOR CORTICOLUS* LIPASE TO A HYDROPHOBIC SUPPORT INCREASES THE TEMPERATURE OPTIMUM OF THE ACTIVITY

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Immobilization of enzymes has many advantages; besides allows their reusability in several reaction cycles and facilitates the separation of the catalyst from the product, it can enhance the enzyme stability under harsh reaction conditions (high temperature, presence of organic solvents etc.). These circumstances could alter the protein structure decreasing the enzyme activity. Industry has high demand for immobilized enzyme preparations since their use can reduce the cost of the biocatalyzation process. Among the techniques applied nowadays (entrapment, crosslinking, etc.), one of the most efficient method to immobilize lipase enzymes is the adsorption onto hydrophobic matrices, such as to the Accurel MP-1000 (particle size <1,500 μm) polypropylene porous carrier. In our current work, the previously purified *Mucor corticolus* (SZMC 12031) extracellular lipase was

immobilized on Accurel MP-1000 support. The adsorption was monitored by measuring the residual hydrolytic activity in the supernatant, then immobilization efficiency (IE, %) and activity yield (AY, %) were calculated. Time course of the adsorption showed very fast initial loading of the lipase onto the support. IE proved to be above 90% after 6 hours of incubation, indicating high affinity of the lipase to interact with the hydrophobic surface. The enzyme-support preparation showed 0.14 U/mg of support specific activities for pNP-palmitate hydrolysis.
In reusability studies, the immobilized lipase was stable up to 3 cycles. Besides, improved storage stability for the enzyme-carrier complex was identified at 5°C. Examination of thermal optimum and stability revealed a significant increase in the activity of enzyme-support complex at elevated temperatures. Optimal temperature for the hydrolysis of pNPP substrate proved to be 60 - 70ºC compared to the free enzyme which showed highest activity at 30ºC. The effect of organic solvents with different LogPow values, such as ethanol, methanol, butanol, heptane, hexane etc. on the activity of the immobilized enzyme preparation was also investigated.

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THE EFFECT OF CONJUGATE VACCINES ON THE SEROTYPES OF CARRIED VERSUS CLINICAL PNEUMOCOCCAL ISOLATES, COLLECTED IN THE SAME TIME PERIOD

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The conjugate vaccine PCV-13 against *Streptococcus pneumoniae* (pneumococcus) became obligatory in Hungary in July 2014, replacing its predecessor, PCV-7. The PCVs mean a huge selection pressure on pneumococci, resulting in a massive serotype rearrangement. The vaccine types (VTs, i.e. the 7/13 types found in the vaccines) are vanishing and the vacated niche is filled by newly emerging serotypes. In the current project, we wanted to compare how clinical versus carried pneumococci respond to vaccine pressure. 522 healthy children aged 1 - 7 years were screened between March 2015 and May 2016, resulting 206 carried isolates (carriage rate 39.1%). In the same time period, 146 clinical isolates were also obtained from Semmelweis University, mainly from mucosal infections. For a more direct comparison, patients were divided into two age groups: <7 years (P1) and >7 years (P2). No data on the vaccination status of the patients were available, but members of P2 probably had low-level vaccination rate, unlike the other two groups. Serotypes, antibiotic susceptibility and clonality of the isolates was determined and compared in the three groups (carriers, P1 and P2). The leading serotypes were 15B, 11A, 24F among the carried isolates; 23B, 15B, 24F among younger patients and 3, 11A, 15A among older patients. The PCV-13 serotype coverage was 5.8%, 17.5% and 32.6%, respectively. Whereas serotype 3 was still leading in P2, it (together with 6A) completely disappeared from carriage. Serotype 19A, a famously resistant and invasive type had emerged markedly after the introduction of PCV-7 all over the world, including Hungary, but now we could observe its significant decrease in all groups. Full susceptibility was observed only to imipenem and vancomycin, in all other cases the clinical isolates were more resistant than carried ones. The following resistance rates were observed among carriers, in P1 and in P2, respectively: levofloxacin: 0% - 4.8% - 8.4%; erythromycin: 17.5% - 19.0% - 28.9%; cefotaxime: 0% - 0% - 4.8%; and finally penicillinI: 13.1% - 17.5% - 25.3% (we found no penicillin resistant isolates). Regarding clonality, serotype 3 showed the lowest level of diversity, in contrast with

serotype 19A. Of note, we found one 19A and one 19F isolate belonging to the world-wide prevalent resistant clone ST320. In conclusion, conjugate vaccines have a remarkable effect on reducing the prevalence of vaccine serotypes. The serotype rearrangement appears immediately among the carriers (start points of the infection chain), but only with a delay among the clinical isolates. Fortunately, the replacing serotypes are less invasive and less resistant. However, continuous monitoring of the changes is still necessary. New 15-valent and 20-valent conjugated vaccines are under development, which were designed to cover some of the emerging serotypes, such as 11A or 15B.

ROOT COLONIZING NONPATHOGENIC FUNGI – DIVERSITY AND FUNCTION

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The vast majority of the plants form mutualistic symbioses across their roots with different nonpathogenic fungi. These mycorrhizal and endophytic fungi play a crucial role in ecosystem functioning. When abiotic stress is strong, like in arid environments, the importance of mutualistic plant-fungal interactions increase. Although, the effects of these symbioses on the drought tolerance of plants have been studied, a mycocentric approach is still needed when mycorrhizae and endophytic fungi of arid environments are studied. In the last fifteen years, the main aim of our studies presented in the lecture was to gain data about the key players of the root colonizing fungal communities, about their diversity, possible functions and the different methods of the research. Ectomycorrhizal (EM), arbuscular mycorrhizal (AM), dark septate endophytic (DSE) fungi and desert truffles were studied applying diverse methods: e.g. isolation of fungi, *in vitro* experiments, enzyme assays, molecular screening (direct sequencing, clone libraries, NGS), in planta diversity and visualization, genome sequencing and comparative genomics. Taxonomic revisions were also carried out if needed. Beside revealing the compositional diversity of certain fungal communities and identification of common generalist lineages of those root colonizers, we formally described 10 new genera and 27 new species. We developed, improved and/or adopted several 'wet-lab' methods, in some cases first time in the study of those fungi and analyses technique for our research. Our results also showed the roots colonizing fungal communities were functionally diverse and that diversity might had a crucial importance in the role of those fungi in the ecosystems.

THE EFFECT OF NIKKOMYCIN Z IN COMBINATION WITH ECHINOCANDINS AGAINST *CANDIDA ALBICANS* AND *CANDIDA PARAPSILOSIS* BIOFILMS

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Echinocandins may be potential candidates for anti-biofilm alternative therapies such as antifungal lock therapy; however, treatment may fail especially against *C. parapsilosis* biofilms due to the previously well-documented intrinsic fks mutation. Nikkomycin Z is a nucleoside-peptide, which inhibits chitin synthesis by acting as competitive analogue of the chitin synthase substrate UDP–N-

acetylglucosamine. Previously *in vitro* synergistic interactions have been already described with various antifungals including echinocandins against *Candida* species; however, these studies focused on exclusively planktonic forms. Potential synergy was examined between two echinocandins (caspofungin and micafungin) and the chitin synthesis inhibitor nikkomycin Z against *Candida albicans* and *C. parapsilosis* biofilms including an echinocandin-resistant *C. albicans* strain. Antifungal susceptibility was evaluated using the XTT colorimetric assay-based chequerboard microdilution method, while the nature of interactions was assessed by calculating fractional inhibitory concentration indices (FICI) and using the Bliss independence model. For *C. albicans* isolates, nikkomycin Z caused 2 - 16-fold and 16 - 128-fold decrease in median sessile MICs (minimum inhibitory concentration) for caspofungin and micafungin, respectively. Regarding the echinocandin-resistant *C. albicans* strain, 64-fold and 4-fold sessile MIC decrease was observed for caspofungin and micafungin, respectively. For *C. parapsilosis* biofilms, the median sessile MICs observed for caspofungin and micafungin in combination with nikkomycin Z showed 2 - 4-fold and 2 - 64-fold decrease for caspofungin and micafungin, respectively. Based on FICI values, micafungin showed higher synergism combined with nikkomycin Z against biofilms compared to caspofungin/nikkomycin Z combination, which was also confirmed by the Bliss independence model. The results obtained by statistical interaction analyses correlated well with the fluorescent LIVE/DEAD viability assay. LIVE/DEAD viability staining revealed that micafungin-exposed *C. albicans* and *C. parapsilosis* biofilms exhibited increased cell death in the presence of nikkomycin Z compared to untreated biofilms, micafungin-exposed sessile populations or nikkomycin Z treated biofilms. Caspofungin showed highly similar pattern to micafungin both alone and in combination with nikkomycin Z. The present study is the first analysis evaluating the interaction of echinocandins and nikkomycin Z against *Candida* biofilms.

We have shown that nikkomycin Z is capable of causing a striking synergy in combination with caspofungin or micafungin against *C. albicans* and *C. parapsilosis* biofilms. This synergy was more pronounced for micafungin combined with nikkomycin Z. It is highlighted that synergistic interaction was observed not only for echinocandin-susceptible strains but also for the echinocandin-resistant *C. albicans* isolate. Our results support the simultaneous use of nikkomycin Z and caspofungin or micafungin in alternative therapies as antifungal lock therapy.

IN VIVO APPLICATION OF *NEOSARTORYA FISCHERI* ANTIFUNGAL PROTEIN 2 (NFAP2)

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The emerging number of drug-resistant opportunistic human pathogenic fungal strains urges the development of new antifungal agents differing in mode of action from the clinically applied conventional drugs. Based on recent findings, the small, cysteine-rich and cationic antifungal proteins from filamentous ascomycetes (AFPs) are considered as safely applicable compounds to fight against mycoses caused by filamentous species. However, there is no information about their *in vivo*

applicability against *Candida* isolates. The *Neosartorya fischeri* antifungal protein 2 (NFAP2) represents a unique member of AFPs as it shows high *in vitro* antifungal activity against clinically relevant *Candia* species. In the present work we evaluated its antifungal potency in the treatment of vulvovaginal candidiasis (VVC). In the CLSI-M27A3 susceptibility test method the minimal inhibitory concentrations (MICs) of NFAP2 proved to be 800 µg/ml for both the planktonic, and sessile biofilm cells of a fluconazole (FLC)-resistant *Candida albicans* strain isolated from human VVC. Fluorescence-activated cell sorting analysis indicated death of the *Candida* cells by pore formation, which was visualized with scanning electron microscopy. Electronic circular dichroism spectroscopy indicated that structural change of NFAP2 is not required for its pore formation activity on *Candida* plasma membrane. *In vitro* cytotoxicity tests excluded any toxic effects of NFAP2 on primary human keratinocytes and dermal fibroblasts even when applied at twice the concentration of the MIC. NFAP2 could significantly reduce the number of FLC-resistant *C. albicans* cells *in vivo* in a murine vulvovaginitis model compared to FLC, and its combination with FLC enhanced the antifungal activity. Histological examinations showed neither morphological alterations, nor pathological reactions of the vagina and vulva after NFAP2 treatment. Results suggest that NFAP2 is a potential mono- or polytherapeutic candidate to overcome drug resistance of *Candia* species.

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EFFECT OF AGRICULTURAL PESTICIDES ON A *BACILLUS VELEZENSIS* STRAIN WITH BIOCONTROL POTENTIAL

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There is an emerging need for new, environment-friendly approaches of plant protection in order to achieve the control of plant diseases with a minimized negative impact of synthetic pesticides. Integrated Pest Management (IPM) is an ecologically friendly agricultural approach, which combines biological, chemical and physical means of control, resistant crop cultivars and modification of cultural practices in order to realize a stable, long-term protection and prevent or reduce pest damage. The application of biological control has a word-wide increasing importance for the reduction of plant diseases. In IPM strategies there is a preference for pesticide-tolerant biocontrol agents, as such strains can be applied in combination with lowered amounts of pesticides, or after pesticide treatments. Biocontrol agents are environmentally friendly and can remarkably control plant diseases, however, they may be sensitive to the different xenobiotic used in the agriculture. Data about the pesticide sensitivity of potential biocontrol agents reveal important information for planning their eventual combined application with particular compounds. In this study we examined the effects of widely used herbicides and fungicides on the growth of a *Bacillus velezensis* strain selected as a potential biocontrol agent. The presence of sulfonylurea herbicides, like bensulfuron-methyl, cinosulfuron, chlorsulfuron, ethoxysulfuron, triasulfuron and primisulfuron-methyl strongly inhibited the biomass production of the strain at the concentration of 6.25 mg/L. Contact fungicides like captan, maneb, mancozeb and thiram resulted in total inhibition at the concentration as low as 6.25 mg/L, while the demethylation inhibitors (DMI fungicides) fenarimol, imazalil, penconazole and tebuconazole showed inhibition in a concentration dependent manner.

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INVESTIGATION OF THE PHYTOHORMONE PRODUCTING CAPACITY OF EFFECTIVE PLANT GROWTH-ENHANCING ABIOTIC STRESSTOLERANT RIZOBACTERIA FROM DETERIORATED SOILS

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Diminishing water resources, extreme temperature fluctuations and environmental pollution became the main obstacles of effective intensive agricultural production to date. The various abiotic stress effects - extreme temperatures, soil salinity, heavy metal pollution, drought and flood - influence the yield and quality of crops and drastically reduce the area of arable land. Sustainable agricultural technology solutions are therefore necessary in the production in order to increase the yield and abiotic stress tolerance of crop plants paralleled with reduced usage of fertilizers and pesticides. Microbial soil inoculation is an opportunity to improve the fertility of plants. Several studies have proven that rhizosphere colonizing microorganisms can provide tolerance against abiotic stress by the production of exopolysaccharides (EPS), phytohormones, volatile organic compounds (VOC), osmolytes, accumulation of antioxidants and regulation of stress-responsive genes. Auxins, gibberellins, and other phytohormones produced by plant growth promoting rhizobacteria (PGPR) induce cell proliferation and elongation and therefore increase the root surface, resulting in increased absorption of nutrients and water, thereby increasing tolerance of crops during abiotic stress. PGPR isolated from extreme environmental conditions may possess stress tolerance capacity and therefore are potential candidates as microbial soil inoculation components for deteriorated soils. The aim of presented work was the selection and test of osmotolerant, salt and presumably drought-tolerant rhizobacteria which, by phytohormones producing ability could promote stress tolerance of crop plants grown on deteriorated soils. The obtained experimental results have shown that approximately 10 PGPR species were able to produce higher levels of phytohormones.
The tested *Kocuria* and *Azospirillum* strains produce indole-3-acetic acid and gibberellic acid effectively, however they grow slower under unfavorable conditions. Some outstanding gibberellic acid producer *Pseudomonas* strains grow well in cold and acidic environment, but are sensitive to high salt content. Based on the results, the best cold and osmotolerant PGPR strains, that grow well under acidic or alkaline conditions belong to the *Arthrobacter* genus. These bacteria represent promising candidates for a soil inoculate used on deteriorated soils because of the efficient indole-3-acetic acid production under stress conditions.

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LACTOBACILLUS SALIVARIUS CAN AFFECT AFLATOXIN PRODUCTION OF *ASPERGILLUS FLAVUS* AT DIFFERENT TEMPERATURES

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Aflatoxins are the most toxic and carcinogenic of the mycotoxins that contaminate agricultural products. Aflatoxin (AFB1) is produced by *Aspergillus flavus* growing in feedstuffs. Laboratory-scale silos were prepared to evaluate the efficacy of lactic acid bacteria (*Lactobacillus salivarius*) on the fermentation quality and mycobiota of corn silage. Their influence on *Aspergillus flavus* toxin production and the expression of the aflatoxin synthesis related genes was studied by using the HPLC and qRT-PCR techniques. Silage inoculated with *Lactobacillus salivarius* was incubated at 20°C, 30°C, and 37°C. Samples were prepared and analyzed at different time points (7, 12, 16, and 21 days of the experiment). During silage production, temperature and oxygen availability are most likely to change. Using a silage model, the development of these parameters was examined for the AFB1 production capacity of *A. flavus*. In parallel, some of the important aflatoxin biosynthesis related genes' mRNA expressions were studied at different temperatures and *Lactobacillus salivarius* supplementation. The amount of AFB1 detected in *A. flavus* samples increased significantly during the 30°C incubation. At 20°C incubation, a significant AFB1 level decrease was measured. The AFB1 production of *A. flavus* incubated at 37°C did not change significantly. Incubation in a closed vessel and Lactobacillus salivarius supplementation significantly reduced the toxin production. The strongest effect on the AFB1 production was found for the *Lactobacillus salivarius* supplementation. Among the examined genes, the mRNA expression of *aflU* (*cypA*) and *aflM* (*ver-1*) genes decreased with decreasing AFB1 concentration at 20°C. The AFB1 production significantly increased at the 30°C incubation and also *aflU* (*cypA*) and *aflQ* (*ordA*) mRNA expressions increased, while *Lactobacillus salivarius* supplementation reduced AFB1 production parallel decreasing expression of both genes' mRNA levels. The AFB1 production of the *A. flavus* incubated at 37°C did not change significantly, however, *Lactobacillus salivarius* supplementation reduced AFB1 production while simultaneously decreasing the expression of *aflR*, *aflP* (*omtA*), and *aflM* (*ver-1*) at mRNA levels.

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LONG TERM SEROLOGICAL SURVEY OF TWO HUMAN PATHOGEN HANTAVIRUS INFECTION AMONG RODENTS IN HUNGARY

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Worldwide, hantaviruses are emerging zoonotic infectious agents. The hosts of these viruses are rodents, soricomorphs and bats. Two human pathogenic hantaviruses are circulating in Hungary, Dobrava and Puumala which are carried by mice and voles, respectively. In Hungarian forests, the dominant rodent species are yellow necked mouse (*Apodemus flavicollis*), striped field mouse (*Apodemus agrarius*), wood mouse (*Apodemus sylvaticus*) and bank vole (*Myodes glareolus*), which are natural reservoirs of different hantaviruses. The aim of the study was to survey the prevalence of hantaviruses among rodent populations and examine the potential correlation between population densities, years, genders and seroprevalence. The rodents were trapped at 13 sampling plots in a Forest Reserve in Mecsek Mountains, Hungary from March to October between 2011 and 2014.

Rodent sera were tested for antibodies against Dobrava- Belgrade (DOBV) and Puumala (PUUV) viruses by ELISA. During the 4-year sampling period, 2,491 animals were examined and 254 were positive for antibodies against hantaviruses (DOBV, PUUV). In 2011, the seroprevalence among *Apodemus* spp. was 17.25% (114/661), while it was 3.9% (3/77) among *Myodes glareolus* voles. By 2014, this rate was reversed, the seroprevalence among *Myodes* was 18.46% (12/65) and among *Apodemus* spp. it was 3.62% (13/359). Despite the fact that in 2012 there was a significantly larger number of *Apodemus* spp. compared to 2011, the seroprevalence was significantly lower. In 2012, the number of *Myodes* specimen was significantly increased. This ecological change had a delayed and long lasting effect on the prevalence of the PUUV.
This is a proof of the existence of a viral dynamics between different related hantaviruses. In the sampling area, the seroprevalence decreased to half despite of the higher number of specimen compared to the previous year of 2012. This result may indicate that the number of infected individuals and the size of the community are not directly proportional.

PHENOTYPIC CHARACTERIZATION OF *STARMERELLA BACILLARIS* (SYN. *CANDIDA ZEMPLININA*) FROM OENOLOGICAL ASPECTS

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Starmerella bacillaris (syn. *Candida zemplinina*) is a wine related yeast species, commonly found on grape. It is particularly associated with botrytized grapes and wines fermented from these grapes (e.g. Tokaj Aszú). *S. bacillaris* has recently attracted significant attention from oenologists because of some beneficial oenological properties. Our laboratory has an initiative and comprehensive activity in studying these properties under wine conditions. Since *S. bacillaris* coexists and competes with *Saccharomyces cerevisiae* and *Saccharomyces bayanus* var. *uvarum* strains in the spontaneous fermentation of special sweet wines, we focus on a comparative approach. This presentation aims to give an overview on the main findings obtained in natural or synthetic grape juices or solid media simulating wines. All the investigated *S. bacillaris* strains showed a clear fructophilic character – in contrast to the *Saccharomyces* wine yeasts. This property is useful in counterbalancing the sharply decreasing glucose-fructose ratio during grape juice fermentations by *Saccharomyces* strains. *S. bacillaris* was a significant glycerol producer (a positive trait) at any investigated media. We found the volatile acid production (a negative characteristics) moderate, comparable with *Saccharomyces cerevisiae*. Production of other organic acids were medium dependent. In accordance with the smaller biomass production, *S. bacillaris* has relatively low nitrogen demand. Ethanol production (up to 6 - 8 v/v%) and fermentation rate of the strains are moderate. Ethanol tolerance was considerable, although much lower than that of *S. cerevisiae*, with no active growth at 10 v/v% ethanol. It has been known that *S. bacillaris* is an osmotolerant and psychrotolerant species, which was confirmed by our detailed investigations. Biogenic amine production of the yeasts is an important safety issue in winemaking. In comparison with *Saccharomyces* species, *S. bacillaris* produced significantly more tyramine, but the other amines were produced at the same levels. A valuable antioxidant of white wines, tyrosol production of the yeast strains was also studied. *S. bacillaris* produced significantly less tyrosol. As a part of the grape juice microbiota, *S. bacillaris* has significant interactions with the other species. The competitiveness of this yeast with *S. cerevisiae* was studied in fermenting wines monitoring the populations in mixed cultures. At the beginning of fermentation *S. bacillaris* was able

to grow at the presence of *S. cerevisiae*, but its population sharply decreased at 20°C. At lower temperature (12°C) the survival of *S. bacillaris* strains was much better, being present at 10^5 - 10^6 populations at the end of fermentation. The main conclusion of this research is that its distinctive properties make *Starmerella bacillaris* a good candidate as starter culture, used in combination with *Saccharomyces* strains, not only in botrytized wines but in normal wines as well. The fructophilic character and the good sugar tolerance of this species are promising tools in managing sluggish or stuck fermentations.

NEW PEPTAIBOL COMPOUNDS FROM THE GENUS *TRICHODERMA*

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The genus *Trichoderma* is well known for the production of bioactive secondary metabolites, including peptaibols, which are short, linear peptides containing unusual amino acid residues and being synthesized via non-ribosomal peptide synthetases (NRPSs). Certain *Trichoderma* species cause serious losses in mushroom production worldwide. *Trichoderma aggressivum* and *T. pleuroti* are among the major causal agents of the green mold diseases affecting *Agaricus bisporus* and *Pleurotus ostreatus*, respectively. Other species, mainly from section Longibrachiatum of the genus may cause infections in immunosuppressed patients. Our working group established a comprehensive and comparative approach to reveal the structural diversity and bioactivities of *Trichoderma* peptaibols. After HPLC-ESI-MS investigation of the crude extracts from 2 species from clade Harzianum causing green mold diseases (*T. aggressivum* f. *europaeum* and *T. pleuroti*), 16 species from section Longibrachiatum (*T. aethiopicum*, *T. andidense*, *T. capillare*, *T. citrinoviride*, *T. effusum*, *T. flagellatum*, *T. ghanense*, *T. konilangbra*, *T. longibrachiatum*, *T. novae-zelandiae*, *T. pinnatum*, *T. parareesei*, *T. pseudokoningii*, *T. reesei*, *T. saturnisporum*, *T. sinensis*), as well as 2 species from clade Viride (*T. koningiopsis*, *T. gamsii*), several new and already known compounds could be identified from the groups of 18-, 19- and 20-residue peptaibols. The detected peptaibol compounds could be categorized into different groups based on their primary structure. Both similarities and differences could be observed between the examined producer strains. New peptaibol groups were also identified and named as tripleurins and koningiopsins. *In vitro* experiments revealed that peptaibols are potential growth inhibitors of mushroom mycelia, and that the host mushrooms may have an influence on the peptaibol profiles of green mold agents. Further bioactivity tests were carried out on mammalian cells and *Arabidopsis thaliana* seedlings.

Toxicity endpoints could be identified after biotest analysis against fetus feline lung cells and boar semen cells. The treatment with high concentrations of purified peptaibol extracts proved to be a deterrent for *A. thaliana* seedling growth, although, below a certain peptaibol level which was still toxic to mammalian cells, negative effects could not be detected on plants.

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GENOMICS APPROACHES IN THE INVESTIGATION AND UTILIZATION OF MICROALGAL COMMUNITIES

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Photosynthetic microalgae (both cyanobacteria and eukaryotic green algae) represent important component of multiple ecosystems including natural as well as human-constructed environments. Microalgae always live in interactions with other microorganisms in the communities. Various applications utilize the algae-based communities (biohydrogen evolution, wastewater treatment, biostimulant production among others). However, not much is known about the molecular background of the various algal interactions in these communities so far. We are investigating selected algal - bacterial associations at genomic and transcriptomic levels. Genomic data either on simple artificial and on complex natural algae-related ecosystems will be presented and discussed.

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

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Plant stubble and root residues have relevant effect on soil structure and nutrient availability. The large volume of crop plant litter without adequate decomposition may cause severe problems in soil management and can temporarily disrupt nutrient supply. Intact residues and non-degraded organic matter rather deteriorate than improve soil structure or water storage capacity, and may also hinder the agrotechnical operations. Also, the improperly comminuted stalks impair seeding quality and may serve as infection sources for plant pathogenic fungi. Each year about 8 - 10 million tons of corn stalk is created in Hungary, a large part of which is turned back into the soil, therefore the presence of microorganisms able to decompose cellulose, hemicellulose and lignin is relevant. Rapid decomposition of stalk residues provides nutrient supply for crops, improves soil microbial life and diminishes the chance of a pathogenic fungal epidemic next vegetation period. This process contribute to the emergence and maintenance of soil fertility. The corn stem is mainly built of lignocellulose, that is composed from cellulose, hemicelluloses and lignin. The cellulose chains are linked together to form microfibrils, which are bundled to form cellulose fibers. The long-chain cellulose polymers are linked together by hydrogen and van der Waals bonds, which cause the cellulose to be packed into microfibrils. Hemicelluloses and lignin cover the microfibrils. In tillage biomass (corn stalk, straw and grasses), hemicelluloses are composed mainly of xylan. The aim of presented research was the isolation and characterization of cellulose and xylan (NSP, non-starch polysaccharide) hydrolyzing microbes that accelerate crop residue degradation. Samples were taken from Tornyiszentmiklós - pseudogley brown forest soil - and Kiskunlacháza - meadow chernozem - in Hungary, from corn fields after harvest. From Kiskunlacháza, both organically fertilized (stable manure) and unfertilized areas were sampled. Eight different culture media were used for the isolation and purification of cultures. The isolates were screened for polysaccharidase enzyme

activity with Congo red staining. Thirty-two strains were polysaccharidase positive and these strains were identified with molecular biology methods. Twelve bacterial species representing 9 genera were isolated from pseudogley brown forest soil, whereas 20 species of 8 genera were found in meadow chernozem samples. Diversity in fertilized meadow chernozem was lower compared to unfertilized soil. The exact enzyme activity of the positive strains was measured by DNSA method.

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THE ORIGINS OF COMPLEX MULTICELLULARITY IN AGARICO- AND PEZIZOMYCOTINA

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The emergence of complex multicellularity (CM) raises many questions in evolutionary biology. Fungi are one of the five main lineages which can form complex multicellular structures: a three-dimensional organization with cell adhesion, communication and an integrated developmental program. Unlike other lineages, fungi spend most of their life-cycles as simple multicellular vegetative hyphae, therefore, their complex multicellular stages are manifested only in specific structures such as rhizomorphs, ectomycorrhizae, sclerotia or (a)sexual fruiting bodies (FB). The best-known FB-forming fungi belong to the Pezizomycotina (Ascomycota) and Agaricomycotina (Basidiomycota). However, complex multicellular structures also occur in the Mucoromycota, Taphrino-, Puccinio- and Ustilagomycotina. Unlike the other non-fungal lineages, CM evolved independently 8 - 12 times in fungi, which suggests a „noncanonical" convergence process and the importance of studying the origin of CM in fungi. Although several studies deal with the genetic background of FB formation in certain species, our knowledge about similarities or differences of FB formation among the Agarico- and Pezizomycotina is limited. Therefore, the aim of this project is to identify the shared and unique FB development related molecular mechanisms between the two phyla. For this reason, we analyzed developmental transcriptome data of five and four species from Asco- (*Fusarium graminearum*, *F. verticilloides*, *Sordaria macrospora*, *Neurospora crassa*, *Pyronema confluens*) and Basidiomycota (*Armillaria ostoyae*, *Coprinopsis cinerea*, *Hypsizygus marmoreus*, *Flammulina velutipes*), respectively. Published (7 spp.) and new (2 spp.) RNA-seq data comprised 2 - 13 stages, which was used to identify developmentally regulated (DevReg) genes: those that show at least four-fold change in expression between any two FB stages. In addition, a dataset containing 15 and 88 whole genomes were used for an OrthoFinder clustering- and a deltaBLAST- based phylostratigraphic analysis, respectively. The relative age of proteins, gene families and domains was determined using these approaches. In order to determine the phylum-specific and conserved molecular processes we determined the proportions of DevReg genes in different ages and performed domain enrichment analysis across the lineages. Depending on species, 17.5 - 66.3% of genes appear to be DevReg. Proteins of the nine species grouped into 40.084 gene families, from which 21.745 contained DevReg genes. From these, 163, 106 and 33 clusters were shared between the two phyla containing DevReg genes from 7, 8 and 9 species, respectively.
These conserved clusters harbor many fungal cell wall remodeling and membrane transport related proteins, suggesting the universality of these processes in FB development. In contrast to this, remarkable differences have been found between the two phyla in other gene families, such as targeted protein degradation in the Basidiomycota and a putative clr5-based chromatin remodeling in

the Ascomycota. The age distribution, number, and ratio of phylum-specific gene families suggest that the Basidiomycota uses more recent clusters during FB development than the Ascomycota. However, the age distribution of DevReg genes was even, which portrays a gradually expanding molecular toolkit of CM in fungi. Our findings might help to reveal the genetic toolkit and the evolutionary processes that lead to FB formation in Dikarya.

THE RELATIONSHIP BETWEEN ALTERNATIVE RESPIRATION AND STERIGMATOCYSTIN FORMATION IN FILAMENTOUS FUNGI *ASPERGILLUS NIDULANS*

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Alternative oxidase (AOX) (systematic name: ubiquinol:oxygen oxidoreductase, non-electrogenic; EC 1.10.3.11) as terminal oxidase of the alternative respiratory pathway occurs in many organisms, in plants and fungi but also in animals and protists. This cyanide-resistant terminal oxidase is located on the matrix side of the mitochondrial inner membrane and reduces oxygen to water. The alternative pathway moves fewer protons across the inner mitochondrial membrane to generate a proton motive force, and thus provides less ATP by oxidative phosphorylation. Aflatoxins (AT) belong to a large and diverse class of metabolites known as polyketides and are produced by a variety of fungi, mainly from the genus *Aspergillus*. These opportunistic fungi can contaminate cereal crops and other staple commodities before harvest or during storage, leading to famine in tropical countries and can cause acute hepatic failure in humans and animals. A less toxic fungal compound named sterigmatocystin (ST) is the penultimate intermediate in the biosynthesis of AT and produced by *Aspergillus nidulans*. However, the AT/ST biosynthetic pathway is well-characterized in this fungus, but many of the mechanisms by which environmental factors influence mycotoxin formation remain enigmatic. *A. nidulans* owns one gene for alternative oxidase named *aodA*. To investigate the relationship between this mitochondrial terminal oxidase and the formation of the mycotoxin sterigmatocystin, the encoding *aodA* gene was both deleted and overexpressed. Relative to the wild-type, the cyanide-resistant fraction of respiration in the late stationary stage - when sterigmatocystin production occurs - doubled in the overexpressing mutant carrying three *aodA* gene copies but decreased to 10% in the deletant. In contrast, ST yield in the *aodA* deletant was about half of that in the control when grown in the dark, while *aodA* overexpression resulted in up to 70% more ST formed, the yield increasing with alternative oxidase activity. Essentially identical results were obtained regardless whether the cultures were illuminated or protected from light. Data were quite different when cultures were illuminated: ST volumetric yields were significantly lower regardless the presence, absence or the copy number of *aodA*. We conclude that the copy number of *aodA* and, hence, the balance between alternative- and cytochrome-C-mediated respiration appears to correlate with sterigmatocystin production in *A. nidulans*, albeit only in the absence of light.

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NEW GENOTYPES AND DMI RESISTANCE IN HUNGARIAN GRAPEVINE POWDERY MILDEW (*ERYSIPHE NECATOR*) SAMPLES

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Grapevine powdery mildew, *Erysiphe necator*, was introduced into Europe more than 150 years ago. Sympatric populations of this fungal species belong to genetically distinct groups, known from earlier studies as A and B genotypes. Based on sequences of four DNA loci (ITS, *igs, tub2, ef1-α* and *cyp51*), four genotypes have been detected within group B and only one within group A in Europe so far. Intensive fungicide usage in vineyards has often led to reduced sensitivity or resistance to fungicides in powdery mildew and other target plant pathogenic populations. We focused on resistance to DMI (azole) fungicidesin *E. necator*. DMI fungicides are single-site inhibitors, their target is the sterol 14α-demethylase (CYP51) essential for ergosterol biosynthesis in fungi. Recent studies revealed several mechanisms of DMI resistance in grapevine powdery mildew. Most common are single nucleotide substitutions causing target site mutations in the CYP51 enzyme (Type 1), constitutive overexpression of the *cyp51* gene (Type 2) and increased copy number of *cyp51*. The aims of the present study were to (i) genotype grapevine powdery mildew populations in Hungarian vineyards; and (ii) detect the genetic markers of DMI resistance in these populations. We sampled Eger, Tokaj-Mád and Ászár-Neszmély wine regions in 2017. DNA was extracted from single chasmothecia, the sexual fruiting bodies of E. necator developed on grapevine leaves. ITS and partial *igs, tub2, ef1-α* and *cyp51* genes were sequenced. We confirmed that chasmothecia mostly represented genetic group B. However, we have also detected mixed genotypes at certain polymorphic sites in all genes examined. As a consequence of the polymorphic sites in *tub2* and *ef1-α* sequences, new genotypes have been determined in this work. The SNP indicating Type 1 DMI resistance was rarely detected in the samples from Eger and Tokaj-Mád, whereas it was frequently found in Ászár-Neszmély. Type 2 resistance was not detected. Further studies will include samples from other Hungarian wine regions and will focus on the distribution of the different genotypes and DMI resistance in Hungarian *E. necator* populations.

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ANTAGONISTIC ACTIVITY OF YEASTS ISOLATED FROM GRAPE BERRIES AND MUST

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The wine fermentation is a complex microbiological process, and the interaction among the yeasts can influence the process. Investigation of antagonistic activity of yeasts belonging to microbiota of grape and must therefore has a paramount importance. Total 59 yeast strains were isolated from the berries and must originated from different vineyards of Zala, Balatonfüred-Csopak, and Etyek-Buda Wine Regions. The antagonistic effect of the isolated strains were tested on Methylene Blue Medium

at pH 4.5 against *Saccharomyces cerevisiae* S6 sensitive strain, *Zygosaccharomyces rouxii* IV/3/7 and *Z. bailii* I/3/3 strains isolated from wine. 18 out of the 59 isolates (30%) showed antagonistic effect against one or more test strains. Five strains of active isolates were able to inhibit the growth of *S. cerevisiae* and both *Zygosaccharomyces* strains. The occurrence of antagonistic strains was higher in case of grape berries (50% - 55%). All 11 strains isolated from berries inhibited the *Z. bailii* and/or *Z. rouxii*, while only seven strains were active against *S. cerevisiae*. In case of musts 15 - 20 percent of the isolates had antagonistic effect. Most of these isolates were able to inhibit *S. cerevisiae* S6, but two isolates only showed antagonistic effect against *Zygosaccharomyces* strains. Further studies were performed to uncover the background of the antagonistic activity of the strains. The examination of cell free supernatants and the molecular investigation of presence of dsRNA molecules proved that almost 40% of the strains have killer phenotype. The 18 antagonistic strains were grouped on the basis of their morphological and physiological properties. On the basis of the results of traditional characterization the strains were classified into four groups. In order to species identification representatives of these groups were chosen. The identification was performed by sequencing of the D1-D2 region of 26S rDNA. The identified species were *Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, *Saccharomyces cerevisiae* and *Saccharomycopsis crataegensis*. The five strains which inhibited all three test species were identified as *M. pulcherrima*, while the *S. cerevisiae* isolates were only effective against *S. cerevisiae* S6 strain by production of killer toxin. *H. uvarum* isolates had antagonistic activity only against S6, and the presence of cytoplasmic dsRNA suggests the killer activity. One isolates was identified as *S. crataegensis*.
This strain was able to inhibit the growth of *S. cerevisiae* and *Z. bailii* , but not the growth of *Z. rouxii*. Antimicrobial effect is well known in case of *H. uvarum*, *M. pulcherrima* and *S. cerevisiae*. However in case of *S. crataegensis* this phenomenon has not been reported until now and further studies are needed for deeper characterization of antagonistic properties of this species. Nowadays industrial application of starter cultures in wine making is widespread. Most used oenological starters are *S. cerevisiae* strains however non-*Saccharomyces* species are recently also used to enhance the quality of the wine. For that reason further purpose would be the oenological characterization of the isolated *H. uvarum*, *M. pulcherrima* and *S. cerevisiae* strains.

EXTRAORDINARY INCREASE IN THE NUMBER OF HUMAN WEST NILE VIRUS INFECTIONS − CONCLUSION OF THE LABORATORY EXPERIENCES

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West Nile virus (WNV), a mosquito-borne member of the Flaviviridae family is one of the most important viral zoonotic agents in Hungary. However, most of the infections are asymptomatic, 20% of the acute cases are characterized as West Nile fever, which is the milder form of the disease, while approximately 1% of the patients develops severe neuroinvasive symptoms such as meningitis or encephalitis. The laboratory diagnosis of human WNV infections includes both different serological and advanced molecular methods following international diagnostic guidelines. Human sera, whole blood, cerebrospinal fluid and urine samples are mostly appropriate to perform differential diagnostic tests. By the 10th of September 2018, a total of 153 autochthonous and 9 imported cases were laboratory verified and reported to the European Centre for Disease Control and Prevention (ECDC),

albeit the number of cases may continue to increase until the end of transmission season in late autumn. So far, WNV lineage 2 genetic variants have been identified in case of 29 patients. Regarding human infections, data are available from 2004, when the first human neuroinvasive infections were diagnosed by the Hungarian National Reference Laboratory for Viral Zoonoses. So far the largest number (44) of WNV infections were reported in 2016. This year a three-fold growing could be observed, while considering the last year, there is a seven-fold increase in the number of cases. An early occurrence of a large number of cases has been observed, the first cases were confirmed four weeks earlier than in the previous years. Similar extraordinary increase in the number of WNV cases is observed in the most European countries affected by WNV circulation. Clarification of the causes of the present situation requires further investigation.

THE EFFECT OF HUMAN SERUM ON ANTIFUNGAL SUSCEPTIBILITY OF *CANDIDA ALBICANS* AND *CANDIDA PARAPSILOSIS* BIOFILMS

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Biofilm development is a relevant risk factor for morbidity and mortality among hospitalized patients especially in intensive care units. *Candida albicans* remained the most important fungal pathogen in bloodstream infections followed by *C. parapsilosis*. In our study, the activity of fluconazole, amphotericin B, micafungin and caspofungin was determined against *C. albicans* and *C. parapsilosis* planktonic and sessile cells in RPMI-1640 with or without 50% human serum. The *in vitro* activity of different antifungals against *Candida* biofilms were quantified with XTT reduction assay. Antifungal activity was calculated using the following formula: fungal damage (%) = [1-(absorbance of experimental cells (492 nm)/absorbance of control wells (492 nm))] x 100. Fluconazole showed lower activity in RPMI-1640 compared to amphotericin B or to the tested echinocandins against *C. albicans* biofilms. In case of amphotericin B, all isolates were significantly more resistant in 50% serum at concentrations ranging from 0.5 to 1 mg/L as compared to susceptibility in serum-free RPMI-1640 ($p < 0.01$ - 0.05). In case of caspofungin, significantly higher fungal damage was observed in RPMI-1640 compared to serum-containing medium at concentrations ranging from 0.015 mg/L to 1 mg/L for all isolates ($p < 0.05$). Micafungin showed high antifungal efficacy (>50% fungal damage) at concentrations from 0.015 mg/L in RPMI-1640 and from 2 mg/L in the presence of 50% human serum. In case of *Candida parapsilosis* fluconazole did not result significant fungal damage (>50%) at tested concentrations in RPMI-1640. However, enhanced activity was observed in the presence of 50% serum at concentrations starting from ≥0.03 mg/L. Amphotericin B caused at least 50% fungal damage from 1 mg/L and from 0.015 mg/L for in RPMI-1640 and RPMI-1640 plus 50% human serum, respectively. The tested echinocandins showed high activity against *C. parapsilosis* biofilms in the presence of 50% serum. The structural and viability properties of *C. albicans* and *C. parapsilosis* biofilms with and without 50% human serum were demonstrated with fluorescent microscopy. Serum exposure caused a remarkable adverse effect in terms of biofilm structure in case of both species this was more marked in case of *C. parapsilosis*, as confirmed by fluorescent microscopy and flow cytometry. Fluconazole exerted better antifungal activity in serum than biofilm-active antifungals against both examined biofilms, supporting the utility of fluconazole prophylaxis to reduce the risk of catheter-associated fungal infections.

INVESTIGATION OF CARRIAGE OF MULTIRESISTANT GRAM-NEGATIVE ENTEROBACTERIA BY ROOKS (*CORVUS FRUGILEGUS*) WINTERING IN AN URBAN ENVIRONMENT AND COMPARISON TO CONTEMPORARY PATIENT-DERIVED STRAINS

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During winter, trees in the campus of the University of Debrecen serve as a gathering place for a large group of rooks (*Corvus frugilegus*) and their droppings pollute the area. We investigated carriage of multiresistant Gram-negative enterobacteria by these rooks between October 2016 and March 2017. Cloacal swabs were collected from 96 captured rooks. In parallel, all 2,455 human fecal samples sent for routine fecal culture were also examined in the study period. MDR isolates were recovered using eosin-methylene blue agar containing 2 mg/L cefotaxime and identified by MALDI-TOF-MS. Susceptibility testing was performed by EUCAST disk diffusion, production of extended spectrum beta-lactamases (ESBLs) was examined by double-disk synergy tests. In comparison, 32 contemporaneous ESBL-producing *Escherichia coli* from inpatient clinical samples were also examined. Each ESBL-producing isolate was screened by polymerase chain reaction for carriage of *blaTEM*, *blaSHV* and *blaCTX-M*; *blaCTX-M* carriers for CTX-M subgroups 1, 2, 8 and 9 as well. A total of 41/96 (43%) rooks carried cefotaxim-resistant isolates, of which 37 *Escherichia coli* and 4 *Enterobacter cloacae* isolates were found. Four *E. cloacae* and 3 *E. coli* isolates showed AmpC phenotypes, the remaining 34 isolates were ESBL producers. In the 2,455 human fecal samples 25 ESBL producing *E. coli* were found (corresponding to 1% carriage rate). Co-resistance rates among isolates carried by birds, humans and human clinical isolates were 35% (12/34), 64% (16/25) and 91% (29/32) to ciprofloxacin, 47% (16/34), 68% (17/25) and 78% (25/32) to co-trimoxazole, 9% (3/34), 44% (11/25) and 56% (18/32) to gentamicin, 9% (3/34), 60% (15/25) and 56% (18/32) to amikacin and tobramycin, respectively. Group *blaCTX-M-1* was predominant, found in 65% (22/34), 60% (15/25) and 56% (18/32) of rook, human fecal and clinical isolates, respectively; as a sole ESBL gene by 38% (13/34), 36% (9/25) and 56% (18/32) or together with *blaSHV* or *blaCTX-M-9* by 27% (9/34) of bird and 24% (6/25) of human carriers. Group *blaCTX-M-9* was also frequent; alone in 21% (7/34), 20% (5/25) and 37% (12/32) or with *blaCTX-M-1* group in further 21% (7/34), 12% (3/25) and 0% of birds, human carriers and clinical isolates, respectively. Genes *blaSHV*, *blaCTX-M-2* and *blaCTX-M-8* were rarely, *blaTEM* was never found.

In summary, bird isolates showed lower co-resistance rates than human isolates, which were similar regardless of being carried or pathogenic. ESBL gene distributions were similar, though gene diversity among clinical isolates was lower than among isolates carried asymptomatically. These suggest that rooks as well as patients may serve as reservoirs for ESBL-producing *E. coli*, underlying the One Health concept in case of multiresistant bacteria.

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ROUS SARCOMA VIRUS AND THE VIRAL TUMORIGENESIS - IN MEMORIAM JAN SVOBODA (1934 - 2017)

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Svoboda spent most of his career at the Institute of Molecular Genetics, Czech Academy of Sciences. He also served as Director of the Institute from 1991 to 1999. His main scientific achievement came from the infection and transformation by RSV of mammalian hosts, laboratory rats, mice and hamsters. The non-permissive mammalian cells did not support RSV replication, but the virus could be rescued after injection of minced tumor cells into chickens or, later, by *in vitro* induced fusion of transformed cells with permissive chicken cells. The elusive persistence of latent RSV, so called virogeny, was explained by the provirus hypothesis. He also studied this virogenic state in XC rat cells. He was permanent connection to those known names of that time as Howard Temin, Harry Rubin, Peter Voght, Hidesaburo Hanafusa and others. His XC cells became a frequent tool in many laboratories. In his 1975 Nobel lecture, Howard Temin fairly mentioned Svoboda's credit, saying that "Svoboda et al. from studies of RSV-infected rat cells independently postulated the existence of a provirus in RSV-infected cells.". Later he found, that replication-defective virus could be rescued as replication-competent virus by superinfection with helper virus. These findings contributed to the definition of the src oncogene and to the concept of retroviral vectors. This was the time, when he visited Hungary several times, and participated in 1978 at the Tumor Virus Group Conference in Balatonfüred. In 2015, he was elected as Foreign Associate of the National Academy of Science of the USA. At his honorary speech he recalled his scientific career, and took an oath on science. Jan Svoboda was complex, brilliant and deeply dedicated to science, a philosophical man of eclectic interests and a committed debater. He delightfully discussed or argued not only science, but also politics, history, and philosophy. With his death a giant of the science and the retrovirology left us.

COMPLEX MULTICELLULARITY IN FUNGI: HIGH LEVEL CONVERGENCE REVEALED BY GENOME AND TRANSCRIPTOME COMPARISONS

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Mushroom formation is one of the most spectacular and complex processes in the fungal world, comparable to the development of higher plants and animals in terms of its complexity. Yet, its genetic bases, in particular, conserved developmental genetic events are hardly known. Here we set out to identify conserved developmentally regulated genes in mushroom-forming fungi. We identify developmentally regulated genes by comparing transcriptome data across three to eight developmental stages of six Agaricales, Polyporales and Hymenochaetales species. One of the species included in our analysis is *Phanerochaete chrysosporium*, which produces simple, crust-like (resupinate) fruiting bodies, presumably resembling the ancestral fruiting body morphologies in the Agaricomycotina. We find that 10 - 40% of the genes are differentially regulated during fruiting body development in the examined species, comprising functions related to cell wall synthesis and modification, mRNA stability, cell growth and regulation of transcription. By studying the conservation of developmentally genes and their evolution through a comparative genomics analysis of 202 fungal genomes, we aim to understand the origin of fruiting body-related genes and to zoom in on the minimal gene set required to initiate and develop basidiomycete complex fruiting bodies.

ROLE OF SEROLOGICAL CROSS-REACTION AND CROSS-NEUTRALIZATION IN THE DIAGNOSIS OF FLAVIVIRUS INFECTIONS

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Serological diagnosis of secondary or multiple flavivirus infections is often complicated by the cross-reactive antibody response, a characteristic phenomenon of the genus. Even cross-neutralizing antibodies during the early-phase of the immune response are described in the literature. In Hungary, West Nile virus and tick-borne encephalitis virus, two important members of the *Flavivirus* genus cause human morbidity annually, and imported flavivirus infections due to Zika virus and dengue virus have also been diagnosed in the recent years. Moreover, active immunization against yellow fever virus and tick-borne encephalitis virus is prevalent among the Hungarian population. As the majority of flavivirus infections have mild or subclinical manifestation, more seropositive persons are estimated than the reported number of cases.

These undiagnosed infections may lead to unexpected serological cross-reactions and controversial diagnostic results. In our study, we examined the cross-reactive and cross-neutralizing effect of West Nile virus and tick-borne encephalitis virus specific antibodies on the diagnosis of imported flavivirus infections. We used sera of acutely ill patients of the 2017 and 2018 seasonal period of West Nile fever and tick-borne encephalitis. Different diagnostic methods, such as indirect immunofluorescence technique and enzyme-linked immunosorbent assay were used to detect cross-reactivity, while cross-neutralizing effect was tested by plaque-reduction neutralization assay. Furthermore, sera of patients with vaccination history against tick-borne encephalitis- or yellow fever virus were also analyzed by the previously described methods, to determine the cross-reactivity/cross-neutralization caused by flavivirus vaccination during serological diagnosis.

COMPARISON OF THE TRANSACTIVATING POTENTIAL OF THE LONG CONTROL REGION IN HUMAN PAPILLOMAVIRUS 11 INTRATYPIC VARIANTS ORIGINATING FROM MUCOSAL DISORDERS OF THE HEAD-NECK AND GENITAL REGIONS

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Certain nucleotide polymorphisms of the long control region (LCR) of human papillomavirus (HPV) type 11 may influence severity of the caused disease by conferring enhancement or attenuation of the transactivating potential as reported earlier in six patients (Patient 1 - 6) with juvenile-onset respiratory papillomatosis (RP). Seven new patients were tested; three with juvenile-onset (JO-) (Patient 7 - 9) and two with adult-onset (AO-) (Patient 10 - 11) RP, one with cervical atypia (Patient 12) and one with genital papilloma (Patient 13). In case of the three JO-RPs, samples from multiple localizations or episodes and samples from two new episodes of a known patient (Patient 6) were tested. LCR polymorphisms were identified based on the determined complete genome sequences. Unique viral LCRs were cloned into pALuc reporter vector and transfected to HEP-2 cells with Lipofectamine. Transactivating potential was assessed by luciferase activity measurements.

Luciferase activities were compared by ANOVA with Tukey's post-tests. Samples from a single patient always yielded identical sequences. Unique LCR sequence was found in three patients with JO-RP (the known Patient 6 and two new Patients 7 and 9); one new T7331G polymorphism was found (Patient 9), all other polymorphisms, but not their presently found combinations, were previously reported. A patient with JO- (Patient 8), two with AO-RPs (Patient 10 and 11) and one with the genital papilloma (Patient 13) had LCRs identical to a previously published sequence (HE574702). The LCR from the cervical atypia (Patient 12) was identical to the reference sequence (M14119) which originates from a highly aggressive JO-RP. Highest luciferase activity was measured in the LCR from Patient 7, lacking a previously reported attenuating polymorphism (A7413C), but harboring one enhancing and two attenuating polymorphisms found in the four identical LCRs from Patients 8, 10, 11 and 13. However, the difference to the next highest LCR activities of the reference sequence and the LCR identical to the reference was not statistically significant. Luciferase activity of the sequence (from Patient 12) identical to the reference sequence was comparable to that of the reference. LCR from Patient 6, similarly to the earlier measurements, yielded an activity slightly, but not significantly lower than the reference LCR. These four sequences with the highest activity, excepting LCR from Patient 6, produced significantly higher luciferase activity than the remaining sequences, all of which produced similar and statistically comparable luciferase activities. These new data suggest that though individual polymorphisms do have an impact on transactivating potential, transactivating potential of a virus variant is determined by the effect of the combination of harbored polymorphisms.

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INVESTIGATION OF LIQUID EGG PRODUCTS MADE OF EGGS FROM SEVERAL SUPPLIER

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Instead of shell eggs, the use of ready-to-use egg products have a wide expansion in food industry, especially in plants using high quantities of eggs. Eggs don't have to be broken and handled in plants, moreover, if egg yolk and white are used in different ratio, there will be the opportunity to buy these separated (in form of liquid egg yolk and liquid egg white). The aim of our study was to evaluate the effect of shell eggs originated in different laying plants on product quality. The goal was to get a comprehensive analyze of suppliers and product's quality. Methodology used for these purposes are summarized below. Liquid eggs were examined and produced from shell eggs supplied from 2, or 3 plants. The ratios were 50 - 50%, 33 - 33% in products, respectively. Examinations were carried out monthly for six months. Investigation of dry matter content is important, because dry matter content of eggs is decreasing during laying cycle (yolk's ratio is decreasing), during storage time (dry matter content increases caused by desiccation). Sartorius MA30-000V3 was used for determining dry matter content, drying procedure was 120°C for 20 minutes. The most important techno-functional properties of liquid egg white are the foaming ability, foam retention ability and the drop loss. Foaming ability means how many volumes are whipped from a volume liquid egg white. Foam retention ability means the ratio of drop loss and whipped egg white foam during a given storage time. Drop loss means how many liquids is lost from whipped egg white foam during a given storage time. For investigation of foaming ability 500 g liquid egg white was whipped whit kitchen whisks

for 5 minutes and the volume of foam was measured. Foam retention ability was calculated by measuring a volume of egg white foam in a measuring cylinder and the ratio of drop loss was calculated during storage time. Drop loss was evaluated during storage time, when the volume of liquid in the bottom of measuring cylinder was investigated. Microbiological investigations are important parts of evaluation of suppliers in the food industry. 1 - 1 g of liquid egg white were analyzed in sterile conditions using selective media (X.L.D. Agar, Harlequin *Salmonella* ABC, Biokar VRBG Agar; Biokar Nutrient Agar). Incubation time and temperature of inoculated samples were carried out adequate to recommendations of media.
Our results show that quality of liquid egg white (dry matter content, techno-functional properties) is more balanced, if shell eggs of more suppliers are used. Different tendencies are more balanced, if shell eggs are used from different laying farms (e.g. age of the layers). Microbiological food safety requirements are full field independent from weather conditions and independent of the number of suppliers, if hygienic and technological requirements are fulfilled. Liquid egg white products are *Salmonella*-free, count of Enterobacteriaceae is under 10 CFU/mL.

A MOLECULAR GENETIC TOOLBOX FOR *AMPELOMYCES*

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Powdery mildew fungi (Erysiphales) are well known obligate biotrophic pathogens of thousands of angiosperm plants. Pycnidial fungi belonging to the genus *Ampelomyces* often invade powdery mildew colonies in the field and have been used as biocontrol agents against powdery mildew infections of agricultural crops. To perform functional molecular genetic analyses of these mycoparasitic fungi a suitable stable transformation method is inevitable. With such a method the possibility of targeted gene knockout (KO) could also be tested. To achieve this goal, we tested *Agrobacterium*-mediated transformation of some Ampelomyces strains. AGL1 strain of *A. tumefaciens* carrying a plasmid with the hygromycin resistance (hygromycin phosphotransferase; hph) and the green fluorescent protein (GFP) genes was used in this work. Transformants were selected on hygromycin-containing medium; their hygromycin resistance and fluorescence emission were checked after several transfers following the initial selection. To test the homologous recombination-based targeted KO in *Ampelomyces*, the nitrate-reductase gene (*eukNR*) was selected as elimination of this gene was expected to result in an easily detectable, visible phenotype. To achieve KO of *eukNR* in *Ampelomyces*, an *E. coli – Agrobacterium* - yeast shuttle vector was produced first. As a second step genomic regions flanking *eukNR* were cloned upstream and downstream to hph in this vector using yeast recombinational cloning. This was done to guide the insertion into the *eukNR* target locus. The *Agrobacterium*-system proved to be effective to produce high numbers of stable transformants of *Ampelomyces*. With this method we obtained 10 - 30% transformation efficiency. Transformants kept their ability to colonize powdery mildews similar to the wild-type strain. Efficient KO of the target gene was confirmed by PCR, which means that the insertion in *Ampelomyces* can be guided to the desired locus by using homologous DNA fragments.
Visible phenotype caused by *eukNR*– genotype was clearly detected on media with different nitrogen sources. To our knowledge, this is the first report of a successful transformation and gene knockout of *Ampelomyces* strains. Commonly used heterologous promoters and reporter genes are fully functional, which allows the use of existing systems for heterologous gene expression, controlled

overexpression and targeted knockout. Our results pave the way for future molecular studies of this mycoparasite, providing a solid basis for functional genetic analysis.

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DEVELOPING MOLECULAR TOOLS TO STUDY THE VIRULENCE OF THE HUMAN FUNGAL PATHOGEN *CANDIDA PARAPSILOSIS*

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Candida parapsilosis is a significant causative agent of candidemia amongst low birth weight neonates, however little is known about its physiology and pathogenesis mostly due to the lack of appropriate molecular tools. The existing approaches are either time consuming or require a special parental strain. Moreover the properties of the organism like its diploid nature, the absence of sexual cycle and the alternative codon usage inhibit direct adoptations of techniques developed in the intensively studied yeast model *Saccharomyces cerevisiae*. To facilitate genetic modifications in *C. parapsilosis* yeast we decided to set up and optimize the crispr/Cas9 system for this fungus. Crispr/Cas9 is the latest innovation on the field of genome editing, where the endonuclease Cas9 can be directed by a single guide RNA to specific sites of the genome. In the presence of a given template DNA, called the donor DNA different genome alterations can be obtained by repair mediated processes. The main advantage of the system that theoretically it can be used unlimited times and is capable of nucleotide precise editing without leaving any selection marker in the genome. We optimized an already existing approach in *Candida albicans* to GFP-label *C. parapsilosis*, introduce STOP codon, overexpress a gene or remove a complete ORF and perform nucleotide precise reintegration in this species. This was also usable to HIS-tag proteins for potential ChIP-Seq analysis and creating GFP reporter constructs to reveal transcriptional networks and perform epistasis analysis.

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D-GALACTOSE AND L-ARABINOSE CROSS-INDUCE THEIR RESPECTIVE CATABOLIC PATHWAYS IN ASPERGILLI

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Ascomycetes are often saprophytic and/or plant pathogenic fungi, feeding on living or dead plant biomass. Hemicellulose is a major component of the plant cell wall. The pentose L-arabinose and the hexose D-galactose are common monosaccharide units of hemicellulose as well as pectic heteropolymers, like e.g., L-arabinogalactan. The Pentose Catabolic Pathway (PCP) is comprised of alternating reduction and oxidation reactions, ultimately followed by an irreversible phosphorylation to D-xylulose-5-phosphate, which enters the pentose phosphate pathway (PPP). In *Aspergillus*

nidulans, the minor alternative oxido-reductive pathway (ORP) for D-galactose catabolism employs a similar sequence of conversions yielding, eventually, the glycolytic intermediate D-fructose-6-phosphate. By contrast, the Leloir pathway of D-galactose breakdown is effectively epimerizing D-galactose into D-glucose. While there is a genuine similarity and even functional overlap between PCP and ORP (at least one enzyme is shared), the first three enzymes and intermediates of the Leloir path are exclusive to the catabolization of D-galactose. We observed that L-arabinose and D-galactose are simultaneously taken up and catabolized by *A. nidulans*, even at high sugar concentrations; the pentose is consumed somewhat faster than the aldose. We hypothesized that structural genes of the ORP, PCP and Leloir pathways may be responding coordinately to the presence of either one of these sugars, irrespective of whether the enzymes encoded are actually involved in the catabolism of the other sugar. We demonstrate that the *galE* gene, coding for galactokinase (Leloir), is equally well induced by the two sugars. Furthermore, the *galmB* gene encoding D-galactose mutarotase, which was recently shown to contribute to D-galactose breakdown via Leloir, is even hyperinduced by L-arabinose, notwithstanding the fact that *galmB* and *galE* deletion mutants display no phenotype on the pentose. Conversely, the *araA1* mutant strain shows growth phenotypes on both sugars, as L-arabitol dehydrogenase functions in both ORP and PCP, although the gene is much better expressed on the pentose, particularly earlier in culture. Finally, the *xkiA* (xylulokinase) gene is expressed to high basal levels under all conditions, with only some overexpression apparent on L-arabinose early in culture.

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ANTIBACTERIAL EFFECT OF DIAZINE-RING CONTAINING HYDRAZONES AND THEIR METAL COMPLEXES

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The antibiotic resistance is a serious problem for the treatment of bacterial infections due to the appearance of reduced or missing response of microorganisms to the applied antimicrobial agents. Consequently, the discovery of new antimicrobial agents is a major challenge for drug development. The obligate intracellular Gram-negative *Chlamydia trachomatis* invades host cells to replicate inside a membrane-bound vacuole called inclusion. *C. trachomatis* D is of great public health significance because of the impacts of untreated diseases on reproductive outcomes. The aim of our study was to investigate the antibacterial effects of 13 metal complexes against Gram-positive and Gram-negative bacteria. The minimum inhibitory concentrations (MICs) of compounds were determined by two-fold broth microdilution method in 96-well plate on reference Gram-positive (*Staphylococcus aureus*, *S. epidermidis*, *Enterococcus faecalis*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*) strains. Elementary bodies (EBs) of *C. trachomatis* D were incubated with tested compounds at various concentrations (2, 10 μg/ml). To quantify the anti-chlamydial effects of the compounds, HeLa cells were seeded in tissue culture plates with coverslips. After 24h, the confluent cells were infected with EBs or compound-treated EBs. After 48h the cells were fixed and the titer of the infectious EBs was determined by indirect immunofluorescence assay. The number of chlamydial inclusions was counted under a UV microscope. Seven compounds, containing phthalazine and pyridazine fragment,

had antibacterial effect on both Gram-positive and Gram-negative strains, while three complexes showed promising antimicrobial properties against Gram-positive bacteria only. Two compounds containing pyridazine fragment exerted antibacterial activity against *C. trachomatis* D, in particular, the Zn(II) complex caused significant growth inhibition at the concentration of 2 ug/ml, while the ligand was moderately active at the concentration of 10 ug/ml.
These results suggested that diazine-ring containing metal complexes could be used as effective antimicrobial agents against Gram-negative and Gram-positive bacteria.

DIRECTED EVOLUTION OF *CANDIDA PARAPSILOSIS* IN THE PRESENCE OF ECHINOCANDINS INFUENCES VIRULENCE

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Candida parapsilosis is second most common cause of candidaemia after *Candida albicans*. In contrast with *C. albicans*, *C. parapsilosis* clinical strains have higher MIC values to echinocandin type antifungals. However, *C. parapsilosis* infections 100% successfully treated with caspofungin and other echinocandin drugs. To reveal the connection of this two opposing phenomena we aimed to generate three independent *C. parapsilosis* strains each of them resistant to one of the three echinocandin, then we aimed to examine changes of abiotic stress response and virulence of the generated strains. In our microevolutionary study, we have grown the *Candida parapsilosis* CLIB 214 parental echinocandin sensitive strain in the presence of increasing concentration of different echinocandins. Abiotic stress tolerance of generated resistant strains were determined on YPD plates complemented with different osmotic-, oxidative stressors and cell wall-, membrane perturbing agents. To investigate virulence properties of the echinocandin evolved strain we have used the unconventional *Galleria mellonella* model. The three echinocandin evolved strains showed increased MIC for the echinocandin which were used to evolve the particular strain. Additionally, the anidulafungin evolved strain were resistant to all three kind of echinocandins and the caspofungin evolved strain were resistant to caspofungin and micafungin, however the micafungin evolved strain were only resistant to micafungin. The evolved strains were susceptible to cell wall perturbing agents, this phenomena were more prominent on 37°C and the micafungin evolved strain were unable to growth in the presence of calcofluor white and congo red on both 30°C and 37°C.
Interestingly the micafungin evolved strain were the only one (including parental strain) which were able to grow without any defect in the presence of SDS. During *Galleria mellonella* infection we find that the micafungin evolved strains showed attenuated virulence as the infected wax moth larvae survived better than those infected with other echinocandin evolved strains or parental strain. In this study, we have shown that adaptation to different echinocandin forms lead to different patterns of MIC values and abiotic stress tolerance. Our micafungin resistant strain displayed attenuated virulence in a *Galleria mellonella* infection model.

TRANSCRIPTOMIC ANALYSIS OF HYPHAL GROWTH IN *SCHIZOSACCHAROMYCES JAPONICUS*

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Fungal dimorphism (in some cases rather polymorphism) is an ability of an organisms to switch between single cell (yeast) form and filamentous (hyphae) form. Several pathogenic and saprophyte fungi possess this ability, which enables them to survive in various environmental conditions and to colonize/invade their niche. For example, the *Candida albicans*, our commensal - in immunocompromised state - is able to evade the host immune response and colonize the organs by using this switching mechanism, which - in most cases - leads to death. *C. albicans* cells which are blocked in both yeast and filamentous forms, are less virulent or completely avirulent. Although we know a lot about the above-mentioned processes, certain mechanisms, such as transcriptional changes that induce hyphal growth and morphogenesis, are not well understood. Thus, studying molecular background of the dimorphic switch is a very timely and important issue. To study dimorphism, we can use model organisms which are easier to work with. Such a model is the *Schizosaccharomyces japonicus*, which is a dimorphic, haploid, non-pathogenic yeast. We have previously described several external conditions which could influence the hyphal growth of *S. japonicus*. Interestingly, this species was also able to sense fetal bovine serum (FBS), thus it could produce hyphae in the presence of FBS, similarly to the *C. albicans*. Considering these inducing conditions, total RNA from the *S. japonicus* hyphae was extracted and analyzed by RNA sequencing. Later, we functionally categorized the genes which showed altered mRNA levels by GO term annotations. These data were compared to *C. albicans*, *Histoplasma capsulatum* and *Yarrowia lipolityca* data and we found several common genes which seemed to be evolutionarily conserved between these species. Our data also suggested that the transcriptional regulation of hyphal growth in *S. japonicus* is different rather than similar to these species. However, genes which were regulated similarly were also found.

DELETION OF THE DNA LIGASE IV (LIG4) GENE RESULTS IN STRESS SENSITIVITY IN *SCHIZOSACCHAROMYCES JAPONICUS*

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Genetic modification of an organism – nowadays - is tightly related to our daily life. With this method we can produce specific proteins, map molecular background processes or enhance a certain ability of a strain. Integrative transformation is one of the key steps to create modified organisms. In ideal cases, homologous recombination (HR) is responsible for the proper integration of recombinant DNA. However, in some species, such as filamentous fungi or certain plants “refuse” to replace their gene with the introduced foreign DNA, which - in most cases - results in non-specific integrations. This non-homologous recombination (or NHEJ) is more common in these organisms and it can cause negative effects, such as non-desired gene interruptions. Thus, NHEJ can significantly increase the time and therefore the costs of gaining a properly modified organism. Model organisms are very useful in understanding certain, sometimes very complicated processes. The *Schizosaccharomyces japonicus* is a dimorphic fission yeast which is less-known compared to its well-known relative the *Schizosaccharomyces pombe*. While *S. pombe* integrates the foreign DNA in most cases by homologous recombination, this is not true for *S. japonicus*, whose dominant repair pathway is NHEJ (our unpublished data). Our aim was to delete the ligase IV (*lig4*) (the effector enzyme of the NHEJ) gene from the chromosome of *S. japonicus*. The deletion did not alter the cell morphology compared to the wild type. However, the *lig4* deleted cells were sensitive to osmotic stress.

PHYSIOLOGY AND CYTOLOGY OF LACTOSE UTILIZATION AND ß-GALACTOSIDASE PRODUCTION OF *KLUYVEROMYCES WICKERHAMII*

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One of the important and unique characteristics of the species belonging to the *Kluyveromyces* genus is their ability to assimilate lactose, the only exceptions being *K. lactis* var. *drosophilarum* and *K. dobzhanskii*. *K. lactis* var. *lactis* and *K. marxianus* are able to utilize lactose not only by aerobic respiration but anaerobic (ethanolic) fermentation as well. Two other lactose utilizing species of the genus, *K. nonfermentans* and *K. wickerhamii* assimilate but do not ferment lactose. While *K. lactis* var. *lactis* and *K. marxianus* have been widely studied and used in various biotechnological processes, the knowledge concerning the physiological and genetic properties of the other *Kluyveromyces* species - including *K. wickerhamii* - is limited. Lactose utilization of *K. lactis* var. *lactis* and *K. marxianus* is inducible and regulated by glucose repression. Activity of two unique genes, encoding lactose permease and β-galactosidase are essential for lactose utilization of these species. The enzyme ß-galactosidase is responsible for the hydrolysis of lactose to glucose and galactose but under certain conditions it is able to synthesize galacto-oligosaccharide (GOS) molecules as well. Commercial ß-galactosidase products of *K. lactis* and *K. marxianus* are extensively used for these purposes but the potential of *K. wickerhamii* ß-galactosidase has not been investigated from these respects. Our aim was to determine the physiological conditions and cultivation parameters (lactose concentration, nitrogen supplementation, pH) of lactose utilization by *K. wickerhamii* and the cellular localization of the enzyme ß-galactosidase. For these purposes shaken cultures of *Kl. wickerhamii* NCAIM Y.01730T were applied in laboratory scale experiments. Our results indicate that yeast extract as an organic nitrogen source is more favorable for growth and ß-galactosidase production of *K. wickerhamii* than inorganic nitrogen supplementation. Nonetheless, considerable biomass production was achieved in minimal medium containing lactose, inorganic macro-nutrients and vitamins, which is considered as a more favorable condition for enzyme isolation and purification purposes than application of yeast extract or other organic nitrogen sources. We also concluded that fast acidification of the culture broth took place during cultivation, which led to early cell lysis in the cultures. Therefore, buffering of the culture medium was necessary to produce high cell density cultures for enzyme production and isolation purposes. ß-galactosidase activity was measured in the extracellular, periplasmic and intracellular spaces. Majority (approximately 99%) of the ß-galactosidase activity in exponential phase cultures was found in the intracellular space, which decreased slightly as the stationary phase proceeded. This was probably the consequence of the progressing cell lysis in stationary phase cultures. In order to investigate the potential application of *Kl. wickerhamii* ß-galactosidase in lactose hydrolysis and GOS production, enzyme extraction, purification and characterization are necessary and already in progress.

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STUDY OF THE FUNGISTATIC EFFECT OF SOIL BACTERIA AGAINST PHYTOPATHOGENIC FUNGI

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The sustainability of agricultural production and the ever so intensifying demand for healthy and clean foodstuff badly urge the reduction of fungicide usage in field. Environment friendly and also time and cost effective biological solutions are highly required in field crop production to replace synthetic fungicides. Microbial soil inoculants - composed of bacteria able to inhibit soil born plant pathogenic fungi, like *Fusarium* spp., *Sclerotinia* sp. - represent useful tool to diminish pathogen epidemics. Current work aimed the investigation of the biocontrol efficacy of domestic, fungistatic soil bacteria (*Bacillus* sp., *Pseudomonas* sp., *Paenibacillus* sp.), acting separately or in combination against economically relevant plant pathogenic fungi *Sclerotinia sclerotiorum* and *Fusarium graminearum*. *In vitro* fungal growth repression experiments were performed on agar plates, and plant tests were also carried out in greenhouse by controlled fungal infection of potted soil. The biocontrol efficacy has been evaluated by monitoring qualitative and quantitative traits of crops plants grown in the pot trials. The *in vitro* results indicate that the bacterial species of various modes of action repress pathogenic fungal growth and spread, reaching diverse levels of efficacy. In our pot experiments the strain combinations were highly effective against *S. sclerotiorum* starting at 10^4 cells/ml, and 10^5 cells/ml against *F. graminearum*. The strain combinations have notably reduced the severity of pathogenic symptoms, moreover, plant growth promotion effects could be detected, just like enhanced emergence and better biomass production. Based on the results, few bacterial strain combinations have a very promising biocontrol agent potential, worth for bioproduct development.

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A RAPID METHOD TO GENOTYPE GRAPEVINE POWDERY MILDEW AND DETECT THE DMI FUNGICIDE RESISTANCE MARKER A495T

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Grapevine powdery mildew (GPM) caused by *Erysiphe necator*, is one of the most important diseases in grape production. Disease control mostly relies on the use of sterol 14a-demethylation inhibitors (DMIs). These fungicides inhibit the cytochrome P-450 sterol 14a-demethylase, a key enzyme of the sterol biosynthetic pathway [1]. Because of the site-specific mode of action, the intensive use of DMIs has led to the development of resistance in many fungal groups worldwide [1], including GPM [2]. Here we report a newly developed rapid DNA extraction method to (i) reveal the genetic variability among Hungarian GPM samples; and (ii) detect the A495T (Y136F) mutation in *EnCYP51* gene, a common marker of DMI fungicide resistance [2]. The DNA extraction started from single chasmothecium separated from the mycelium with a glass needle and placed into a PCR tube

containing 20 µl of different extraction media. The chasmothecium was crushed and different subsequent protocols were tested with the material. To test the suitability of the DNA extracts obtained with different protocols, PCR amplifications with different target volumes and enzymes were run. In these test reactions different loci were amplified: ITS, IGS, β tubulin, translation elongation factor EF1-α and *cyp51*. Amplicons were verified by sequencing. The high quality sequence results confirmed the suitability of the single chasmothecium DNA extraction method for genotyping of GPM, however, there were differences of the efficiency of the protocols applied. In further tests single chasmothecial DNA samples were tested with quantitative real-time PCR (qPCR)-based genotyping. To detect the A495T marker, the method described by Dufour et al. [3] was applied with minor modifications. Our DNA extracts were successfully used for qPCR diagnostics. The method developed in the present study can be applied as a routine test and will be useful to monitor GPM populations for fungicide resistance and other genetic characteristics.

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FUNGAL STRESS DATABASES

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To help the work of fungal stress biology experts, several databases incorporating fungal stress response protein orthologs and also stress physiology data have been constructed. Fungal Stress Response Database (FSRD) version 2 (FSRDv_2; http://internal.med.unideb.hu/fsrd2/?p=consortium) is based on 1,985 fungal stress response proteins with verified functions and currently includes 43,725 stress protein orthologs in 39 fungal species [1]. Fungal Stress Database (FSD; http://www.fung-stress.org/) is a repository of fungal stress physiology data and currently stores 1,412 photos taken on stress-exposed colonies of 17 *Aspergillus* species [2]. Fungal stress databases may facilitate (i) homology search and annotation work in newly sequenced genomes [1, 3, 4], (ii) comparative genomics research programs [1, 3], (iii) evolutionary biology studies in fungi [5] and (iv) the exploitation of bioinformatics-based data in various „wet lab" projects [6, 7, 8, 9]. Hopefully, the construction of FSD will also contribute to the standardization of fungal stress assays frequently used by the community of mycologists [2]. Importantly, either long-term maintenance or future revision/supplementation of already existing fungal stress databases need further community efforts in terms of both manpower and financing.

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EFFECT OF STRESS-TOLERANT BACTERIAL STRAINS ON PHYSICAL- AND BIOLOGICAL PARAMETERS OF THE SOIL

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The organic material content of soils is significantly reduced by intensive cultivation, which affects negatively the physical and biological parameters of the soil. To date, soil acidification, salinization, and degradation have become emerging issues for the agriculture and the protection of the environment. This problem exists not only in Hungary but also in entire Europe, furthermore it is a relevant worldwide issue. Application of plant-growth-promoting rhizobacteria containing soil inoculants has 30 years old history in Hungary. The inoculants containing bacterial strains with nitrogen fixation, potassium mobilization, phosphate solubilization or siderophore production ability have been used with success on several hundreds of thousands of hectares. The work presented here aimed the compilation of PGP bacterial strain combinations that are able to improve the fertility of deteriorated soils, even under abiotic stress conditions. Strain selection was based on BioFil® Rhizobaceria Screening System. According to our expectation inoculation with the strain combinations will contribute to the enhanced microbial activity of soils by increased enzymatic activity and higher viable cell count. Beside of PGPR effects, the inoculants will be able to improve physical and biological parameters of soils. We carried out lab experiments as well as pot experiments to test three inoculant combinations on two soil types, applying two different doses. Beside of new combinations, we applied the already commercially available BIOFIL ACIDIC soil inoculant as a positive control. Plant experiments were carried out by the University of Debrecen, Institute of Agricultural Chemistry and Soil Science. Our results show that the selected bacterial strain combinations exerted positive effects. Pot trials yielded significant differences.

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CHARACTERIZATION OF AN ATOXINOGENIC *ASPERGILLUS FLAVUS* STRAIN AND TESTS ON CORN HYBRIDS

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Aflatoxins (AFs) due to their toxicity pose significant economic and human health threat; therefore, it is important to avoid this type of contamination in agricultural products. Until now significant AF contamination occurred mainly in foods of tropical and subtropical origin because the optimal growth of the producer *Aspergillus* species is between 32 - 38°C. Nowadays, the AF contamination is becoming higher threat in Middle Eastern Europe, due to the vast imported products and the climatic changes. There is a significant knowledge on the genetic and environmental factors of AF production; however, it is remained a great problem to control the mold contamination and toxin production in farming and stock-raising. Here we present a potential biocontrol agent, an atoxigenic *A. flavus*. Endemic atoxigenic *A. flavus* strains were isolated from feed stocks and characterized by ITS sequencing. Stress resistance against NaCl, Congo Red and SDS were investigated and colonization

tests were performed on corn kernels with different mold resistance and were compared to toxigenic *A. flavus* isolates. On corn kernels the atoxigenic strain was able to take over the niche and therefore to make the AFB1 level much lower, proportionally to the applied spore concentrations. HPLC-MS measurement of the chloroform extracts from molded corn kernels revealed the total absence of AF metabolites but low speradine F presence. Speradine F is an oxindole intermediate secondary metabolite of the cyclopiazonic acid (CPA) biosynthetic pathway with unknown toxicity. No other metabolites were presented by the atoxigenic strain on corn kernels.
Further tests on fields are required to evaluate the performance of the strain and the effect of the climatic conditions on the plant and fungal-fungal interactions.

THE ADVENT OF SEMMELWEIS' RELEVATION IN MEDICAL EDUCATION

BÉLA RALOVICH

Balatonberény

The author reviews the knowledge on microorganisms, their effects and the hygienic practice of medication before 1850 in order to emphasize the real talent of Semmelweis born 200 years ago. Introduces the mental process of Semmelweis, his results and his struggle for the acceptance, and adoption of his justness. Summarizes the appearance of Semmelweis' ideas in the different fields of medical professions. Demonstrates the actuality of the use of chlorine containing disinfectant agents.

EFFECT OF OXYGEN LIMITATION ON MICROBIAL COMMUNITY STRUCTURE OF BENZENE AND TOLUENE DEGRADING ENRICHMENT CULTURES

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In subsurface BTEX-contaminated environments the availability of oxygen is the main limiting factor of quick and complete degradation of all BTEX-compounds. Since availability of oxygen is always restricted in subsurface environments, bacteria adapted to hypoxic BTEX-degradation may have a key role in this process. Previous studies of oxygen-limited, BTEX-contaminated sites of Hungary have shown the large diversity of subfamily I.2.C-type extradiol dioxygenase (*C23O*) genes in these environments. Older literature suggests that such catabolic potentials could be associated to hypoxic degradation. However, recent results of a DNA-SIP-based investigation of an oxygen-limited, toluene-degrading enrichment culture have shown that not all subfamily I.2.C-type *C23O* genes can be linked to hypoxic degradation of aromatic hydrocarbons. To reveal those *C23O* genes which have a key role in the hypoxic degradation of benzene and toluene, aerobic and microaerobic enrichment cultures, degrading solely benzene or toluene, were established and thoroughly characterized. Results of the study have shown that highly different microbial communities were observable under aerobic and microaerobic conditions. While the aerobic benzene-degrading enrichment cultures were overwhelmingly dominated by Betaproteobacteria (95.8%), in the aerobic enrichments only members

of the class Gammaproteobacteria were found (99.8%). At the genus level, members of *Hydrogenophaga* (Comamonadaceae) dominated the aerobic benzene-degrading enrichments, while practically only *Pseudomonas* genus related bacteria prevailed in the microaerobic enrichments. The aerobic toluene-degrading enrichments were dominated by Gammaproteobacteria (66.8%), followed by Betaproteobacteria (22%) and Flavobacteria (9.3%).
At genus level *Pseudomonas* species dominated the community (66%), followed by *Acidovorax* (9.7%) and *Flavobacterium* (9.2%). Although *Pseudomonas* species were also predominant in the microaerobic toluene-degrading enrichment cultures (81%), members of the *Simplicispira* and *Herminiimonas* genera were the dominant betaproteobacteria in these communities (8% and 4.8%, respectively). Regarding subfamily I.2.C-type *C23O* genes it was observed that completely different genotypes were detectable under aerobic and microaerobic conditions, suggesting that ecophysiological fine-tuning, rather than catabolic repertoire contributes to niche definition amongst hypoxic degraders of BTEX compounds in groundwater systems.

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MICROBIAL COMMUNITY ANALYSIS OF CRUDE OIL/GASOLINE MIXTURE AMENDED AEROBIC AND HYPOXIC ENRICHMENT CULTURES

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Aliphatic petroleum hydrocarbons are still among the most common environmental contaminants. Accidental spills and leaks occurring during transport and storage of crude oil and refine products cause the majority of these contaminations. In terrestrial ecosystems petroleum hydrocarbon pollutions considerably threaten subsurface water reservoirs which are often the primary sources of drinking water. Saturated hydrocarbons (alkanes) are quantitatively the most abundant fraction among all petroleum hydrocarbons. Although alkanes show relatively low reactivity, several microorganisms can use them as sole source of carbon and energy coupled with the reduction of different electron acceptors. Nevertheless, the most rapid biodegradation of alkanes can be observed under aerobic conditions due to the fact that under anaerobic conditions the chemical inertness of the carbon-carbon bond retards the degradation. The initial step of aerobic alkane degradation is the incorporation of molecular oxygen into the hydrocarbon molecule by the activity of oxygenases. Nevertheless, in subsurface ecosystems the availability of oxygen is often restricted even in pristine environments. On the other hand, hydrocarbons are potential carbon and energy sources for several aerobic microorganisms. Therefore, contamination increases the microbial metabolism and, consequently, the aerobic microbial respiration and accompanying biological processes decrease the dissolved oxygen concentration in the contaminated environments. However, only a handful of studies have investigated alkane degradation under oxygen-limited conditions or alkane degrading microbial communities in oxygen-limited environments (e.g. oil reservoirs). The hypothesis of the present study was that different microbial communities can be observed in aliphatic hydrocarbon contaminated environments under aerobic and hypoxic conditions. To investigate this hypothesis a pilot experiment was performed by establishing aerobic and hypoxic bacterial enrichments amended with a crude oil – gasoline mixture. Results have shown that aerobic enrichments had much greater microbial diversity than the hypoxic enrichments. The aerobic microbial communities were

dominated by Betaproteobacteria (36.5%) followed by Gammaproteobacteria (28%), Alfaproteobacteria (8.7%) and Actinobacteria (5.6%). The most dominant genus was *Polaromonas* (14%), followed by *Pseudomonas* (10.5%), *Acidovorax* (6.7%) and *Rhodococcus* (5.6%).
Contrarily, the hypoxic communities showed highly reduced diversity and were overwhelmingly dominated by Gammaproteobacteria (79%) followed by Betaproteobacteria (20%). The most dominant genus was *Acinetobacter* (66.3%), followed by *Acidovorax* (11%), *Pseudomonas* (11%) and *Variovorax* (1.8%). Consequently, results shed light on the fact that the excellent aliphatic hydrocarbon degrading *Rhodococcus* species favor clear aerobic conditions, while oxygen-limited conditions can facilitate the high abundance of *Acinetobacter* species in aliphatic hydrocarbon contaminated subsurface environments (e.g. oil reservoirs).

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OPPORTUNISTIC PLUMBING PATHOGENS IN HUNGARIAN WATER DISTRIBUTION SYSTEMS

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In Hungary, most samples from water distribution systems are of adequate quality (in 2017, the rate was 87% with regard to microbiological parameters). Despite this, in some cases tap water can still be a source of infections. In some cases, for example under extreme weather conditions, pathogens can reach the drinking water systems. Moreover, pathogens can proliferate in water systems if they are not operated appropriately. Microbiological proliferation can start in the water distribution system; however, the plumbing systems inside the buildings pose an even greater risk. Especially the hot water systems of large buildings can have ideal circumstances for proliferation of opportunistic pathogens. In this study, we investigated bacteria with high nutrient requirements in Hungarian distribution systems. We investigated the public health risk of these bacteria and the condition that promote their proliferation. 100 water samples were tested from 15 water systems. More than 1,200 stains were isolated from these samples, from these approximately 750 were successfully identified. In drinking water systems inside the buildings *Acidovorax* and *Pseudomonas* genera were the most common and had the highest colony count. In the hot-water samples, the most typical taxa were *Legionella pneumophila*, *Pseudomonas aeruginosa* and bacteria typical on human skin (for example *Micrococcus* and *Staphylococcus* genera). By investigating raw water samples, a complex bacterial community can be observed. In these samples, the above-mentioned bacteria, which are typical in the plumbing systems inside of buildings, were absent or very rarely observed. In the cold-water samples, the most typical bacteria have minimal pathogeny, even if they are in higher concentration. Despite this, potential pathogens are more common in hot water systems. Interestingly, the bacteria from normal human skin are common in both water types; they are present in most samples and reach high concentrations. That means that water systems can be infected from the tap and can become reservoirs for pathogen strains (especially in the case of healthcare buildings).

CLINICAL EXPERIENCES WITH BLOODSTREAM INFECTIONS OF DIFFERENT ORIGIN, WITH SPECIAL EMPHASIS ON INFECTIONS AMONG PATIENTS WITH FEBRILE NEUTROPENIA AFTER CHEMOTHERAPY AND INFECTIVE ENDOCARDITIS

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At the Department of Medicine I (Oncohematology) of the Central Military Hospital of Hungarian Defense Forces was performed a study by Oncohematology team in two periods from the 1995 to 1997 and from 1998 to 2001 to analyze infections among patients with febrile neutropenia after chemotherapy. The aims of the study were: i. To survey the frequency and distribution of microbiologically and clinically defined infections in febrile neutropenic patients after chemotherapy in two study periods. ii.To examine susceptibility conditions of those antibiotics, which we use in the therapeutic protocol. iii. To survey the frequency of pneumonia and his role in the mortality in two study periods. During the 132 febrile neutropenic episodes after chemotherapy we observed microbiologically documented infections in 50.8%, and clinically documented infections in 21.2% of cases. There were 47 bacteremia and 2 fungemia. In the case of bacteremia we verified Gram-positive organisms in 70.2%. Isolates of coagulase-negative staphylococci from blood stream infections were resistant to oxacillin. We have not noticed glycopeptide resistant strains. In one case we found imipenem/cilastatin resistant *Pseudomonas aeruginosa* strain. Among antibiotics which we use in the antimicrobial guidelines we have not noticed antibiotics that are resistant to Gram-negative strains. In our study among 132 febrile neutropenic episodes after chemotherapy we observed pneumonia in 20 cases (15.2% of documented infections) and in 5 cases played role in the lethal outcome. We reported a 43-year-old patient with septic aneurysm complicated by rupture of pyelon due to *Salmonella* Enteritidis, which we treated successfully by combination of conservative method and surgical intervention. We described the epidemiology, microbiological characteristics, and outcome of infective endocarditis (IE) in patients treated in Military Hospital Budapest. The study aimed to compare characteristics of community acquired and health-care associated IE. In our retrospective study we reviewed clinical data of adult patients with the diagnosis of IE between July 2007 and July 2010. A series of 61 patients with 62 episodes were analyzed.
The overall in-hospital mortality rate was 22.6% (14/62). The most common pathogens were *Staphylococcus* sp. (23/62 cases, 46%) and *Streptococcus* sp. (19/62 cases, 38%).

MONITORING OF THE AFLATOXIN B1 CONTAMINATION OF CORN-SILAGES

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In 2012, the Hungarian dairy industry was shocked by the high Aflatoxin M contamination of milks. As it was clarified later, this was caused by the contamination of corn-silage by aflatoxin B1

produced by *Aspergillus flavus* strains. The aim of our research was the elucidation of the mechanism of AFB1 contamination. *A. flavus* can infect corn before the silage-producing state or may colonize the mature, opened silage, and significant increase in the content of aflatoxins may occur. For elucidating the mechanism, two different types of silage were made in model silos of 150 l final volumes with temperature and pH control. In the anaerobic silo model, corn mass was infected with high AFB1 producer *A. flavus* ZT41 in triplicate before silo assembling. For preventing high AFB1 contamination, two parallel silos were co-inoculated with atoxinogenic *A. oryzae*, and with a lactobacillus-rhodococcus mix inoculant. The used lactic acid bacterium strains are highly antagonistic against molds, while *Rhodococcus erythropolis* Ni1 is one of the best AFB1 degrader microbe. During the 5 months anaerobic- and 4 months aerobic silo experiment, the pH, temperature, and AFB1 concentration by HPLC-analytical method were measured. Bacterial and fungal microbiomes were monitored by classical microbiological and NGS methods. Fungal isolates were grown on malt peptone broth and incubated at 25°C for 3 days. DNA was extracted from mycelia using the Masterpure™ yeast DNA purification kit. For species identification, partial calmodulin and ITS sequences were amplified. Species delimitation was based on calmodulin sequences in the case of strains presumably belonging to the genera *Aspergillus* or *Penicillium*, while ITS region was used for the identification of species from others than the abovementioned genera. Sequences were aligned by PRANK v.140603 with default settings. From our results it seems that contamination of the opened silos by *A. flavus* is the main source of the AFB1 contamination. In the anaerobic silos, the concentration of AFB1 remained constant during the experiment and was almost identical with the AFB1 content of the silos with *A. flavus* ZT41 inoculation. The opened and *A. flavus* contaminated silos showed the highest AFB1 concentration - around 1 ppm.

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THREE DECADES IN WINE YEAST RESEARCH

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In spite of all the technological advancements in recent times, quality wine is still produced from fruit (in Hungary mainly grape) juice by fermenting yeasts. Some thirty years ago, a research line focused on wine yeasts was established at the University of Debrecen, Hungary. Since then the team has been working on numerous research projects, occasionally in collaboration with winegrowers, and has published a large number of research papers, reviews and book chapters in the international literature. Over the three decades of research a culture collection has also been developed which is now the largest wine yeast collection in the country with its more than three thousand strains belonging to 35 species. We demonstrated that the continuity of the local yeast microflora over years is partially ensured by the yeasts that overwinter in mummified berries left behind on the vines at vintage. Strains have been isolated in 12 wine-growing regions and identified with molecular taxonomic methods. Two groups of isolates turned out to represent hitherto undescribed species. The analysis of molecular markers (e.g. RAPD, microsatellites, mtDNA RFLP, karyotyping) and physiological properties (e.g. fermentation vigor, utilization of carbon sources, temperature profiles, production of alcohol and other metabolites, antifungal antagonism, killer activity and sensitivity) of the isolates revealed very high diversity in Saccharomyces cerevisiae and a much lower diversity in *S. uvarum* and *Candida zemplinina*. *S. cerevisiae* has a very plastic genome that can change during fermentation

to adapt the physiology of the cells to the drastically changing environment. Molecular genetic tests demonstrated that yeasts previously thought to be *C. stellata* were most probably strains of *C. zemplinina*. The latter is a cryotolerant and osmotolerant species, common mainly in Tokaj. In this wine region the cryotolerant *S. uvarum* is also very common. The *S. bayanus* and *S. bayanus* var. *uvarum* strains found in previous studies most probably belong to *S. uvarum*, as we have never found them in wines. Genetic analysis of selected wine yeasts further revealed that the *Saccharomyces* strains are usually highly heterozygous and thus their single-spore clones (segregants) can show very diverse phenotypes. The technologically favorable elements of these phenotypes can be combined by hybridization. Strains of different species can also be hybridized but the hybrids are sterile. We found that the species are isolated by two postzygotic sterility barriers, the second being specific for yeasts. It turned out that the first barrier can be overcome by spontaneous tetraploidisation and the second barrier by malsegregation of the MAT-carrying chromosomes. After the breakdown of the sterility barriers the hybrid genome undergoes rapid reduction and allosyndetic recombination, leading to chimeric genomes in which genes of both species can be present in various proportions. The process allows gene transfer between species without the application of GMO methods.

METSCHNIKOWIA POSES A CHALLENGE TO RDNA-BASED BARCODING: BIRTH-AND-DEATH EVOLUTION OF THE ITS SPACERS

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Pulcherrimin-producing antagonistic *Metschnikowia* strains are common components of the yeast communities colonizing ripening fruits including grape and are usually present also in the yeast populations of the early phase of wine fermentation. Their taxonomic affiliation cannot be determined by sequencing the D1/D2 domains and ITS regions of the nuclear repeats coding for rRNA molecules widely used for barcoding of fungal species because of the single-nucleotide polymorphism (SNP) at certain positions of the sequences. We found that the polymorphism is due to an extremely high intragenomic diversity of the repeats [e.g. 1, 2]. In contrast to other yeasts, these *Metschnikowia* strains do not homogenize their rDNA repeats. Here we show that within the genomes of the type strains of *M. andauensis* and *M. fructicola* the ITS segments of certain repeats differ from each other at up to 38 polymorphic sites which is a difference much higher than the taxonomic thresholds proposed by Vu et al. [3] to discriminate yeast species and yeast genera. The nucleotide differences in these sites are entailed by drastic differences in the secondary structures of the transcripts, indicating that not all rDNA repeats may be functional. By searching the genome sequence of *M. fructicola* for rDNA repeats, we found that in contrast to most other yeasts, its repeats are not organized in continuous arrays, and many of them have truncate structures. We assume that the latter are most probably inactive, decomposing derivatives of once-functional rDNA units. The very high sequence heterogeneity, the scattered locations of the rDNA units in the genome and the presence of truncate units indicate that these species maintain the sufficient number of active rDNA units by the birth-and-death mechanism rather than by concerted evolution. Besides, the ITS segments of the two species appear to have evolved in interaction by reticulation. These peculiarities make the pulcherrimin-producing *Metschnikowia* species unique among the ascomycetous yeasts for which rDNA data are available. Their ITS sequences are not suitable for barcoding.

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EXAMINATION OF THE CONTENT OF THE MAIN ANTIBIOTIC RESISTANCE GENES OF INTESTINAL *BACTEROIDES* STRAINS FROM FIVE EUROPEAN COUNTRIES

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In an earlier study coordinated by ESGAI the assessment of the antibiotic resistance and the corresponding resistance gene levels of European clinical *Bacteroides* strains were determined. We aimed to record the similar data for intestinal isolates to see the situation and to gain information for the source of clinical strains. Intestinal *B. fragilis* group strains strains (n = 184, 25 *B. fragilis* and 159 non-fragilis *Bacteroides*) were isolated from stool samples of healthy and carbapenem-treated patients in five European countries using the novel *Bacteroides* chromogenic agar (BCA). Antibiotic susceptibilities were determined by agar dilution and carriage of eight antibiotic resistance genes (*cepA*, *cfxA*, cfiA, *erm(F)*, *nim*, *bexA*, *tetQ* and *tetX*) were assessed by previously established RT-PCR methods. The following prevalences were detected: *cepA* (10.9% for all isolates, 8.0% for *B. fragilis* strains) for *cfxA* (47.3%), *cfiA* (3.8% for all isolates, 28.0% for *B. fragilis* strains), *erm(F)* (39.7%), *nim* (0%), *bexA* (13.6%), *tetQ* (79.3) and *tetX* (26.6%). We found three *cepA* - *cfiA* double positive strains whose existence is curiosity. The resistance rates well correlated with the corresponding resistance genes for cefoxitin (*cfxA*, $r = 0.425$, $p < 0.001$), imipenem (*cfiA*, $r = 0.283$, $p < 0.001$), clindamycin (*erm(F)*, $r = 0.242$, $p < 0.001$) and tetracycline (*tetQ*, $r = 0.416$, $p < 0.001$).
Comparison of the gene content with clinical strains showed that the frequencies of *cfxA*, *cfiA*, *erm(F)*, and *tetX* were statistically higher in the normal flora group with the following p values <0.001, 0.18, 0.003 and <0.001. The occurrence of *erm(F)* and *tetX* correlated well ($r = 0.389$, $p < 0.001$) indicating possible common genetic carrier(s). The higher prevalence of some genes in the normal flora group may be explained by that there are two different *Bacteroides* populations in the gut, e.g. one the luminal from which the stool samples may originate and second the mucosa associated population from which the infections may arise. The intestinal *Bacteroides* population may be a reservoir for shedding antibiotic resistance genes as well.

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GENETIC VARIABILITY OF GRAPE BLACK ROT (*GUIGNARDIA BIDWELLII*) POPULATIONS IN HUNGARY

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Grape black rot, caused by the pathogen *Guignardia bidwellii*, is an economically important polycyclic disease affecting grape leaves and berries in most viticultural regions with humid growing seasons. *G. bidwellii* produces both ascospores and conidia in infected berries that have mummified and overwintered in the vineyard. Primary black rot infections on young leaves are caused by

ascospores liberated from mummies infected in the previous years. Conidia released by pycnidia are responsible for the rapid disease spread in the vineyard during the season. The aim of the present study was to investigate the genetic variability the populations of grape black rot in Hungarian vineyards, by comparing their polymorphism. We successfully amplified the intergenic spacer region of the nuclear ribosomal DNA repeat, portions of the translation elongation factor 1 alpha, calmodulin, and chitin synthase 1 genes, and two other genes encoding actin and ras protein. All amplicons were sequenced and determined to amplify the target gene. These regions are a potentially rich source of characters for grape black rot populations. However, the successful amplification of all loci was dependent on the quality of the template. We have collected environmental samples in the wine-country of Eger since 2010. Microsatellite markers (SSRs) have been one of the most widely used markers for genotyping eukaryotes, because they are highly informative, codominant, multi-allele genetic markers that are experimentally reproducible and transferable among related species. The DNA from environmental samples including mummified berries, foliar lesions and from fungal cultures was analyzed using 11 pre-selected SSR primers [1]. The all microsatellite loci was successfully detected in all examined *G. bidwellii* samples by Agilent 2100 Bioanalyzer automated electrophoresis system. Our observations are consistent with the existing data in the literature. Our results show that SSRs can be useful for characterization of grape black rot at molecular level. In additional experiments we would like to involve further wine-countries of Hungary in our sampling. Furthermore we would like to use the QIAxcel Advanced automates sensitive, high-resolution capillary electrophoresis system and the high-resolution DNA melting analyis, because these methods provide more accurate results for genotyping.

Acknowledgements: Supported by the GINOP-2.3.2-15-2016-00061 project.

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GENOTYPING OF *MYCOPLASMA HYOPNEUMONIAE* STRAINS FROM CENTRAL EUROPE

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Mycoplasma hyopneumoniae is a world-wide prevalent member of the class Mollicutes and the causative agent of porcine mycoplasma-pneumonia. *M. hyopneumoniae* infection can result in significant economic losses for the pig industry through growth retardation and the consequential secondary infections. Since vaccination is not able to completely inhibit the colonization of the respiratory tract, antimicrobial treatment may be also necessary to control the infection. Epizootiological investigations also support the prevention and eradication strategies, through tracing the spreading of *M. hyopneumoniae* strains between different swine herds. Isolation of *M. hyopneumoniae* is a time-consuming and fastidious process, therefore the polymerase chain reaction (PCR) based typing methods are practical tools of epizootic investigation. The aim of the present study was to compare the molecular typing methods multi-locus sequence typing (MLST), multiple-locus variable-number tandem repeat analysis (MLVA) and analyzing gene *p146*, and to evaluate the mentioned assays for phylogenetic and epidemiological purposes. Forty-four *M. hyopneumoniae* strains isolated from different Hungarian, Czech and Slovakian farms in 2015 and 2016 were

involved in the investigations. MLST schemes are based on housekeeping genes, which are relatively conserved regions in the genome. The conventional *M. hyopneumoniae* MLST uses the genes *efp*, *metG*, *pgiB*, *recA*, *adk*, *rpoB*, *tpiA*, but the use of a reduced set of three of the listed genes (*adk*, *rpoB*, *tpiA*) was suggested also earlier. In the present study, the seven- or three-gene based MLST assays resulted in the same resolution (27 sequence types) of the examined isolates. MLVA method is based on the analysis of highly variable tandem repeat regions.
The MLVA assay utilizing the loci P97 - RR1 and Locus1 extended with the analysis of the serine repeat numbers of gene *p146* revealed the highest discriminatory power among the compared assays, with 40 different types of the studied strains. The analysis of gene *p146* can also be accomplished independently or as part of the MLST assay. The independent analysis of gene *p146* distinguished 31 different types of the studied strains, but the clustering of the strains proved to be less reliable according to the comparison of all examined methods. According to the results, MLST based on the genes *adk*, *rpoB*, *tpiA* is suitable for phylogenetic purposes. Tandem repeat sequence analysis of loci P97 - RR1 and Locus1 and gene *p146* is appropriate for the resolution of common MLST sequence types, refining closer genetic relationships. The analysis of gene *p146* is suggested for epizootiological investigations in local outbreaks.

BACTERIOPHAGE C130_2 IS A MYOVIRUS REPRESENTING A NEW GENOTYPE AND INFECTING PATHOGENIC ENTEROBACTERIA

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Several members of the Enterobacteriaceae bacterial family are important enteral pathogens; many of them cause foodborne infections. In light of the serious problem of multidrug resistance, several enterobacterial pathogens, including *Escherichia coli* O157:H7, *Shigella* species and various *Salmonella* serovars have been targeted in studies involving bacteriophages as biocontrol agents to be applied on foodstuff. In the current study we characterized a bacteriophage isolated from cattle cheese. This bacteriophage, termed C130_2 shows Myoviridae morphology with an approx.75 x 78 nm icosahedral head and a 115 nm long contractile tail. C130_2 is capable of lysing *E. coli* K-12, EHEC O157:H7, enteropathogenic (EPEC), enteroinvasive (EIEC) and *Shigella* strains with efficiency of plating (EOP) between approx.10^{-2} to 2 x 10^{-8}. The genome of the phage C130_2 is a 41,775 bp long, linear double-stranded DNA, with a GC content of 55.4%. We identified a total of 59 protein-coding sequences (CDSs), but no tRNA genes. At the nucleotide-level, the genome does not show close homology to any other previously sequenced bacteriophage.
Protein homology searches indicated that the predicted proteins show homology to genes of Enterobacteria phage IME_EC2 and *Klebsiella* phage vB_KpnS_IME279. For the PSI-BLAST hits, CDSs showed an average coverage of 93.3% a low average homology of 47.4%. The Uniprot hits showed 99% average coverage, and 76% average amino acid identity. Whole genome-based phylogenetic analysis has shown that C130_2 indeed represents a completely new genotype of bacteriophage within the order Caudovirales. Our study showed that food could contain unknown phages, which could be effective agents to be used against foodborne pathogenic bacteria. Genomic characterization of such phages could reveal hitherto unknown genes regulating host specificity, or playing key role in the lytic cycle and the survival in the environment. Given its host spectrum,

bacteriophage C130_2 could be a promising candidate for experiments aiming at the eradication of EHEC O157:H7 and *Shigella* strains from foodstuff and other environment.

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IDENTIFICATION AND CHARACTERISATION OF TWO RV5-LIKE COLIPHAGES DIRECTED AGAINST A WIDE RANGE OF ENTEROBACTERIA

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Foodborne bacterial pathogens, especially highly virulent species and pathotypes like enterohemorrhagic *Escherichia coli* (EHEC) and *Shigella* still pose a significant public health problem and are a serious concern in food safety. The counter-indication of antibiotic usage against EHEC and the rising prevalence of antibiotic resistance in several other enterobacterial pathogens prompted an interest in alternative antibacterial agents. Research efforts have renewed on bacteriophages capable of lysing foodborne pathogens, and their possible therapeutic or preventive application, the latter of which is often termed biocontrol. In the current study We isolated and characterized two novel lytic bacteriophages from independently collected food samples. The phages produced clear plaques with a burst size of approx. 1,000 viral particles and a latent period of 60 minutes. Morphological investigation indicated that the new phages, termed C203 and P206 are members of the family Myoviridae with an approximate head length of 85 nm, tail length of 75 nm, and a head width of 96 nm. C203 and P206 exhibit a broad and uniform host range, which included EHEC strains of serogroup O157, multi drug resistant (MDR) *E. coli* strains of various sero- and pathotypes, and both *Shigella sonnei* and *S. dysenteriae* strains. C203 and P206 both effectively reduced the number of living EHEC O157:H7 Sakai in experimentally inoculated minced meat. Nucleotide sequence analysis revealed that these phages have linear double-stranded DNA genomes comprising 138,073 bp with 213 CDS and 5 tRNA genes. The two genomes contain completely identical nucleotide sequence, albeit there is a 10,718 bp-long shift in the sequence. The GC content of the phage genomes was 43.7% and they showed high general homology to rV5-like bacteriophages. The genome of both phages contains a unique ORF that encodes for a putative phage homing endonuclease. The broad host range, the lack of any virulence related genes, their stability and short latent period suggest that these newly found phages could be suitable candidates as a bio-control agents against food-borne pathogenic Enterobacteria.

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UNEXPECTED DOMINANCE OF THE ACIII ACTINOBACTERIAL LINEAGE IN PLANKTONIC BACTERIAL COMMUNITIES OF ALKALINE SODA PANS CAUSED BY ZOOPLANKTON GRAZING

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Astatic soda pans of the Carpathian Basin are unique environments regarding their physical and chemical characteristics (high turbidity, pH and salinity coupled with ionic composition distinct from other saline lakes). According to current knowledge, soda pans in Europe are restricted to this area. Fish are absent from these lakes, but microcrustaceans, especially the natronophilic *Arctodiaptomus spinosus* (Copepoda: Calanoida) and *Moina brachiata* (Cladocera) can be abundant by late spring. Unlike other soda lakes, these pans are relatively easily accessible, which provides a unique opportunity to investigate the seasonal dynamics of soda lake planktonic bacterial communities. In our study, two alkaline hyposaline soda pans were sampled monthly between April 2013 and July 2014 to reveal seasonal changes in the structure of bacterioplankton and related these to changes in biotic and abiotic parameters. Sós-ér pan has 'non-turbid, colored' water with high amounts of dissolved humic substances, and has extensive marshland vegetation around its shoreline. Zab-szék pan represents the 'turbid-white' type of soda pans, dominated by large amounts of suspended clay particles with a macrovegetation-free shoreline. By late spring/early summer in both years, a sudden change in the community structure was observed in the two lakes. The previous algae-associated Flavobacteriia-, *Cytophaga*- and Rhodobacterales-dominated communities collapsed, and Actinobacteria, characterized by the acIII lineage, became dominant, with sometimes up to 88% relative abundance within the communities. Before the actinobacterial peaks, extremely high abundance (>10,000 ind./L) of microcrustaceans were observed. Previous studies showed that planktonic actinobacteria are more resistant to grazing than other freshwater bacteria due to their small cell size and the presence of S-layer in their cell wall. Although little is known about the acIII lineage of Actinobacteria, we additionally identified this group from other soda lakes situated in the Carpathian Basin and different regions of the Eurasian Steppe.
OTU-based statistical approaches showed that besides algal blooms and water-level fluctuations, zooplankton densities had the strongest effect on the composition of these bacterial communities, which implies a strong community shaping role of microcrustacean grazers.

MYCOTOXIN ANALYSES OF HUNGARIAN BREAST MILK SAMPLES

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The *Aspergillus* fungi produced aflatoxin B1 (AfB1), and ochratoxin A (OTA) mycotoxins have outstanding importance. While the previous one is a cytotoxic, and human mutagenic and carcinogenic compound, the latter has verified neurotoxic and mutagenic effect, and classified as possible human carcinogenic (IARC Group 2B) agent. Mycotoxins are stress produced compounds, thus their production will be intensified as the environmental impacts are changed, but have no effect

on the growth of producing microscopic fungi. Environmentally hazardous aflatoxins nowadays are presented in the far Southern and Eastern regions of Europe what could be one of the negative effects of climate change. The orientation of the mycotoxin problem headed toward north, thus it is becoming a forbidding source of health risking hazard in Hungary. The growth of hazardous fungi and OTA production are favored by inadequate methods of storage and drying of agricultural products (eg. cereals). Based on these information breast milk samples from Hungarian volunteering mothers were collected to measure AfM1 and OTA exposure of new born babies. With regard to that AfM1 in mother's milk is indicator of B1, because this is the end product of AfB1 metabolism, but OTA is not degraded in human body. The members of Hungarian Child Care Officers Association (MAVE) were connected to the scientific work. Until July 15, 2018 around 150 breast milk samples were collected from volunteering lactating mothers as a result of our cooperation. The AfM1 and OTA content of milk samples were analyzed by the experts of Wessling Nonprofit Ltd. Based on our former results HPLC method was chosen for breast milk sample analysis. One third of collected samples, were originated from the capital city of Hungary, Budapest, others are from different regions of Hungary. In one hand AfM1 was not detected in our samples by the results of the laboratory (detection limit value was 8 ng/kg). On the other hand OTA was detected in several cases. The results are typically not exceeded significantly the detection limit value (1 ng/kg), but one donor sample contained hazardous concentration of OTA according to the EFSA recommendations.

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MOLECULAR AND FUNCTIONAL ANALYSIS OF THE COTH GENES ENCODING SPORE COAT-LIKE PROTEINS IN THE ZYGOMYCETE FUNGUS *MUCOR CIRCINELLOIDES*

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Some members of the order Mucorales, such as *Rhizopus oryzae* and *Lichtheimia corymbifera* can be agents of opportunistic human infections, known as mucormycoses. *Mucor circinelloides* is a broadly used model organism in the studies on this fungal group. CotH proteins are widely present in Mucorales. Previous studies showed the importance of one of these proteins in the pathogenicity of *Rhizopus*. Construction of strains, in which the *cotH* genes are disrupted or overexpressed can provide an excellent tool to investigate the relevance of *cotH* genes in the pathogenicity and other biological mechanisms. The purpose of the present study was to disrupt the *cotH1*, *cotH2*, *cotH3* and *cotH4* encoding possible spore coat proteins in *M. circinelloides*. The applied plasmid free CRISPR/Cas9 system proved to be efficient and reliable; the RNA guided mutagenesis could be achieved by transforming the target fungus with the linearized deletion cassette, the *in vitro* synthesized guide RNA (gRNA, i.e. the CRISPR-RNA (crRNA) and the trans-activating crRNA (tracrRNA) complex) and the Cas9 enzyme without an *in vitro* RNP formation and the using of plasmids. In this strategy, the components of the CRISPR/Cas9 system were introduced together into the fungal cells by the polyethylene glycol (PEG)-mediated protoplast transformation method. The linearized deletion cassette was used as a template DNA for the HR repair. During the targeted mutagenesis, the *pyrG* gene was used as a selection marker and to disrupt the *cotH* genes. The

mutation was proven for each targeted gene by PCR analysis and the *pyrG* gene was found to be integrated at the expected positions in all isolated disruption mutants.
Lack of the appropriate transcripts was proven by qRT-PCR. Micro- and macromorphological characterization of the resulting strains were carried out. Growth ability of the mutants, spore germination and the effect of cell wall stressors calcofluor white (CFW) and Congo red (CR) on fungal growth were tested. Phagocytic assay was performed with the standard macrophage-like cell line J774.16. An alternative invertebrate model, *Drosophila melanogaster* was also involved to study the pathogenicity of the mutant strains.

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CHARACTERISATION OF NOVEL SURFACTINS AND EFFECTS OF DIFFERENT CULTIVATION PARAMETERS ON THEIR PRODUCTIONS

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Surfactins are cyclic lipopeptide-type biosurfactants produced mainly by *Bacillus subtilis* strains consisting of a β-hydroxy fatty acid of various chain length and a peptide ring of seven amino acids linked together by a lactone bridge, forming the cyclic structure of the peptide chain. Surfactins are proved to exhibit various biological activities including their antimicrobial and anti-inflammatory activities. Based on these properties, their therapeutic and environmental applications are considered. In this presentation, our recent results on the investigation of these molecules will be summarized. In our studies a mixture of surfactins were extracted from *Bacillus subtilis* strain SZMC 6179J and these extracts were examined by HPLC-ESI-IT-MS technique to reveal novel lipopeptide varieties with altered peptide sequence and to characterize their structures. Firstly the full scan MS measurements were used to identify the potential surfactin variants, then the MS2 spectra analyses of the sodiated molecular ions were applied for the structural elucidations. Altogether, four new, previously unknown groups of surfactins were discovered containing the replacement of the leucine residue by valine in position 2 ([Val2]) an a glutamic acid residue replacing aspartic acid in the fifth amino acid position ([Glu5], [Glu5,Val7], [Lxx4,Glu5]). Thus, until now, altogether, 33 variants were described based on their chemical compositions varying in the length of the fatty acid chain and in the sequence of the amino acids of the peptide ring. Since there was no information available about dependence of their productions from different cultivation parameters, the effect of both the different carbon sources and various metal ions were examined. Based on our investigations, the lipopeptide profile is changed due the altered cultivation conditions resulting in varying rates of the different isoforms produced, which showed selectivity for specific variants in certain cases. Furthermore, the application of metal ions in the media leaded also to the appearance of new surfactin variants.

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SELECTIVE ENRICHMENT AND ISOLATION OF PAH-DEGRADING AND BIOFILM FORMING BACTERIA

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Polycyclic aromatic hydrocarbons (PAHs) are recalcitrant, ubiquitous polluting compounds released into the environment either from natural or anthropogenic sources. According to U.S. Department of Health and Human Services 15 PAHs are listed as being carcinogenic. Hence elimination of PAHs from the environment is a must. Bacteria able to use PAHs as carbon and electron source under strict aerobic conditions are widespread and have been widely described. However, little is known about PAH biodegradation under oxygen-limited or strict anoxic conditions. Therefore, in this study under aerobic and oxygen-limited conditions the enrichment of PAH-degrading bacteria was aimed. The research is based on a bacterial biofilm, developed in an oxygen-limited hydrocarbon contaminated groundwater, which previously served as a reservoir for isolation of aliphatic and simple aromatic hydrocarbon degrading bacteria. The initial bacterial community alteration which occurred due to the selective enrichment was followed by terminal restriction fragment length polymorphism. The isolation of PAH-degrading bacteria from the enrichments also took place.
The biofilm forming potential and PAH-degrading (naphthalene) ability of the isolates was determined. Prolific biofilm forming and effective PAH-degrading isolates can be used in the future for bioremediation purposes in form of reactive biobarriers.

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SIZE-DEPENDENT ACTIVITY OF SILVER NANOPARTICLES ON THE MORPHOLOGICAL SWITCH OF DIMORPHIC YEAST

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The morphological switch between yeast and hyphal form is one of the most important virulence factors of opportunistic pathogenic yeasts. This conversion is necessary for the formation of a biofilm either on inert or on biological surfaces. Sessile cells in the biofilm are protected from antifungal agents and are therefore intrinsic sources of recurrent infections. In line with this, there is an ever increasing demand to identify novel antifungal agents or develop methods to inhibit the morphological change and the biofilm formation of such pathogens. The potent antimicrobial activity of silver nanoparticles (AgNP) has already been demonstrated against approximately 650 different pathogenic species, thus nowadays nanosilver is considered as the most viable alternative to antibiotics. The inhibitory effect of AgNPs on biofilm formation of *Candida albicans* has also been recently confirmed. Therefore, in this study, we aimed to investigate the potential of AgNPs on the morphological switch of dimorphic yeasts and the dependence of this biological feature on the nanoparticle size. AgNPs in three different sizes were prepared by chemical reduction method and

characterized by transmission electron microscopy, dynamic light scattering and ultraviolet–visible spectroscopy. Microdilution assay was carried out to determine the minimal inhibitory concentration of AgNPs on yeast cells. The morphological state of the cell populations was detected by flow cytometry and by scanning electron microscopy. Biofilm formation of the AgNP-treated and control cells was detected by XTT assay. The same method was applied to check the biofilm degradation ability of AgNPs. Citrate stabilized AgNPs were successfully synthesized and the average diameter of the obtained particles was proven to be of 5, 10 and 20 nm, respectively. AgNPs of 5 nm diameter exerted the most effective biological activities since these were highly cytotoxic to all the tested species. It is also noteworthy, that *Pichia membranifaciens* and *Lodderomyces elongisporus* proved to be the most susceptible to the AgNP-induced toxic effects. Hyphae development as well as biofilm formation was inhibited to the greatest degree by the 5 nm AgNPs compared to larger counterparts, however, the biofilm degradation capacity of the nanoparticles was found to be negligible. These results suggest that 5 nm AgNPs exhibit considerable potential to modulate morphological switch and thereby the virulence of several opportunistic pathogenic yeasts.
Ongoing research is focused on HaCaT human keratinocytes, co-cultured with the examined fungi and treated with differently sized AgNPs, to find out whether AgNPs are able to influence the viability of host cells by modifying the morphology of pathogenic yeasts.

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SHEEP RUMEN CONTENT AS A NOVEL AGENT FOR INTENSIFYING OF BIOGAS FERMENTATION

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Fossil fuels are the most common energy sources all over the world. Nowadays, the extensive usage of energy sources has been leading to serious environmental problems such as air, water or soil pollution and it has been causing greenhouse effect promoting climate change. Moreover, these non-renewable energy sources are being exhausted and available for limited time in the future. Therefore, finding novel, renewable environmentally sound energy sources/carriers replacing fossil fuels is amongst the most urgent scientific, economic and social challenges. Biomass is an intermediate energy store of the Sun energy which can be converted into various biofuels such as biogas, bioalcohols or biohydrogen. Biogas is one of the most common biofuels produced worldwide. Biogas is also produced by natural procedures including the digestive processes of ruminants e.g. sheep, cows, goats, etc.. Approximately 40 millions of sheep are slaughtered in the European Union every year according to Eurostat data. It produces huge amount of rumen content which is considered as waste. In this study, we examined the microbial composition of sheep rumen content and its effect on biomethane production in anaerobic digestive reactors. The samples were collected immediately after slaughtering and transferred into the laboratory for further usage. The genomic DNA samples from rumen content were isolated via ZymoResearch® Quick-DNA Fecal/Soil Microbe Miniprep Kit and used for metagenomic analyses (complete shotgun and 16S rDNA metagenomic analyses). The sequencing was performed on Illumina MiSeq® platform. The raw sequencing data were analyzed with CLC genomics workbench, MEGAN and Qiime Software. The composition of sheep rumen content microbial community provides a deeper insight into the rumen fermentation processes. Furthermore, we examined the effect of rumen content on biomethane production in anaerobic

digestion systems. We found that sheep rumen contents substantially intensified the biogas formation likely due to their high organic acid (acetic, butyric, propionic, valeric acids, etc) content. A comparison of the data obtained with cow and sheep rumen contents clearly indicated that sheep rumen materials had significantly higher effect on the biogas yield. Our experiments clearly proved that sheep rumen could substantially promote biogas yield in commercially used biogas reactors.

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COMPARISON OF CATABOLIC ACTIVITY PROFILES OF SOIL MICROBIOTA IN A GRASSLAND, A FALLOW AND MAIZE-WHEAT DICULTURE BY APPLYING MICRORESP AND MULTIRESP METHODS

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Land use change from the former grasslands or forests to arable land and the intensification of the crop production is considered to lead the loss of biodiversity and functions in soils. Nevertheless, results in this subject are quite controversial. The approach of the functional traits using catabolic activity profiling of the soil microbial communities is a simple and high throughput method and can be combined with the genetic diversity approach. In the present study, five long-term experimental fields on loess soils near Martonvásár were examined: 1) grassland with natural vegetation, 2) fallow with spontaneously developed vegetation on a former arable soil, and arable soils with 3) no fertilization, 4) NPK fertilization, 5) manure fertilization 4-yearly. The arable soil is a long-term maize-winter wheat diculture experiment changing crops after 2 years. Soil samples were taken from the top layers in July 2017. MultiResp method using gas-chromatographic detection of evolved CO_2 after substrate addition and Microresp method using colorimetric microplate-based detection of evolved CO_2 were applied using the same 15 substrates (sugars, amino-acids and carboxylic acids). There were obvious differences in the results by comparing the two methods. To show the differences of soil samples according to their catabolic activity profiles, principal component analysis was used. The grassland, fallow and NPK fertilized arable soil samples clearly separated from all other soil samples; however, the unfertilized and manure fertilized arable soil samples did not separated by any of the two methods. To evaluate the responsibility of substrates for the soil groups' separation, SIMPER test was used. Generally, only one or a few, somewhat different substrates were responsible for the sample distinctions using multiResp and microResp methods. To explore the influence of soil physical or chemical properties on the catabolic activities, redundancy analysis was applied.

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INVESTIGATION OF LCR ACTIVITY OF HUMAN PAPILLOMAVIRUS 6 VARIANTS IN THE HEAD-NECK AND GENITAL REGIONS

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Our research group has previously found that polymorphisms of the long control region (LCR) as well as unique amino acid polymorphisms in virus proteins may be related to differences in severity of respiratory papillomatosis (RP) caused by human papillomavirus (HPV) 11. This led to the assumption that similar associations may also be applicable to HPV6. The aim of our work was to amplify and sequence LCRs of HPV6s found in samples from the head and neck or genital mucosa in order to identify nucleotide polymorphisms which may affect transactivating potential. HPV6 sequences from thirteen patients were analyzed; three HPV6b (from adult-onset RP) and ten HPV6vc (two from juvenile-onset RP, four from adult-onset RP, one from condyloma acuminatum, two from cervical atypia and one from a leukoplakia penis). LCRs were sequenced by the Sanger method; identified unique viral LCRs were cloned into pALuc reporter vectors and transformed into *Escherichia coli* XL-1 bacteria. Plasmids were purified, then transfected into HEP-2 cells and LCR activity was assessed by measuring luciferase activity in triplicates. HPV6b LCRs exhibited six polymorphisms (G7373T, A7585C, G7653C, C7654A, G7815C, G7860A), all of which excepting G7815C were present in all three LCRs sequenced. Seven of the ten HPV6vc LCRs were identical to the reference sequence (AF092932), two harbored single unique polymorphisms (A7332C and T7909G), while a single sequence from cervical atypia showed four unique polymorphisms (C7613G, T7626C, C7669G, C7900A). The transactivating potential of all three HPV6b LCRs were comparable to the reference and to each other. In case of HPV6vc, as expected, sequences identical to the reference LCR showed similar LCR activities. The A7332C polymorphisms did not influence LCR activity markedly. The T7909G polymorphism was associated with increased LCR activity (HPV6vc from a recurring vulvar condyloma). The sequence with four unique LCR polymorphisms (from cervical atypia) showed markedly decreased LCR activity. Sequence similarity of Hungarian HPV6b sequences suggest that these may represent the wild type LCR. However, this does not seem to differ functionally from the reference, thus it is unlikely that LCR plays a major role in determination of disease severity. The unique polymorphisms in HPV6vc LCRs, in contrast, were associated with altered LCR activity, suggesting that, similarly to that found in case of HPV11, LCR activity may have an impact on the severity of the disease caused.

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BACTERIA CONTRIBUTE TO THE BIOGEOCHEMICAL CYCLES OF NITROGEN AND SULFUR IN LAKE FERTŐ

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Lake Fertő, the westernmost steppe lake (pH 8.3 - 9.7) of Europe is located in the Hungarian - Austrian border and 85% of the area is covered by reed (*Phragmites australis*). In the macrophyte-dominated littoral systems, where the considerable amount of the DOC originates from aquatic macrophytes, the knowledge of the role of the bacteria is limited. The aim of the present study was to reveal the bacterial communities in a macrophyte-dominated shallow lake and to elucidate their role in the biogeochemical cycle of nitrogen and sulfur. Therefore the bacterial community of the water and sediment of an open water region and two macrophyte-associated areas (an inner pond and a

reed-covered area of the external belt) were investigated in November 2015 and in July 2016 using 16S rRNA gene amplicon sequencing and cultivation techniques. Bacterial communities of the sediment and water samples showed significant differences, although the relative abundance of Proteobacteria was high in all samples. Several uncultivated taxa were detected. In the open water, the acTHI clade, the acIV clade (Actinobacteria) and genera *Synechococcus* were dominant. Planktonic bacterial community of the inner lake and the reed-covered area showed significant similarities: genera *Flavobacterium*, *Fluviicola* (Bacteroidetes), members of '*Candidatus* Aquiluna' and an unclassified member of family Comamonadaceae (Betaproteobacteria) were identified with high relative abundances. In the sediment samples Anaerolineaceae, group GIF9 (Chloroflexi), *Thiobacillus* and the unclassified group SVA0485 (Deltaproteobacteria) were dominant. The MPN of heterotrophic bacteria were always higher in the reed-covered areas. As the members of N-cycle, N_2-fixing (*Synechococcus*, *Prochlorotrix*, *Pseudanabaena*), ammonia-oxidizing (*Nitrosomonas*, *Nitrosococcus*), nitrite-oxidizing (*Nitrospira*) and nitrate-reducing (*Thiobacillus*, *Pseudomonas*) bacteria were identified. Both in the water and sediment samples sulfide/sulfur-oxidizing bacteria were detected: members of aerobic chemolitotrophs (*Thiovirga*, *Thiobacillus*, *Thiocapsa*, *Beggiattoa*) and anoxigenic phototrophs (*Chlorobium*, *Roseiflexus*, *Chloronema*, *candidatus* Chlorotrix, *Rhodobaca*, *Rhodobacter*) and in the sediment samples, sulfate-reducing bacteria were identified (*Desulfobulbus*, *Desulfocapsa*, *Desulfobacca*, *Desulfatiglans*, *Desulfatirhabdium*, *Sulfuricurvum*, *Sulfuritalea*). Overall we can state that bacteria present in the Fertő strongly contribute to the transformation of the elements in the water as well as sediment of the lake.

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INVESTIGATION OF THE ZINC UPTAKE SYSTEM OF THE HUMAN FUNGAL PATHOGEN *CANDIDA PARAPSILOSIS*

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The *Candida* species are among the most common causes of diseases that specially affects immunocompromised and hospitalized patients with serious underlying conditions worldwide. *Candida albicans* is reported as the dominant species isolated from these conditions and the most extensively studied of them all. However over the past three decades, the incidence of *Candida parapsilosis* has extremely increased. Many studies show that *C. parapsilosis* outbreaks of infection in neonatal intensive care units, where the role of healthcare workers were established in transferring the pathogen from patients to patients. Both species has several virulence factors to adapt the host's environment. These factors include adhesins and invasins, as well as the ability to form biofilms and hydrolytic enzymes, such as the acidic proteinases, phospholipases and lipases. It was established that *Candida* species are able to obtain growth-limiting heavy metals (such as copper, iron, zinc ions) from their environment, so it can be considered as a virulence factor as well. One of these trace elements is zinc, that plays a very important role as a cofactor of proteins and also play an important role in the elimination of microorganisms in the phagocytosis, where in most cases a high zinc ion concentration occurs. Nevertheless during host - pathogen interaction the host is keeping the concentration of free zinc ions as low as possible to defend, so it is not available for most microbes. Therefore, pathogens need a zinc transport system that allows them to access bound zinc ions from the hosts environment during the infection process.

In the case of *C. albicans*, the relevance of the mechanism of zinc ion recruitment is intensively studied, but there is less information available on the similar process in *C. parapsilosis*. Thus we aimed to *in silico* predict the zinc transporters in *C. parapsilosis* and create homozygous knock out mutants and expose them to various type of stressors and zinc limiting conditions. We have identified six genes, but only two (CPAR2_210740 and CPAR2_212100) showed difference in zinc uptake compared to the wild type strain. In addition, we analyzed these mutants kinetics of uptake by murine macrophages and their killing efficiency during *in vitro* infection. Our work is the first that provides data on the components of *C. parapsilosis* zinc ion uptake.

PRODUCTION AND PARTIAL CHARACTERIZATION OF IMMOBILIZED *RHIZOMUCOR MIEHEI* BETA-GLUCOSIDASE

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Enzymes as biocatalysts make many industrial processes more economical and ecofriendly; however, the main limitations of their applications are the moderate-efficiency of conversion and the recovery of the enzyme. Immobilization of enzymes to an appropriate carrier can improve the activity and stability and allow the reusability in multiple reactions. Therefore, enzyme-carrier complexes could be applicable more economically in the industry than the free enzyme preparations. Beta-glucosidase enzymes are frequently used biocatalysts in both hydrolytic and synthetic processes; they have been extensively utilized in the synthesis of many biologically active compounds and natural products. In our previous works, a *Rhizomucor miehei* isolate was identified as high-yield beta-glucosidase producer under wheat bran-based solid conditions. Then, the extracellular enzyme was successfully purified through sequential chromatographic steps. Here, our aim was to produce an immobilized *R. miehei* beta-glucosidase biocatalyst that possesses improved reusability with utilizable biochemical properties. For this purpose, two approaches were tested: i) entrapment in calcium-alginate beads and ii) covalent binding to different particle-size corncob granules using glutaraldehyde as crosslinking reagent. Both the applied techniques proved to be efficient to immobilize the enzyme. The *R. miehei* beta-glucosidase-corncob granule complexes showed high hydrolytic activity, proved to be reusable, and the temperature and pH optimum of their activity were comparable to those determined for the free enzyme. After optimization of the process, entrapment in calcium alginate beads also resulted in stable and reusable enzyme-carrier complexes. Using 1.5% alginate and 1 M $CaCl_2$ as reaction surrounding, the obtained enzyme-support complex was stable for up to five cycles retaining 65% of its hydrolytic activity at the fifth recycle step. Till date, as we know, there are no data about the immobilization of *R. miehei* beta-glucosidase to a support.

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CARBOHYDRASE-ASSISTED EXTRACTION OF BIOACTIVE PHENOLIC COMPOUNDS FROM FRUIT RESIDUES

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Application of plant derived phenolics in functional foods has increased on the past years. Though many of these phenolics have antioxidant and antimicrobial capacities, their bioavailability is often limited due to the glycosidic complexes formed. Carbohydrate-cleaving enzymes, however, can hydrolyze these bonds releasing the phenolic aglycone. Fruit residues are excellent substrates for the production, thus, we aimed to mobilize such bioactive phenolic compounds from oven-dried and lyophilized grape, apple and pitahaya (known as dragon fruit) residues via *in vitro* enzymatic treatment using *Rhizomucor miehei* cellulase and *Aspergillus niger* pectinase cocktails. Positive correlation between the total phenolic content and antioxidant activity was generally found after the enzymatic treatments. However, the antioxidant activity increase depended on the substrate pretreatment technique as well. Concentration of the major individual phenolics determined by HPLC changed by different degrees after the enzymatic treatments depending on the substrate and the pretreatment. In further studies, the antimicrobial and antibiofilm activities of the extracts were evaluated against foodborne pathogens and food spoilage bacteria. Then, we studied the anti-quorum sensing potential of the samples using the model organism *Chromobacterium violaceum*. We found that the overall inhibitory effect of grape extracts against most pathogens was slightly higher than that of apple and pitahaya samples. Furthermore, carbohydrase treatments have increased the initial antimicrobial and antibiofilm activity in many samples.
In our experiments, the most sensitive bacteria against the extracts were among the *Bacillus*, *Pseudomonas* and *Staphylococcus* strains tested. In general, carbohydrase treatments influenced positively the anti-quorum sensing properties of the extracts as well. But the effects were varied according to the type of the fruit, the pretreatment and the enzymatic treatments. Extracts with effective activity can be used as natural additives in food preservative development processes.

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EXAMINATION OF THE VIRULENCE OF *ACTINOBACILLUS PLEUROPNEUMONIAE* SEROTYPE 16

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Actinobacillus pleuropneumoniae is the causative agent of porcine hemorrhagic, necrotic pneumonia and fibrinous pleuritis causing high economic losses. It has two biotypes, 18 serotypes and several virulence variants. Serotype 16 of *A. pleuropneumoniae* was described in Hungary, and in has not been detected in other countries yet. The aim of the trial was examination of the virulence of the reference strain of serotype 16, *A. pleuropneumoniae* 85/14. Thirty 12-week-old piglets were divided in three groups and they were infected intra nasally with 10^8, 10^7 or 10^6 colony forming units of the bacterium. The clinical signs were scored for 6 days, their body mass was measured, the post mortem

and the histological lesions were evaluated. Clinical signs could be seen in all three groups already 6 hours after the infection; however they were not very serious.
Severity of the clinical signs and the number of febrile days was parallel with the infective dose and significant differences in the body mass were also evident. Typical post mortem and histological lesions could be observed in all groups, while piglets left at the place of origin remained healthy. The examination confirmed that the type strain of serotype 16 *A. pleuropneumoniae* 85/14 is able to cause typical clinical signs and post mortem lesions in 12-week-old piglets.

IN VIVO ANTIOXIDANT ACTIVITY EXAMINATIONS OF TOMATO EXTRACTS USING *SACCHAROMYCES CEREVISIAE*

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Tomato contains a favorable composition of phytonutrients, having a positive impact on human health. *In vitro* general antioxidant methodologies (e.g. FRAP, DPPH, TPC) used widely differ in sensitivity and selectivity, the applied chemical reactions are different to a natural system. In order to model biochemical and molecular mechanisms, lower organisms can be applied. Biological studies were done on the eukaryotic cells of *Saccharomyces cerevisiae* (NCAIM Y.00801) type strain. The hypothesis of the study was that when the yeast cells are supported by the antioxidants of the tomato extracts before the exposure to a stressing agents (H_2O_2) an increase can be experienced in the survival of the strain. The tomato contains a notable amount of β-carotene, the precursor of vitamin A, which is responsible for the functioning of eye. Ascorbic acid present in tomato is hypothesized to have a supportive role of human immune system against certain cancer types and cardiovascular diseases, as well as against DNA mutation induced by oxidative stress. The amount of ascorbic acid and polyphenolic compounds predominantly defines the water-soluble antioxidant status of a tomato sample. The aim of the *in vivo* methods is to overcome to the disadvantages of *in vitro* assays. The focus of such investigations is on the effect of a sample on a living organism. The *S. cerevisiae in vivo* test is a traditional method for modelling the reaction of eukaryote cells. In case of *in vivo* investigations, mortality rate following to stress factors (e.g. heat stress, UV radiation) are measured, which is capable to provide information about the protective effect of antioxidants against free radical generation. The *in vivo* methods are cheap, and less environment-dependent, however direct use of the results for monitoring the impact on human health is not possible, since it ignores the whole digestion system and the metabolic/detoxifying processes. There are limited sources about the comparison of the above mentioned antioxidant capacity assay types available. Thus, the extent of the correlation between *in vitro* and *in vivo* methodologies is unclear. In our case the *in vivo* survival tests are executed following to previous studies with the application of *Saccharomyces cerevisiae* strain NCAIM Y.00801. Hydrogen peroxide is used as a stressing agent, performed in three replicates. We aimed to develop and apply those *in vivo* methodologies, which can overcome the differences in selectiveness and sensitivity of *in vitro* antioxidant assays through the simulation of natural environments. It is believed, that the *S. cerevisiae in vivo* biological activity assays can model cellular physiological conditions, absorption, bioavailability and metabolic issues. We assume, that the results will be of higher relevance with regards to human utilization of antioxidants and contributes to the understanding of interdependence of different antioxidant assays.

CHANGES IN POPULATION STRUCTURE OF MULTIDRUG-RESISTANT *ACINETOBACTER BAUMANNII* IN HUNGARY BETWEEN 2010 - 2017

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Multidrug-resistant *Acinetobacter baumannii* (MACI) is one of the most difficult antimicrobial-resistant Gram-negative pathogens to control and treat. In MACI the prevalent carbapenem resistance mechanism is the production of carbapenem-hydrolyzing Ambler class D β-lactamases (CHDLs). The main acquired CHDLs belong to the OXA-23, OXA-40 and OXA-58 groups. Three prevalent, distinct lineages of MACI have been described in Europe termed International clone I (IC-I), IC-II, and IC-III. We analyzed the national genomic surveillance database for MACI in order to explore its population structure in Hungary during 2010 - 2017. MACI isolates from patients over the entire country submitted to the National Reference Laboratory (NRL) between 2010 - 2017, as well as two collections from two medical universities (Semmelweis University – n = 117, University of Debrecen – n = 22) from 2010 - 2012 and 2016 - 2017 were investigated. Based on pulsotype analysis and antibiotic resistance data we selected 53 MACI isolates for whole genome sequencing (WGS) performed by Illumina 250-bp paired-end method. Genomes were searched for MLST-Pasteur, MLST-Oxford, core genome (cg)MLST by SeqSphere+ (Ridom) and for identification of acquired antimicrobial resistance genes by ResFinder tool. Among 922 MACI isolates genotyped in the NRL IC-II clone (54%), IC-I clone (23%) and ST636 as singleton clone (19%) proved prevalent in this period. Five different sequence types (STs) associated with variable CHDL-group distribution (OXA-23 44%, OXA-58 26%, OXA-72 (belonging to OXA-40 group) 19%, OXA-23 + OXA-58 8% and OXA-23 + OXA-72 3%) consisted of 85% of all investigated isolates. IC-II was the most prevalent clonal lineage between 2010 - 2015, and ST2/IC-II was the dominant sequence type. AC033 pulsotype/ST2/OXA-23 (6%) and AC053 pulsotype/ST2/OXA-23 + OXA-58 (8%) uniformly harbored *armA* gene encoding 16S rRNA methyltransferase. ST636/OXA-72 emerged in 2015 and became widespread by 2016. In 2017 62% of all MACI isolates belonged to this clone. MACI isolates carrying two CHDLs (OXA-23 + OXA-58 in ST2, OXA-23 + OXA-72 in ST636) appeared first in 2015 and their proportion rose to 41% by 2017. Similar changes were found in strain collections from both medical universities comparing the periods 2010 - 2012 and 2016 - 2017. ST2/OXA-23 (40%) and ST1/OXA-23 (39%) were the prevalent types in 2011/2012, while ST1 disappeared and ST636/OXA-72 (82%) and ST2/OXA-58 (8%) became dominant types by 2016 in the Semmelweis University. Although ST2/OXA-23 was the prevalent clonal lineage in the University of Debrecen in 2010 it belonged to a different cluster from other ST2 strains investigated in this study by WGS and harbored armA. In 2017 this unique ST2/OXA-23 cluster with *armA* remained prevalent, but ST636/OXA-72(+/-OXA-23) clone also became dominant and a new IC-II clone, ST492/OXA-72 with *armA* emerged. Major changes were observed at institutional and at national level in population structure of MACI during the study period. Emergence and spread of ST636 singleton clone with OXA-72 as well as double-carbapenemase harboring strains in a short time is of great concern. Investigations are needed to ascertain the reasons for their emergence.

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ACTIVATION OF HUMAN NEUTROPHIL GRANULOCYTES IN RESPONSE TO THE MELANIN PRODUCING FUNGUS, *CURVULARIA LUNATA*

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Members of the genus *Curvularia* are melanin producing filamentous fungi that can cause both plant and human infections. *C lunata*, *C. spicifera* and *C. hawaiiensis* are opportunistic human pathogenic species typically causing phaeohyphomycoses, which can manifest as local infections (e.g. keratitis, sinusitis and cutaneous lesions) in immunocompetent or invasive mycoses with frequent involvement of the central nervous system in immunocompromised patients. Although their plant-fungal interactions have been intensively studied, there is only little information available about the host response to these fungi in human infections. The aim of this study was to investigate the neutrophil granulocytes' response to the hyphal forms of *Curvularia* in comparison with that to *Aspergillus fumigatus*. In the present study, *C. lunata* SZMC 23759 and *A. fumigatus* SZMC 23245, both isolated from human eye infection, were examined. Release of hydrogen peroxide by neutrophil granulocytes were measured in the presence and the absence of the supernatant of germinating conidia and after serum treatment. Activation and survival of neutrophils and killing efficiency towards *C. lunata* were checked after the interaction. ROS production of neutrophils in interaction with the fungi were compared. It is already known that *Aspergillus* species induce ROS production of neutrophils only after serum treatment. Similarly, *C. lunata* was also able to induce hydrogen peroxide release only after serum opsonisation. However, viability of *C. lunata* did not decrease when the hydrogen peroxide production was detected. Activation of neutrophils was observed only when the supernatant of the germinating conidia was added to the cells. It seems that recognition of *C. lunata* by neutrophil granulocytes is dependent on serum opsonisation, but activation is induced by a soluble factor produced by the fungus, which does not generate ROS production.

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THE EFFECT OF CHANGES IN ANTIBIOTIC CONSUMPTION ON RESISTANCE EVOLVES OVER TIME

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Trends in antibiotic consumption have a fundamental effect on antibiotic resistance patterns. It was documented earlier that antibiotic use and resistance evolve in a spiral-like manner; spread of resistance induces increased use of broader-spectrum drugs, which will provoke resistance against these, which in turn leads to preference and increased use of new drugs. Present work strives to follow up associations between drug use and resistance over time in order to capture changes in these relationships between 2004 and 2017. Monthly cephalosporin, carbapenem, fluoroquinolone, aminoglycoside and colistin consumption data were collected in defined daily doses/100 occupied bed-days (DDD/100 OBDs). Resistance levels were characterized by incidence densities/1,000

occupied bed-days (IDs). Resistance of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* was examined; these were added up to form a cumulative resistance for all major Gram negative pathogens. The resistance spiral was reconstructed as follows: cephalosporin consumption leads to cephalosporin resistance, which provokes carbapenem use leading to carbapenem resistance, which in turn results in colistin use. Associations were identified in vector autoregressive models using impulse-response functions. First we estimated associations identified in time series between October 2004 and August 2016 (143 months), then we compared these to those found when the study period was extended to September 2017 (156 months). All models systems were built in a rolling-window manner, i.e. series were truncated by the last years' months and models were built on all truncated series as well. An effect was accepted if significant in more than half of the rolling windows for a period. Considering all Gram negative bacteria together, the resistance spiral could be followed up from cephalosporin consumption to colistin use; in the later period the effect of cephalosporin resistance on carbapenem use became weaker, while the provoking effect of carbapenem use on carbapenem resistance was more marked. When considering different species separately, important changes were found in case of *K. pneumoniae*, *P. aeruginosa* and *A. baumannii*. An earlier absent effect of carbapenem consumption on carbapenem resistance of *K. pneumoniae* appeared in the later period. In parallel, this effect became weaker in case of *P. aeruginosa*. The effect of carbapenem use on carbapenem resistance of *A. baumannii* became more marked, and its effect on colistin use remained significant. Carbapenem resistance gained further importance as compared to the earlier (2004 - 2016) period, at least in *K. pneumoniae* and *A. baumannii*. Further increase in colistin use may be anticipated. Colistin resistance was present, but remained unchanged by colistin use in the models constructed.

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EXAMINATION OF ANTIFUNGAL ACTIVITY AND MECHANISM OF DE NOVO DESIGNED Γ-CORE PEPTIDE MOTIFS FROM *NEOSARTORYA FISCHERI* NRRL 181 ANTIFUNGAL PROTEINS

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Filamentous fungal infections of agriculturally important plants cause enormous crop losses worldwide in every year. The prevention of emergence and spread of pathogenic fungi represent a serious challenge for the agriculture both in pre- and postharvest conditions. This problem is further exacerbated by the emerging number of pesticide-resistant strains. There is therefore an urgent need to develop new, alternative pesticides. The evolutionary conserved γ-core motif constituted by [GXC]-[X3-9]-[C] of cysteine-rich antimicrobial proteins provide a feasible base for this purpose. Previous studies already demonstrated that the γ-core motif plays an important role in the antimicrobial activity and/or folding of antifungal plant defensins, and the synthetic γ-core peptide variants are active alone. This activity highly depends on the physicochemical properties of the constituting amino acids. The *Neosartorya fischeri* NRRL 181 antifungal proteins contain the γ-core

motif, which differ in physicochemical properties. The aim of the present work was to investigate the *in vitro* antifungal activity and mechanism of their synthetic variants. Peptides according to the native γ-core motif and their rationally designed variants to improve the antifungal activity were also examined in broth microdilution tests alone or in combination with succinate dehydrogenase inhibiting pesticides such as carboxin and fluopyran. Sixteen filamentous fungal isolates representing eight ascomycetous pre- and postharvest pathogenic species were involved in the tests. The native γ-core-containing peptides did not inhibit the fungal growth, but their positively charged and more hydrophilic rationally designed variants proved to be antifungal active, and they showed synergistic or additive interaction with the investigated pesticides.
Disruption of germ tubes is supposed as main antifungal mechanism based on the microscopic observations. Our results indicate that the de novo rationally designed γ-core peptides from this study provide promising bases to develop novel antifungal strategies in the agriculture.

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COMPARISON OF KILLING ACTIVITY OF MICAFUNGIN AGAINST SIX *CANDIDA* SPECIES ISOLATED FROM PERITONEAL AND PLEURAL CAVITIES IN RPMI-1640, 10 AND 30 PERCENT SERUM

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Echinocandin antifungals are the drugs of choice in cases of invasive *Candida* infections including those with pleural or peritoneal involvement, however pharmacokinetic and pharmacodynamic parameters of the aforementioned drug class in body sites other than the circulation are almost non-existent. In our study, we determined the *in vitro* activity of micafungin against *Candida* isolates originating from pleuritis and peritonitis cases in RPMI-1640 medium and RPMI-1640 supplemented with human serum (10% and 30%), simulating the protein environment of the pleural and peritoneal cavities. The micafungin concentrations we used were 0.25, 0.5, 1 and 2 mg/L based on previously published *in vivo* results of others. In RPMI-1640, micafungin was fungicidal against *C. glabrata*, *C. krusei* and *C. kefyr* within 2.27 ± 10.68, 2.69 ± 10.29 and 3.10 ± 4.41 h, respectively, while showed fungistatic activity against *C. albicans*, *C. tropicalis* and *C. parapsilosis* isolates. In 10% serum, ≥0.25, ≥0.5, ≥0.5 and ≥1 mg/L micafungin produced positive k values (killing) for all *C. albicans*, *C. glabrata*, *C. kefyr*, and *C. krusei* isolates, respectively. In 30% serum 2 mg/L micafungin produced killing against all *C. albicans*, *C. glabrata* and *C. kefyr* isolates, but was ineffective against *C. krusei*, *C. parapsilosis* and 2 of 3 *C. tropicalis*. Micafungin concentrations should exceed 2 mg/L at the site of infection to safely eradicate non-albicans species from peritoneal and pleural cavities.

GENETIC DIVERSITY AND METABOLIC ACTIVITY OF BACTERIAL COMMUNITIES ASSOCIATED WITH THE RHIZOSPHERE SOIL OF A MAIZE (*ZEA MAYS*) MONOCULTURE

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The recent challenges of society, agriculture and environment give the relevance of the sustainable agriculture in the 21st century. Nevertheless, a comprehensive and deep basic knowledge is needed for the large-scale applied solutions in the field of soil microbiology. Therefore, the present study, as a part of a comprehensive research project, aimed to examine the genetic diversity and metabolic potential of bacterial communities inhabiting the soil of an unfertilized maize monoculture. The samples were collected from a long-term field experiment maintained in Martonvásár (Hungary). Soil samples from a close-to-nature grassland were applied as controls. A and C layers were studied in each case. Next generation sequencing (NGS) on the gene of small subunit of prokaryotic ribosome (16S rDNA) and MicroResp™ substrate induced respiratory activity measurement were implemented to study and compare the microbial communities of a cultivated, but non-fertilized maize plot with the control area. Based on the analysis of an NGS dataset of 9,059 operational taxonomic units (OTUs) and 1,711 identified taxa, differences were found in the genetic diversity of bacterial communities according to both the sampling sites and soil layers. Using the non-metric multi-dimensional scaling (NMDS) method, the tilled layers of the maize monoculture clustered together and separated from the group containing layers A and C of the grassland; while the layer C sample of the maize monoculture diverged from all the others. In the soils of the grassland no higher bacterial taxonomic diversity was revealed than in cultivated soils. Our results only partly verified the decisive role of bacteria highlighted by the literature. Considering the results of MicroResp™ measurements, microbial communities from the surface layer (A) of grassland soils showed an outstandingly high metabolic activity. Nevertheless, there was low difference between the layers of maize monoculture than between the layers of the control area. The samples originated from soil layer A in Martonvásár grouped together using principal component analysis (PCA). According to our results, the genetic diversity and metabolic potential of bacterial communities of cultivated soils examined in this long-term field experiment showed remarkable differences compared to fallow land and grassland samples.

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OPTIMIZATION OF DNA ISOLATION VIA A PILOT NGS FOR STUDYING FUNGAL DIVERSITY IN THE (SEMI)ARID SANDY AREA OF A LONG TERM ECOLOGICAL RESEARCH IN THE KISKUNSÁG

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A multiannual extreme drought and rain manipulations (ExDRain-project) have been started recently to study the effect of local climate change in a multidisciplinary way in a sandy grassland near Fülöpháza. It is important to monitor fungal diversity during the experiment, as fungi have important roles in ecosystem functioning of these areas. Therefore our present aim was to reveal the diversity of soil fungal communities before the manipulation started, where the first step was the optimization of the community DNA extraction verified with a pilot Next Generation Sequencing (NGS). The

experimental site was sampled extensively exactly before the manipulations resulting in 12 composite samples. Community DNA was extracted from every composite soil sample with MoBio PowerSoil® DNA Isolation Kit in triplicate using about 0.3 g samples and MoBio PowerMax® Soil DNA Isolation Kit using 5 g samples. In this way we could test whether DNA extraction from smaller amount in triplicate can compensate one-fold DNA extraction from about 10-fold larger sample amount. For understanding of fungal communities, a pilot NGS was carried out with ITS1F-ITS4 primer pairs on Illumina platform using the above mentioned 4 parallel DNA extracts from 3 different samples. The raw sequences were processed and analyzed using the pipeline of the SEED sequence editor. The NGS resulted in 6,000 – 39,000 pruned ITS2 reads passing the pipeline per samples. Their rarefaction curves showed saturation for all the samples, but the 5 g samples revealed higher number of OTUs even if the OTUs of 3 parallel 0.3 g samples were averaged artificially per samples. Most of the sequences were classified to Ascomycota with Dothidemycetes and Sordariomycetes as the dominant classes and Eurotiomycetes, Leotiomycetes and Pezizomycetes as minor classes. Basidiomycota represented a less diverse group with an uncultured Tricholomataceae environmental sequence as the major dominant OTU in more samples, whereas 2 - 6% of the sequences were classified to Glomeromycota. Based on this result the DNA isolation kit processing 5 g samples will be used to analyze samples after manipulations. The changes in the fungal community composition due to the manipulations will help us to understand the role of fungi in ecosystem functioning accommodating to extreme drought and rain conditions.

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THE EVOLUTION OF RHIZOMORPHS IN FUNGI: PRELIMINARY RESULTS OF TRAIT ANALYSIS

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With the evolution of complex multicellular life, organisms had to circumvent of the limitations of diffusion. Plants, animals and even brown algae have developed special cells or tissues that facilitate long distance nutrient transport, yet how fungi overcame the constraint of diffusion is barely known. One complex multicellular structure of fungi that potentially ease the transportation of nutrients is rhizomorphs, which is an aggregation of hyphae with varying degrees of anatomical complexity across lineages. These structures can be found all over complex multicellular fungal lineages and in many ecological groups including parasites, saprotrophs and ectomycorrhizal symbionts. For example, rhizomorphs enable some *Armillaria* species to efficiently parasite trees or *Serpula lacrymans* to invade an entire house in a week. These examples show that the rhizomorphs have an essential role in the ecosystem, but questions such as how these structures evolved and are they homologous or they are results of convergent evolution, haven't been examined yet. Here, we provide a summary on the evolution of rhizomorphs based on morphological features. We reviewed more than 200 literature sources and assigned one of the following phenotypic categories to fungal species: (1) no rhizomophs, (2) loose aggregates of hyphae, (3) hyphal aggregations with inner anatomical differentiation and (4) hyphal aggregations consisting of wide vessel hyphae. Overall, we could code 469 species, all belonging to the subkingdom Dikarya. We found that out of the two phyla of this subkingdom, Basidiomycota contains more rhizomorph producing species than Ascomycota does. To test evolutionary hypotheses, we needed a robust and comprehensive phylogeny, thus we used a previously published mega-phylogeny containing 5,284 species from the subphylum

Agaricomycotina. We extrapolated our morphological data to the five thousand species so that we could infer transition rates between the four rhizomorph types by both maximum likelihood and Bayesian analysis in BayesTraits V3.0.1. The results suggested that the transition rate towards loose hyphal aggregates is higher than towards any other types of rhizomorphs. Also, we have found that the most advanced rhizomorph type could have independently evolved from non-rhizomorphic ancestors. These findings suggest that the simplest rhizomorph have frequently evolved relative to other types and that the most advanced type of rhizomorph is the result of convergent evolution.

FUNCTIONAL CHARACTERIZATION OF A NOVEL HYDROPHOBIC SURFACE BINDING PROTEIN IN MUCORALES

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Hydrophobic surface binding protein A is a small secreted protein found in eukaryotes. HsbA proteins typically range between 171 to 275 amino acids in length. The protein was firstly isolated from the culture broth of *Aspergillus oryzae* RIB40. That protein was found to be able to recruit cutinase 1 (CutL1) to the surface of hydrophobic solid materials (PBSA) and could promote the activity of the degradative extracellular enzymes. This protein also participates in fungal resistance to stress that could be caused due to toxicity of some aromatic compound or reactive oxygen species released during the degradation process. During infection of MH-S macrophages, *Lichtheimia corymbifera* expressed an Hsb-A like protein at high level. Hsb-A protein is functionally uncharacterized in Mucorales and its role in the host-pathogen interactions is yet unknown. The main objective of the current study was to characterize Hsb-A proteins and the encoding genes in *Mucor circinelloides*. We found six *hsb-A* genes in the *M. circinelloides* genome, which are homologous to the *L. corymbifera hsb-A*. Two genes (*hsb-A1* and *hsb-A2*) was found to be highly expressed during the life cycle of the fungus which was analyzed through qRT-PCR analysis. Hence, these two genes was used for our further studies. To determine the possible role of Hsb-A1 and Hsb-A2 in the pathogenesis of *M. circinelloides*, deletion and overexpression mutants were constructed. For overexpression, the gene was placed under the regulation of the strong gpd1 promoter. To create *hsb-A* knock out mutants, a recently developed CRISPR-Cas9 system was used. We conducted micro- and macro morphology assays on the deletion and overexpression mutants. Our other objective was to express Hsb-A proteins heterologously in *Pichia pastoris* and to purify the proteins. Hence, *Pichia* expression systems was obtained by transforming *Pichia pastoris* with pPICZα with *hsb-A* vector construct by electroporation and the positive transformants expressed Hsb-A1 and Hsb-A2 proteins in the culture supernatant which were then purified for further analysis.

ORIENTATION-DEPENDENT TOXIC EFFECT OF HUMAN PAPILLOMAVIRUS TYPE 33 LONG CONTROL REGION DNA IN *ESCHERICHIA COLI* CELLS

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For certain high-risk human papillomavirus (HPV) types, intra-type sequence variation was shown to be related with variable oncogenic potential. The functional analysis of HPV sequence variation requires the molecular cloning of different genomic regions of virus variants. Here, we report an unexpected difficulty experienced when trying to clone HPV 33 long control region (LCR) variants in *E. coli*. Standard cloning strategies proved inappropriate to clone HPV 33 LCR variants in the forward orientation into a eukaryotic reporter vector (pGL2-Basic). When a TA-cloning vector (pCR 2.1-TOPO) was used to clone HPV 33 LCR, only constructs were obtained that contained the LCR in the reverse orientation. However, by slight modification of culture conditions (incubation at 25ºC instead of 37ºC), constructs containing the HPV 33 LCR variants in the forward orientation were also obtained. These data indicate that there is a sequence element in the LCR of HPV 33 causing orientation-dependent toxic effect in *E. coli* depending on culture conditions. Sequence analysis revealed the presence of a putative open reading frame (ORF) in the 5' part of HPV 33 LCR potentially encoding a 116 amino-acid polypeptide.
Sequence similarity searches and protein structure prediction analysis suggest that this putative protein might have a structural similarity to transmembrane proteins. Even a low-level expression of this protein can be assumed to cause significant toxicity in the host bacteria. At the moment, we do not know whether this putative ORF is expressed in virus infected human cells.

TESTING THE MOLECULAR HOURGLASS HYPOTHESIS IN FRUITING BODY FORMING FUNGI

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Complex multicellularity evolved several times across the tree of life resulting in an enormous number of well-defined shapes. Although these shapes differ highly and they are established in many different ways, recent studies suggest that a common gene expression pattern may exist throughout the development of complex multicellular organisms. In animals, an hourglass-shaped gene expression pattern was reported to exist with genes expressed in the mid-developmental period being more conserved than the genes expressed in the early and late developmental periods. Also, between species within a phylum, the gene expression similarity of the "mid-developmental genes" is reported to be much higher than in the previous and in the subsequent periods. This phenomenon is called the molecular hourglass model. Comparing the relative age of the transcriptome of each developmental stages, the same hourglass-like pattern was detected at the molecular level in *Arabidopsis thaliana* and in the model fungus *Coprinopsis cinerea*. However, these studies failed to identify the factors that define this pattern. Moreover, they considered only one species for each group, which can lead to biased results about the existence of the molecular hourglass pattern in groups other than animals. Here, using RNA-seq data from generated from three to six developmental stages of the five fruiting body forming fungi, we have tested the molecular hourglass model in multiple fruiting body forming fungi with different levels of complexity. We used a BLAST-based phylostratigraphic approach to infer the relative age of each gene of *C. cinerea*, *Armillaria ostoyae*, *Lentinus tigrinus*, *Schizophyllum commune* and *Phanerochaete chrysosporium*, then computed the relative ages of the trascriptomes of each developmental stages of these five fungi using the Transcriptome Age Index (TAI). To find out what shapes the observed ontogenic expression patterns take in our analysis, we applied several approaches to infer TAI, such as different E-value cut-offs when inferring the relative gene ages, the transformation of the expression data, and the removal of the non-developmentally regulated genes from the analysis. We found that these alterations can change the observed ontogenic expression

patterns and/or the TAI values of the developmental stages. Furthermore we identified genes which could potentially drive hourglass patterns. These investigations will help us to test the molecular hourglass model and to understand factors laying behind this pattern in fruiting body forming fungi.

MOLECULAR IDENTIFICATION OF A NOVEL HANTAVIRUS IN BRONZE TUBE-NOSED BAT (*MURINA AENEA*) IN MALAYSIA

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Hantaviruses (Hantaviridae) cause two types of life-threatening human diseases, hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. To date, as a consensus, wild rodents were believed as natural hosts of hantaviruses. However, recent studies described several novel hantaviruses in shrews, moles and bats, suggesting the dispersal of hantaviruses in several animal taxa during their evolution. Interestingly, the co-evolutionary analyses of most recent studies have raised the possibility of bats and/or soricomorphs may have served as the primordial mammalian host and harbored the ancestors of rodent-borne hantaviruses. The aim of our study was to investigate the presence of hantaviruses in bat lung tissue homogenates originally collected for taxonomic purposes in Malaysia, 2015. Hantavirus specific nested PCR screening of 116 samples targeting the L segment of the virus have revealed the positivity of two lung tissue homogenates originating from *Murina aenea* bat species.
The obtained results indicate the first molecular evidence for hantavirus in *Murina eanea* bat species. Preliminary sequence analysis of the PCR amplicon suggest the identified virus may represents a novel species within Orthohantavirus genus. Furthermore, our results provide additional genomic data to help extend our knowledge about the evolution of these viruses and we present the first hantavirus sequence from *Murina* bat genus.

IN VITRO SURVEY OF MOSQUITO-RELATED VIRUSES ON C6/36 *AEDES ALBOPICTUS* CELL LINE

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Several mosquito-transmitted pathogens pose general threat to human health (such as Zika virus, Dengue virus and Yellow Fever virus) while, in recent years, a novel group of viruses classified into different viral families had been identified, called mosquito specific viruses, with inability to replicate on vertebrate cell lines and showing large-scale distribution around the world. The aim of this study was to investigate the presence of mosquito-related viruses in field collected female mosquito homogenates via *in vitro* virus isolation procedures. We also conducted genomic characterization and phylogenetic studies on the detected strains. Our major goal was to generate an isolate bank of mosquito viruses that would serve as a basis for future experiments. We inoculated 440 mosquito pools onto C6/36 cell line of which 42 showed cytopathic effect after the second

passage. PCR screening of supernatants targeting different viral families has revealed the presence of West Nile virus (Flaviviridae) in one sample, Negev-like virus in 33 samples, Guaico culex virus (Flaviviridae) in 2 samples, Koyama Hill virus (Reoviridae) in 5 samples and we found coinfection of Marisma Mosquito virus (Flaviviridae) and a sigmavirus (Rhabdoviridae) in one sample. Results of recent study have revealed a huge geographic expansion of Guaico Culex virus compared to our recent knowledge and the presence of presumably human pathogenic Marisma Mosquito virus among Serbian mosquitoes. Furthermore, we successfully identified a tentatively new sigmavirus (Rhabdoviridae), previously described only in drosophila species, suggesting the evolution of this virus group in other dipterans and broader host range.