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WHOLE GENOME SEQUENCE ANALYSIS OF THE HEAVY METAL RESISTANT BACTERIUM *CUPRIAVIDUS CAMPINENSIS* S14E4C

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Increased use of metals and chemicals in the industrial processes has resulted in generation of large quantities of effluents containing toxic heavy metals into the environment, and these effluents pose environmental disposal problems due to their non-degradable and persistent characters. Microorganisms can develop different mechanisms to adapt to the heavy-metal stresses. The bacterial strain S14E4C was isolated from a playground of a former industrial city (Salgótarján, Hungary) by enrichment method in a 200 ppm cadmium (Cd) containing broth media. Identification of the strain happened by the 16S rDNA and based on sequence analysis it belonged to the species *Cupriavidus campinensis*. The Maximum Tolerable Concentration (MTC) of the bacterium was analysed by increasing concentration of $Pb(NO_3)_2$, $CdSO_4$ and $HgCl_2$ salt solutions in media which was 3,000 ppm, 15,000 ppm and 1,500 ppm, respectively. The full genome sequence of the strain S14E4C was analysed: genomic DNA of the strain was isolated using a DNeasy Power Lyzer Microbial Kit (50) (Germany) according to the manufacturer's instructions. Whole-genome sequencing was performed in the Genomics Facility RTSF, Michigan State University (USA), on an Illumina MiSeq platform using the MiSeq standard v2 chemistry and annotated by Rapid Annotation Subsystem Technology (RAST) server. The draft genome sequence of *Cupriavidus campinensis* strain S14E4C constituted a total of 56 contigs with 6,375,175 bp, and a GC content of 66.3%. PlasmidSPAdes 3.5.0. revealed that the sequence contains 2 plasmids with 295,460 bp (GC content 59.9%) and 50,483 bp (GC content 63%). In total 4,496 and 1,520 coding sequences were assigned as non-hypothetical and hypothetical, respectively. The genome was shown to encode 6 rRNAs and 58 tRNAs.
The strain possesses a substantial number of genes which are responsible for membrane transport (188 genes, including ABC and cation transporters), detoxification (7 genes) and metabolism of aromatic compounds (108 genes), as well as, 112 genes encoding proteins, resistance to antibiotic and toxic compounds, including BLc (beta-lactamase), fluoroquinolones, CzcCBA (cobalt, zinc, and cadmium resistance), MerRTPCDAB (mercury resistance operon), ChrAB (chromate transport and resistant), a lead-, cadmium-, mercury-, and zinc-transporting ATPase, a copper-translocating P-type ATPase, etc. Additionally, there are several putative proteins believed to contribute to the heavy-metal resistance of this bacterium, such as putative copper-binding protein ScsA, putative silver efflux pump SEP, etc. The genome data show that the strain is particularly well equipped to live in contaminated sites that are rich also in heavy metals. Hence, *Cupriavidus campinensis* strain S14E4C may have high importance in further bioremediation processes.

STWINTRON (SPLICEOSOMAL TWIN INTRON) DIVERSIFICATION: THREE TYPES OF [D] STWINTRON EVOLVED AT THE SAME INTRON POSITION IN LIPOMYCES SPECIES

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Previously, we demonstrated the existence of a [D5,6] stwintron at the first intron position in the gene encoding a fungal reticulon-like protein (RtnA), where the internal U2 intron is nested in the donor of the external U2 intron between nt 5 and 6 (5'GUAAG|U in *Aspergillus nidulans*). True orthologs of the *rtnA* gene are present in the Lipomycetaceae family of the Saccharomycotina subphylum of the Ascomycota, but the [D5,6] stwintron is unique to the Pezizomycotina subphylum. The ortholog *rtnA* genes in 9 *Lipomyces* species carry one to five introns: the first intron position, shortly after the start codon, is always occupied. Intriguingly, in 7 species, the first intron position is occupied by three different complex intervening sequences (CIS) consistent of nested standard U2 introns (none being a [D5,6] stwintron). In *L. lipofer*, it is a [D4,5] stwintron, where the internal intron is nested in the donor of the external intron between nt 4 and 5 (5' GUGA|GU). In contrast, in *L. suomiensis* and *L. japonicus*, the internal intron is localised behind the nt following the 6-nt donor element of the external intron (*L. suomiensis*: 5' GUGAGUGGUAGGU; *L. japonicus*: 5' GUAAGUGGUAAGU). In both fungi, a pair of canonical 3'splice sites (acceptor & associated lariat branch point motif) is available halfway the CIS. Although this intron nesting ([D7,8]) is not a stwintron *sensu stricto*, it is likely that the fully canonical splice sites of the internal intron are paired according to intron definition for the first of the consecutive splicing reactions to remove the whole CIS. Moreover, in four *Lipomyces*, the donor elements of the CIS-constituent U2 introns are abutting (*L. arxii*, *L. starkeyi*, *L. mesembrius* & L. *kononenkoae*: 5' GUACGUGUAAGU), leading to a stwintron sensu latu [D6,7] configuration in the *rtnA* transcript. Interestingly, the CIS in these four species can be removed alternatively. The whole CIS can be excised in one reaction using the (proximal) perfect donor (5'-GUAAGU) and the distal pair of canonical 3' splice sites, leaving the distal, less canonical donor (5'-GUACGU) exonic. Alternatively, it could be removed in two consecutive splicing reactions as a [D6,7] stwintron, using the imperfect internal 3'splice sites halfway the CIS for the definition of the internal intron, and the distal donor (5'-GUACGU) at the 5' splice site of the external intron. We have proven that the alternative splicing paths are used concurrently in *L. starkeyi* and *L. kononenkoae*. The existence of three different types of [D] stwintron at the same intron position in species of one genus provides clues to how these complex structures evolved endogenously, involving a duplication of intron donor elements at the 5' splice site.

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OBTAINING OF *hxnSΔ hxnTΔ hxnRc7* AND *hxnSΔ hxnTΔ hxnYΔ hxnRc7* MULTI-DELETION MUTANTS IN *ASPERGILLUS NIDULANS*

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The investigation of the first eukaryotic nicotinic acid catabolic pathway established by our research group had already revealed the genetic background and regulation of the pathway in the model organism *Aspergillus nidulans*. We have identified eleven co-regulated *hxn* genes organized in three gene clusters. Nicotinic acid Degradation Cluster 1 (NDC1) and NDC2 comprised of 6 (*hxnS*, *hxnR*, *hxnP*, *hxnT*, *hxnY* and *hxnZ*) and 3 genes (*hxnX*, *hxnV* and *hxnW*), respectively are located on chromosome VI, separated from each other by 40 kb. The third cluster (NDC3) composed of 2 genes (*hxnN* and *hxnM*) is located on chromosome I. All cluster genes are regulated by the cluster-specific

transcription factor, HxnR and a yet-unknown pathway metabolite compound downstream from nicotinic acid. Through the study of *hxn* gene deletion mutants we revealed that the eukaryotic nicotinic acid catabolic pathway is split to alternative routes, however in order to determine the fine details of the pathway, multideletion mutants were needed and the application of analytical methods, such as LC-MS. In most of the mutants the nicotinic acid catabolism is blocked before the metabolic inducer of the pathway is produced, therefore the pathway genes cannot be expressed in these mutants, except in the case the mutants carry the constitutive allele (*hxnRc7*) of the transcription factor, HxnR. Henceforth, the deletion mutants have to be placed in *hxnRc7* background. When the *hxn* deletion is not or loosely linked to *hxnR*, the application of genetic cross for obtaining the desired strain for analytical purposes is doable. However, when the candidate hxn deletion is in the close proximity of *hxnR*, the transformation of an integrative expression vector carrying the *hxnRc7* allele into the recipient hxn deletion strain is the most applicable method. In this work we present the construction of the *hxnRc7* expression vector (pAN-HZS-17) and the obtaining of *hxnSΔ hxnTΔ hxnRc7* and *hxnSΔ hxnTΔ hxnYΔ hxnRc7* multi-deletion mutant strains with constitutive *hxnRc7* background by the transformation of the developed pAN-HZS-17 vector. Integration of the transformed vector was checked by conducting vector-specific PCR reactions and the number of integrations was determined by qPCR carried out for *hxnR* and *actA* (gamma-actin).

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FLAVONOL 7-O-GLUCOSIDE HERBACITRIN INHIBITS HIV-1 REPLICATION THROUGH SIMULTANEOUS INTEGRASE AND REVERSE TRANSCRIPTASE INHIBITION

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Here we report the evaluation of the antiretroviral effect of two flavonoid 7-O-glucosides, herbacitrin (1) and gossypitrin (2), together with quercetin (3), a well-studied flavonol. Antiviral activity of the flavonoids was assessed by analyzing HIV-1 p24 core protein levels in the supernatants of HIV-1 infected MT-4 and MT-2 cell cultures. The compounds showed mild to weak cytotoxic activities on the host cells; herbacitrin was the strongest in this regard (CC50 = 27.8 and 63.64 μM on MT-4 and MT-2 cells, respectively). In nontoxic concentrations, herbacitrin and quercetin reduced HIV-1 replication, whereas gossypitrin was ineffective.

Herbacitrin was found to inhibit reverse transcriptase at 21.5 μM, while it was a more potent integrase inhibitor already active at 2.15 μM. Therefore, our observations suggest that herbacitrin exerts antiretroviral activity through simultaneously acting on these two targets of HIV-1 and that integrase inhibition might play a major role in this activity.

MICROBIOLOGICAL PROPERTIES OF RAW EWE MILK AND UDDER SURFACE SAMPLES IN A HUNGARIAN DAIRY SHEEP FARM

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Sheep milk is an excellent source of nutrients and is mainly used for cheese production due to its high total solids content, contributing to high cheese yield. The primary prerequisite for making high-quality sheep milk product is the production of high-quality raw sheep milk by dairy farms. Thus, the aim of this study was to examine the microbiological status of udder surface (US), individual ewe raw milk (IERM) and bulk tank milk (BTM) samples from a Hajdú-Bihar County dairy sheep farm which milking Lacaune breed. Total plate count (TPC), Enterobacteriaceae count (EBC), *Staphylococcus aureus* count (SAC) and coagulase-negative staphylococci count (CNSC) in IERM, in BTM and in US samples, furthermore somatic cell count (SCC) with California mastitis test (CMT) in IERM were examined according to ISO standards. In this study 15 US, 15 IERM and 6 BTM samples were examined from May to July 2018. The change in the microbiological status of IERM, BTM and US samples between the months (May, June and July) of sample collection was considered. The mean values of TPC, EBC and CNSC were 2.7, 1.0 and 2.6 lg CFU/cm^2 in US samples, respectively. The mean values of TPC, EBC and CNSC were 3.3, 2.2 and 3.1 lg CFU/mL in IERM samples, respectively. In the case of BTM, the mean values of TPC, EBC and CNSC were 7.4, 5.1 and 5.3 lg CFU/mL, respectively. In this study, the mean value of TPC in BTM exceeded the European Parliament and of the Council 853/2004 standard, for milk from animal species other than cow must not exceed 6.2 lg CFU/mL. *S. aureus* count (SAC) was less than 10 CFU/mL in all samples. TPC, EBC and CNSC mean values were significantly higher ($p<0.05$) in May compared to June and July in US samples. In the case of IERM, the TPC, EBC and CNSC mean values were significantly higher in June compared to May and July. There was no significant ($p>0.05$) difference for TPC and EBC mean value between the months of sample collection in BTM. CNSC mean value has got a significant difference between May and June in BTM. The correlation between the mean values of US and IERM was very weak for TPC ($r = -0.14$) and for CNSC ($r = 0.17$), and moderate for EBC ($r = 0.58$). The SCC was between 0 and 200,000 cells/mL (-) in 28 (93.3%), between 150,000 and 500,000 cells/mL (+) in 1 (3.3%), and between 400,000 - 1,500,000 cells/mL (++) in 2 (6.7%) of udder half milk samples. The relatively low EBC of US samples indicates a good hygienic condition of ewe housing. The relatively low microorganisms counts of IERM and low SCC indicates good health condition of ewes. The presence of microorganisms in BTM above the limits indicates high microbial contamination due to poor hygienic condition during milking and milk handling.
Therefore, it is necessary to reduce the microbial contamination of bulk tank milk by the respect of strict hygienic measures at the examined dairy sheep farm.

ANDROGEN BIODETOXIFICATION POTENTIAL OF *RHODOCOCCUS* AND *COMAMONAS* SPECIES

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Androgens can be frequently detected in surface waters where their presence can be harmful to aquatic organisms. Elimination of these compounds from the environment is a crucial task to reach and maintain good status of surface waters. For this purpose it is important to select microbes with excellent androgen degrading potential. Members of *Rhodococcus* and *Comamonas* genera are known for their efficient xenobiotic biodegradation capacity and many steroid degrading genes have already been identified from some strains of these genera. The aim of this study was to further investigate the steroid biodetoxification potential of 8 *Rhodococcus* strains (*R. erythropolis* AK35, *R. globerulus* AK36, *R. pyridinivorans* AK37, *R. gordoniae* AK38, *R. ruber* AK41, *R. aetherivorans* AK44, *R. coprophilus* N774, *R. pyridinivorans* K402) that showed excellent 5α-dihydrotestosterone (DHT) biodegradation and biodetoxification potential in previous experiments. In this study their androst-4-ene-3,17-dione (AD) biodetoxification ability was tested. Furthermore DHT and AD biodetoxification potential of 3 *Comamonas* species (*C. aquatica* B194, *C. thiooxidans* B207, *C. thiooxidans* B211), isolated from a natural lake, was also determined. Biodegradation experiments were carried out in Erlenmeyer flasks containing LB medium and bacterial inoculum. Concentration of DHT and AD was set to 0.1 mg/L and 1 mg/L, respectively. Flasks were incubated at 28°C and were shaken at 170 rpm for 72 hours. Samples were taken every 24 hours and after centrifugation supernatant and pellet samples were separately stored at -20°C until further analysis. Biodetoxification was followed up in supernatant samples by *Saccharomyces cerevisiae* BLYAS and BLYR strains that can measure androgenic activity and cytotoxicity, respectively. In the case of AD all studied *Rhodococcus* strains and the two *C. thiooxidans* strains were able to eliminate androgenic activity in the supernatant samples already in 24 hours according to the results of the BLYAS test. In the case of DHT *C. thiooxidans* B207 and B211 strains showed excellent biodetoxification efficiency in 24 hours. In spite of this *C. aquatica* B194 could not eliminate androgenic activity of the samples containing either DHT or AD even at the 72nd hour of the experiment.
Cytotoxicity was not experienced in any of the samples according to the BLYR strain.

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EXPLORING THE DIVERSITY OF XYLENE-DEGRADING BACTERIA IN GROUNDWATER OF THE SIKLÓS BTEX-CONTAMINATED SITE

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The monoaromatic hydrocarbons like the carcinogenic benzene, and toluene, ethylbenzene and xylenes (BTEX-compounds) are frequent groundwater contaminants in Hungary. Several microbes can use these compounds as sole source of carbon and energy, but mainly under aerobic conditions. Therefore, in subsurface ecosystems the contamination relatively quickly decreases the availability of oxygen. In shallow, hydrodynamically active groundwater this leads to the formation of microaerobic conditions, while in deeper layers strictly anaerobic conditions evolve due to the contamination. Under these conditions, xylenes can be persistent contaminants as it can be observed in case of

Hungarian contaminated sites as well. It is known that microbial communities of oxygen-limited subsurface ecosystems with a persistent BTEX contamination are usually dominated by Betaproteobacteriales. Moreover, in layers where low amount of oxygen is present catechol 2,3-dioxygenase (C23O) genes encoding subfamily I.2.C-type extradiol dioxygenases can be found in large diversity. These enzymes, which play key role in the cleavage of the aromatic ring, are believed to function under microaerobic conditions. However, it is still unknown whether these enzymes play any role in the microaerobic degradation of xylenes. To overcome this limitation, we started to investigate the diversity of xylene-degrading bacteria at the Siklós BTEX-contaminated site, where xylenes are among the main contaminants. Accordingly, in the first step of investigation aerobic enrichment cultures were set up by using groundwater sample of the Siklós site with either para-, meta- or ortho-xylene as sole source of carbon and energy.
After seven consecutive transfers, microbial strains were isolated from the enrichments. The meta-xylene-degrading enrichment cultures yielded strains belonging to the genera *Acidovorax*, *Pseudacidovorax*, *Achromobacter* and *Pseudomonas*. Strains isolated from the para-xylene-degrading enrichment cultures belonged to the genera *Hydrogenophaga*, *Acidovorax*, *Flavobacterium*, *Pseudomonas* and *Enterobacter*. In case of the ortho-xylene-degrading enrichment cultures different subspecies of *Pseudomonas chlororaphis* were the dominant isolates, but members of the genera *Rhodococcus*, *Microbacterium* and *Achromobacter* were also isolated.

IDENTIFICATION OF MONOAROMATIC- AND POLYCYCLIC AROMATIC HYDROCARBON DEGRADING COMMUNITY MEMBERS OF A BACTERIAL BIOFILM DEVELOPED IN A PETROLEUM HYDROCARBON CONTAMINATED GROUNDWATER

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Biofilms are ubiquitous. Most of the time their presence is associated with non-desired phenomena; in hospital settings biofilms can lead to nosocomial infections, in industry are responsible for biocorrosions and/or biofouling. However, biofilms under appropriate circumstances can be put in the service of ecosystem. These matrix-enclosed microbial accretions developed in contaminated environments (e.g. with petroleum hydrocarbons) can be sources of biotechnologically relevant bacteria (e.g. bacteria successfully used for bioremediation purposes). In the recent years, by using a polyphasic approach biofilms developed in a petroleum hydrocarbon contaminated groundwater have been thoroughly investigated. First, the phylogenetic and functional diversity of the initial biofilm communities were determined. Second, by initiating aerobic and oxygen-limited selective enrichment cultures the identification and isolation of aerobic / oxygen-limited BTEX (monoaromatic) and naphthalene (polycyclic aromatic hydrocarbon - PAH) degrading biofilm bacteria took place. We aimed at obtaining a strain collection of prolific biofilm producing and BTEX/PAH-degrading bacterial strains. During the experiments conventional cultivation dependent microbiological and cutting-edge cultivation independent molecular biological approaches were combined (e.g. T-RFLP, 16S rRNA and functional gene based cloning and sequencing, Illumina 16S amplicon sequencing etc.). The biofilm bacterial isolates were subsequently screened for their BTEX and naphthalene degradation abilities and for their biofilm producing capabilities. Regardless of the sampling campaign the initial communities of the biofilm samples were overwhelmingly dominated by Betaproteobacteria, followed either by Gamma- and Deltaproteobacteria or Actinobacteria; at genus

level higher dissimilarities were encountered. Representatives of the genus *Malikia* proved to be the most dominant community members of the aerobic BTEX-degrading enrichments. *Acidovorax* spp. dominated the oxygen-limited BTEX-amended microcosms. *Pseudomonas* species proved to be the most dominant community members in both aerobic and oxygen-limited naphthalene amended setups. In total 109 species level identified biofilm bacteria were obtained. Some members of this bacterial strain collection exhibited extreme biofilm producing potential and intensive BTEX/naphthalene biodegradation ability.
Subsequently, members of this strain collection can be used for the development of biofilm based biobarrier systems used in the containment and decontamination of water streams contaminated either with monoaromatic- or polycyclic aromatic hydrocarbons.

TETRAMIC ACID ALKALOIDS OF *FLAVOMYCES FULOPHAZII*, A COMMON ROOT ENDOPHYTE OF SEMIARID SANDY GRASSLANDS

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Endophytic fungi are generally considered to be valuable sources of structurally and functionally diverse natural products, since they interact with other organisms at least partially by their secondary metabolites (SMs). During investigations on root endophytic fungal communities of semiarid sandy grasslands of Hungary and Mongolia, from roots of *Festuca* and *Stipa* species (Poaceae), we frequently isolated the recently described *Flavomyces fulophazii* (Pleosporales). Since its isolates secrete significant amounts of yellow pigments into their media, based on which the name „flavo" was given, we aimed to identify these remarkable SMs. Methanolic extracts of lyophilized media were prepared and analyzed using high-performance liquid chromatography hyphenated with diode array and high-resolution mass spectrometric detection (HPLC-DAD-HRMS). The chemical structures of the detected compounds were determined by HRMS and nuclear magnetic resonance spectroscopy (NMR). As a result, for the first time, we identified tetramic acid alkaloids in the extracts of *F. fulophazii*, i.e., vermelhotin and its four related compounds.
These alkaloids, with the single exception of vermelhotin, were determined to be new natural products. Further experiments are in progress to test, whether these SMs might have a role in the interaction of this fungus with other microorganisms.

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VERIFICATION OF BACK-CONVERSION OF 6-HYDROXYNICOTINIC ACID TO NICOTINIC ACID IN THE NICOTINATE CATABOLIC ROUTE

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The nicotinic acid (NA) degradation pathway was only studied in prokaryotes so far. In eukaryotes the degradation process was completely unknown. We started to unravel the NA catabolic route in the model microorganism *Aspergillus nidulans*. Eight genes were found that take part in the degradation process, two genes that codes for transporters and one gene with regulatory function. By generating and analysing simple- and multiple-deletion mutants we found that *hxnS*, *hxnT* and *hxnY* genes take part in the first steps of the pathway. The first step of NA degradation is the hydroxylation of NA to 6-hydroxynicotinic acid (6NA), which is carried out by the purin-hydroxylase II enzyme, HxnS. This enzyme also participates in the further conversion of 6NA. An *hxnS* deletion mutant cannot utilize NA as sole N-source and the strain shows a slightly reduced growth on 6NA N-source. Although, we do not know the exact position of *hxnT* and *hxnY* in the pathway yet, we suppose that 6NA is the substrate of HxnT. An *hxnT* deletion mutant can utilize NA as sole N-source (indicating a split of the pathway), however the strain shows a reduced growth on 6NA N-source. One would expect that a double deletion *hxnS*/*hxnT* mutant shows a reduced growth on 6NA N-source similarly or stronger than the single mutants. Remarkably, an extremely improved growth of *hxnS*/*hxnT* deletion mutant was observed on 6NA N-source. For the explanation of this paradoxical growth we hypothesize that HxnS and HxnT might convert 6NA back to NA, therefore they normally interfere with the further conversion of 6NA. According to HPLC-MS analysis carried out on cultures of different *hxn* deletion mutants with constitutive *hxnR* background (*hxnRc7*), which were incubated on 6NA substrate, NA is produced. This result strongly indicates the back-conversion of 6NA to NA. In order to experimentally prove back-conversion, we used a NA auxotrophy (*nicB8*) as a tool for *in vivo* detection of back-conversion. We generated *hxnSΔ nicB8*, *hxnTΔ nicB8*, *hxnSΔ*/*hxnTΔ nicB8*, and *hxnSΔ nicB8 hxnRc7*, *hxnTΔ nicB8 hxnRc7*, *hxnSΔ*/*hxnTΔ nicB8 hxnRc7* strains.
We carried out growth tests with the generated mutants and control strains on media that contained NA or 6NA as sole N-source, or as supplements with neutral N-source. Based on our results we proved that both HxnS and HxnT enzymes convert 6NA back to NA.

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GENERATION OF WHOLE-CAPSID NUCLEOTIDE SEQUENCES WITH NEXT GENERATION SEQUENCING FOR MOLECULAR CHARACTERISATION OF ECHOVIRUS 9 STRAINS DETECTED IN HUNGARY IN 2018

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Echoviruses belong to genogroup B enteroviruses and are common human pathogens causing a wide range of symptoms from minor febrile illness to rash and severe, potentially fatal conditions (aseptic meningitis, encephalitis, paralysis, myocarditis). In 2018 the National Enterovirus Reference Laboratory detected Echovirus 9 in 13 clinical specimens from 9 patients between May and September. Seven patients had meningitis, one patient suffered cardiomyopathy and one patient showed skin related symptoms. Initial genotyping was done by partial sequencing of VP1 coding region of the viral capsid. Four viruses from 3 cases were further characterised using next generation

sequencing (NGS) for generating whole-capsid nucleotide sequences. Resulting sequences were also compared to other Echovirus 9 sequences available in GenBank for phylogenetic analysis.

EXPRESSION OF CYTOPLASMIC PROTEIN TYROSINE PHOSPHATASES IN CERVICAL CANCER

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Recently identified cellular targets of HPV E7 are the cytoplasmic protein tyrosine phosphatases PTPN14 and PTPN21. Previously we have demonstrated using cell culture experiments, that the E7 proteins of both high-risk and low-risk mucosal HPV types can interact with these phosphatases and high-risk HPV E7 can cause PTPN14 degradation in a proteasome-dependent manner. Here we show that surprisingly, in severely dysplastic cervical epithelium PTPN14 protein expression seems to be elevated compared to normal epithelial regions of the same histological preparation. There is no detectable PTPN21 protein expression neither in normal nor in dysplastic cervical epithelium. Furthermore, analysis of RNA sequencing data in TCGA database revealed that elevated PTPN14 gene expression is an unfavourable prognostic marker in cervical cancer.
Our ongoing experiments aim at revealing the mechanisms behind this unexpected expression pattern of PTPN14 and PTPN21 phosphatases in cervical cancer tissues.

USING GENOMICS TO STUDY ANTIBIOTIC RESISTANCE

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The cumulative prevalence of multidrug-resistant gram-negative bacteria (MDRGNB) is a growing concern of healthcare systems worldwide. This rising prevalence presents a serious danger as the therapeutic options left are limited and costly. Current routine surveillance and monitoring programs for MDRGNB based on established phenotypic and sub-genome molecular typing only recognize only the presence/absence of phenotype or target genes, and limit their use. The introduction of whole genome sequencing (WGS) for MDRGNB surveillance has allowed characterization and typing as well as accurate epidemiologic analysis to a hitherto unprecedented level. Known antibiotic-resistance genes and virulence factors can be determined, and a holistic analysis that integrates deep genome, epidemiology with patient centric data could be used to identify high-risk clones for dissemination, persistence, or even invasive disease. In addition WGS could assist in the development of new or enhanced diagnostic assays by providing sequence information to enable pathogen-specific or multiplex primer -based assays.
I will describe our efforts and the challenges in establishing microbial next generation sequencing within the context of a clinical diagnostic laboratory.

PREVALENCE OF HUMAN POLYOMAVIRUS 11: IS IT TRANSMITTED VIA RESPIRATORY ROUTE?

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Human polyomavirus 11 (HPyV11) – Saint Loius polyomavirus – was discovered from stool sample in 2013. The complete genome of the virus was described, but virion has not been isolated yet. Although prevalence and seroprevalence studies have been started, the numbers of publications are low, and little is known about this virus. The viral DNA was detected in stool, urine and tonsil samples, and the prevalence rate was low. Based on two publications the adulthood seropositivity is high. One of our aims was to study the DNA prevalence of HPyV11 in respiratory samples. Nucleic acid was isolated from throat swab samples, tonsils, adenoids and middle ear fluid samples. Quantitative, real-time PCR method was optimized to detect viral DNA. HPyV11 DNA was detected in one adenoid sample (1/100), in two middle ear fluids (2/15) and in five throat swab samples (5/156). Complete genome sequence was amplified by primer walking from two samples, non-coding ctrol region and/or VP1 sequence was obtained and analysed from the other samples. The other aim was to express VP1 capsid protein of HPyV11 to develop an indirect ELISA method for seroprevalence study. Codon optimized, 6XHis tagged VP1 gene with restriction endonuclease recognition sites was synthesized and cloned into modified pTriEx™-4 Neo vector. Protein expression is carried out in Origami™ B(DE3) bacterium.
Following qualitative and quantitave analysis, the purified, polyhistidine-tagged protein was used to develop and optimize an indirect ELISA methods. Serum samples from healthy adults, children, and immunocompromised individuals were collected. Seroprevalence study is in progress.

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EFFECT OF AMINO ACID SUPPLEMENTATION ON PIGMENT PRODUCTION OF *METSCHNIKOWIA* SPECIES

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Pre- and postharvest diseases caused by fungal pathogens result in the major losses of fruits. Consequently, protection against these fungal diseases is quite important. Synthetic fungicides are the primary means to control fungal infections. At the same time, there is an increasing public demand to reduce fungicides and produce pesticide-free food. Use of the antifungal microorganisms as biocontrol agents to reduce or inhibit growth of destructive fungi can be a promising alternative solution. Many bacteria and yeasts, such as *Metschnikowia* sp. can produce a red pigment, pulcherrimin which has antimicrobial effect. Pulcherrimin accumulates in the cells and/or in the medium around the colony. This pigment is a large complex formed by a non-enzymatic reaction between ferric salt and pulcherrimic acid, which is present in the medium and extracted by the cells. According to our knowledge, pulcherrimic acid is derived from L-leucine, which was converted to an

intermedier cyclo (leucyl-leucyl). Our aim was to examine possible effects of the external amino acids supplementation on the pigment production in the case of *M. andauensis* type strain (CBS 10809T). Leucine, glutamic acid and arginine supplementation were used in PDA and minimal SMA media at different pH values. According to our results, the precursor molecules do not diffuse into the medium at lower pH, except for the arginine supplemented media. In contrast, higher pH value favoured diffusion of the red pigment. Our results revealed that amino acid supplementation increased size of the pigmented halos around the colonies. Furthermore, pigment production was greatly depended on the composition of medium. We assume that some components of the minimal salt medium SMA have effect on the chelate complex formation of the pulcherrimic acid.

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OUT OF SYNC - FUNGAL PLANT BIOMASS DEGRADING ENZYMES AND THEIR RELATED REGULATORY SYSTEMS DISPLAY DIFFERENT EVOLUTIONARY PATTERNS

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Plant biomass is the main carbon source for many fungal species. To degrade this substrate into metabolizable sugars, fungi produce a wide range of plant biomass degrading enzymes that target the various polymers and linkages. These enzymes can be divided into families based on their amino acid sequence similarity and have been catalogued in the Carbohydrate Active Enzyme database (CAZy, www.cazy.org). The availability of fungal genome sequences has revealed that despite high variation in the numbers of genes/family, overall these families show strong conservation across the fungal tree of life and the changes in genome content can often be linked to the biotope of the species. To be able to produce the right set of enzymes to match the prevailing substrate, the production of these enzymes is under control of a complex regulatory network. This network consists of a number of transcriptional activators that respond to the presence of specific monomeric or small oligomeric compounds (inducers) together with more general transcriptional repressors. Interestingly, the regulatory network shows strong diversity across the fungal kingdom, with many regulators being restricted to subgroups of fungi. This suggests that the origin of the regulatory network may be more recent than the origin of the enzyme families. Examples of these differences will be presented.

DIVERSITY OF THERMOPHILIC FUNGAL COMMUNITIES IN MUSHROOM COMPOST PROCESSING

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According to the practice common nowdays, mushroom production requires nutrition compost of standard quality, with precise processing. The composition of mushroom compost is highly influenced by the activity of different colonizing microfungal communities. At high temperatures due

to concentrated decomposition, the presence and role of thermophilic fungi is outstandingly significant. According to the results of culturing with quantitative mycological methods at 46°C, the dimension of thermophilic mycota of variant technological phases differed. While the total number of thermophilic fungi had been 4.2 x 10^4 CFU/g (CFU: colony forming unit) in the introducing fermentation phase, it has decreased to 5.9 x 10^2 CFU/g following an 8 days long thermal treatment. Surprisingly, in the final phase, when the mushroom mycelialisation is carried out, the dimension of the thermophilic mycota has reached a high level again (3.2 x 10^5 CFU/g). The thermophilic fungi inhabiting raw materials i.e. horse manure (2.6 x 10^3 CFU/g), poultry manure (8.0 x 10^2 CFU/g) and wheat straw (1.7 x 10^2 CFU/g) can be considered as the sources of compost colonization.
Furthermore, the diversity of the thermophilic fungal compositions have dramatically changed during the composting process. Seven species were isolated from the fermentation phase with the dominance of *Rasamsonia emersonii* (24.0%) and *Thermothelomyces thermophila* (24.5%), and it was characterized by high diversity (Shannon's index 1.78, Simpson's index 0.81). The samples of the next phase contained reduced thermophilic mycobiota following the thermal treatment: only two species, with the dominance of *Thermoascus aurantiacus* (98.5%) were found. The final stage, when the mushroom mycelialization takes place, is also characterized by reduced thermophilic mycobiota and low diversity (Shannon's index 0.33). The unambiguous prevalence of these species suggest their ecologically determined coexistence with the mushroom mycelia.

IN SEARCH FOR TREATMENT OPTIONS AGAINST URINARY TRACT INFECTION: CHARACTERIZATION OF *E. COLI* FITNESS TRAITS AND ADAPTATION IN THE URINARY TRACT

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Urinary tract infections (UTI) are a wide spread public health problem. Antibiotic treatment is often still effective, but with recurring symptomatic episodes or long-term prophylaxis often problematic side effects occur. The high proportion of antibiotic-resistant pathogens requires rapid establishment of alternative treatment strategies for UTI. Besides provoking symptoms, *E. coli* can also asymptomatically colonize the human bladder. During asymptomatic bacteriuria (ABU), high bacterial numbers are present in the urinary bladder without causing symptoms of UTI. ABU does, with a few exceptions, not require treatment and can prevent symptomatic UTI by more virulent bacteria due to bacterial interference. As an alternative to antibiotic treatment, deliberate bladder colonization with ABU *E. coli* isolate 83972 has been established and can be carried out over longer periods without side effects. To improve patient safety, we analysed the genomes and selected phenotypes of 21 consecutive re-isolates of *E. coli* 83972 from nine different patients. Understanding bacterial mechanisms critical for growth and competitiveness in urine provides a basis for improved bacterial interference, and thus, we aimed at the determination of hot spots of genomic variability and searched for *E. coli* 83972 genes under selection upon growth in the urinary tract. We saw similar mutation patterns in some hosts indicating patient-specific adaptation. The number of mutations in each strain did not correlate to the colonization time, and the percentage of mutations in non-coding (intergenic) regions was higher than in coding regions. Genes involved in iron uptake, metabolism and stress response displayed sequence variability in multiple patient re-isolates. Our data indicate that adaptation of the central carbon metabolism contributes to bacterial competitiveness in urine. We also searched for ABU isolates with superior fitness in urine and a reduced pathogenic potential as possible alternatives to *E. coli* 83972. Genomic and phenotypic characterization of nine ABU *E. coli*

isolates from diabetic patients identified several strains with superior fitness relative to model isolate 83972, and two of them also outcompeted uropathogenic *E. coli* strain 536 in pooled human urine. The strains belonged to different phylogenetic lineages and differed markedly in their virulence-associated genome content. The most promising strains were characterized by low *in vivo* virulence in a murine sepsis model, susceptibility to antibiotics and increased competitiveness in urine relative to *E. coli* 83972. They all lacked marked cytotoxic activity and exhibited a high LD50 in the sepsis model. Antagonistic activities (bacteriocin production) and efficient growth in urine may contribute to their competitiveness. Detailed knowledge on bacterial adaptation and fitness in the urinary tract will help to improve therapeutic bacterial interference against a broad spectrum of uropathogens.

DOES MYCORRHIZATION ALLEVIATE NEGATIVE EFFECTS OF COMBINED DROUGHT AND HEAT STRESS ON TOMATO PLANTS?

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Global climate change represents a serious threat to natural and agricultural ecosystems, which is predicted to largely impact on agriculture in the future. Under field conditions, plants are often exposed to multiple stresses simultaneously rather than a single stress alone. Water stress together with heat cause series of physiological and molecular alterations that negatively influence plant productivity. Hence, there is a pressing need to develop strategies to make agriculture more resilient. Among these strategies, there has been an increasing interest in beneficial microbes due to their immense potential to improve plant tolerance to abiotic stresses. The aim of this study was to examine the impact of arbusculat mycorrhizal (AM) colonization on tomato plant response to combined drought and heat stresses. Our preliminary results indicate that under drought + heat stress, both applied fungal symbionts (*Septoglomus constrictum*, *S. deserticola*) could alleviate oxidative stress by decreasing the lipid peroxidation and hydrogen peroxide level due to improved antioxidant enzyme activities. However, better results were obtained in plants inoculated with *S. constrictum* manifested in enhanced stomatal conductance, leaf water potential and relative water content. In addition, elevated Fv/Fm and biomass production of the hosts were observed compared to non-inoculated plants under drought + heat stress. Under combined stress, inoculation of *S. constrictum* did not change the expression of SlNCED (ABA biosynthetic gene) and SlPIP2.7 (aquaporin gene) while the expression of SlLOXD (JA biosynthetic gene) in root were upregulated. Further studies are required to clarify molecular mechanisms of mycorrhizal plant tolerance to combined stress.

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COMPARISON OF LOW-RISK AND HIGH-RISK HPV E7 ONCOPROTEINS FOR ASSOCIATION WITH PTPN14

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The HPV (human papillomavirus) E7 oncoprotein is essential for the proliferation of infected epithelial cells and carcinomas induced by high-risk HPV genotypes. One of the recently discovered

interacting partners of E7 is the cytoplasmatic protein tyrosine phosphatase PTPN14. The PTPN14 can associate with cytoskeleton and cell membrane, therefore affects the structure of actin cytoskeleton, cell-adhesion and cell-proliferation. Thus, PTPN14 could be an important factor in life-cycle of HPV and also in the malignant transformation of infected cells. Based on the previous results of our research group, the E7 oncoprotein of HPV-16 - the most frequent high-risk HPV genotype - can bind to PTPN14 phosphatase and significantly decreases its expression. The aim of our project was to investigate whether the expression of PTPN14 phosphatase is decreased only by the high-risk HPV E7 protein, or the low-risk HPV E7 can also have the same effect. First of all, specific primers were designed and then we constructed pCMV eukaryotic plasmid expression vectors which contain HA/Flag-tagged E7 protein sequences of low-risk HPV-11, or high-risk HPV -18, HPV-31. Then we transfected HPV negative C33A cells derived from cervix carcinoma with HA/Flag-tagged HPV-11, HPV-16, HPV-18 and HPV-31 E7 proteins. Western blot method was carried out to show that the presence of high-risk HPV E7 proteins highly downregulate the expression of PTPN14. However the low risk HPV-11 E7 had only a minor effect on PTPN14 expression. Moreover, using pull-down assay we found that the interaction between low-risk E7 and PTPN14 was weaker than between high-risk E7 proteins and PTPN14 phosphatase. Our study demonstrates that PTPN14 binding and degradation is more prominent in the presence of high-risk HPV E7 proteins.
This raises the possibility that degradation of PTPN14 may contribute to the transformation and immortalization of the infected epithelial cells in high-risk HPV-associated cancers.

COMBINATORIAL STRESS RESPONSES IN FUNGI

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In standard stress biological experiments, the behavior of fungal cultures exposed to one type of stress is compared to that of untreated cultures. Unfortunately this experimental design does not reflect well how fungi behave in a complex environment, like the human body, where they have to cope with different stresses concomitantly. Studying of the interactions between different stress treatments (studying “combinatorial stress” responses) can provide us useful information on how the *in vitro* determined stress tolerance attributes, drug susceptibility or virulence change when fungi grow *in vivo*. In this presentation the main effects of combinatorial carbon stress - oxidative stress, menadione stress, betamethason stress and iron starvation stress - oxidative stress treatments on the transcriptome of *Aspergillus nidulans*, *Candida albicans* and *Aspergillus fumigatus*, respectively, will be summarized. The presentation suggests that: 1) A tiny stress even if it has no real effect on unstressed cultures can significantly modify the response generated by another stresses. Therefore, modeling *in vitro* how fungi behave *in vivo* is rather challenging. 2) A compound which has no strong antifungal activity *in vitro* (on unstressed cultures) may have a strong effect *in vivo* (when fungi suffer from stresses). Improving of *in vitro* tests, based on the results of combinatorial stress response studies, to reflect better the *in vivo* behavior of fungi can help us to find new antifungal agents.

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GEOMICROBIOLOGICAL STUDY IN A CARBONATE CAVE OF THE AGGTELEK KARST, HUNGARY

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The Baradla Cave represents one of the longest caves in Hungary where numerous speleothem formations can be found with varied colors and shapes. Although the cave has been visited for centuries and studied from archaeological point of view, detailed geomicrobiological researches have not been conducted. The cave can be characterized with specific environmental parameters, such as limited bioavailable organic carbon and nitrogen sources, high humidity (96 - 100%), relatively low temperature (9.8 - 10.5°C) and high calcium content. Therefore, it can offer a long-term reservoir for extremophile microorganisms. Biofilm-forming bacteria are presumed to contribute to speleothem formation (calcium-carbonate precipitation) throughout their metabolism and production of extracellular polymeric substance by which they can alter the physical and chemical parameters in their micro-environments. Carbonate precipitating bacteria might take part in crystal phase selection and stabilization, as well. The aim of this study was to gain information about the structure of the prokaryotic communities inhabiting the speleothem surface using scanning electron microscopy, cultivation and next generation amplicon sequencing. In the Baradla Cave, samples were taken from the surface of stalagmites, stalactites, soda straws and the dripping water. The surface of speleothems was covered by bacterial cells with elongated morphology adapted to the low nutrient content. Bacteria isolated from different subsurface-environment specialized media containing minerals, low amount of nutrients, and extract of soda straws showed high similarities to environmental sequences, particularly from soil and water samples. Among the identified bacteria, there were several psychrotolerant and mesophilic oligotrophic heterotrophic species participating in the nitrogen biogeochemical cycle or microbially induced carbonate precipitation.

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CALCIUM-CARBONATE PRECIPITATING BACTERIA FROM CSODABOGYÓS CAVE

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The Csodabogyós Cave is situated at Balatonederics, in the Keszthelyi Mountains which belongs to the authority of the Balaton-felvidéki National Park. The cave was formed in the Upper Triassic period in the Ederics Limestone formation and represents a 5,200 m long and 121 m deep multilevel system of fissures. The cave chambers are decorated with various sizes and shapes dripstone

formations, some of which are still actively developing today. This cave (discovered in 1990) has never been studied microbiologically before. The aim of this study was to reveal the cultivable bacterial community in Csodabogyós Cave and observe the bacterial involvement in the $CaCO_3$ precipitation using an *in vitro* experiment. The surface of speleothem (stalagmites, stalactites, soda straws) and the dripping water were sampled in two chambers of the cave. Serially diluted samples were plated onto different subsurface-environment specialized culture media containing low amount of nutrients and extract of cave sediment. Following 2 weeks of incubation at 21°C, altogether 138 bacterial strains were isolated. The isolates were grouped by amplified rDNA restriction analysis (ARDRA), and the 16S rRNA genes of the representatives with distinct ARDRA patterns were sequenced. Most of the 58 representatives were identified as members of phyla Actinobacteria, Firmicutes, Proteobacteria (classes Alpha- and Gammaproteobacteria) and Bacteroidetes. Due to the specific cultivation, strains belonging to species *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Brevibacterium*, *Micrococcus*, *Paeniglutamicibacter*, *Rhodococcus*, *Streptomyces* spp. were found in the highest number among the 28 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 (modified B4) for weeks at 21°C and observed regularly by light and electron microscopes. Majority of the strains were able to precipitate calcium-carbonate minerals with different morphology from the second weeks. Scanning electron microscopy and X-ray diffractometry demonstrated that the observed calcite crystals were produced during these processes. Previously unknown, new carbonate precipitating strains (e.g. *Stenotrophomonas rhizophila*, *Microbacterium azadirachtae*, *Oerskovia enterophila*, *Pseudomonas cichorii*, *Rhizobium nepotum*) were also detected among the bacterial strains.

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SEASONAL DYNAMICS OF PELAGIC BACTERIAL COMMUNITY IN LAKE BALATON

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The Lake Balaton with surface area of 594 km^2 is the largest central European shallow lake. As a result of high external nutrient load the lake was hypertrophic in the ’70s, therefore strict regulations were introduced. At the last decade the water quality stayed between meso- and eutrophic values. Due to the changing trophic level and the touristic significance of the lake numerous ecological research were carried out, but none of them was focused on the seasonal and spatial variability of the bacterial community. At the first year of the project the spatial heterogeneity of planktonic and benthonic bacterial community was investigated at 16 sampling areas with molecular technics. To reveal the seasonal heterogeneity of planktonic bacteria according to our preliminary results 4 sampling areas and 4 time points were marked out on Balaton and the inflow Zala River. Bacterial diversity was investigated by 16S rDNA amplicon sequencing. The bacterial composition of the Lake Balaton markedly different from main inflow Zala River. Mostly well-known freshwater bacteria such as actinobacterial ‘*Candidatus* Nanopelagicus’, cyanobacterial Synecoccales, Nostocales or proteobacterial LD12 group were abundant in the planktonic samples. While some bacterial lineages

showed seasonal dynamics (e.g. members of Verrucomicrobia, Cyanobacteria) some genera (e.g. actinobacterial '*Candidatus* Nanopelagicus', unknown Ilumatobacteraceae) were constantly dominant members of the community. The riverine nutrient input has a detectable microbial effect (e.g. higher cyanobacterial abundance) on the western basin of the lake which is close to the estuarine area.

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PRELIMINARY DATA CONNECTED TO MICROBIOLOGICAL INVESTIGATIONS AT TWO DRINKING WATER SUPPLY SYSTEMS IN HUNGARY

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Bacteria occurring in drinking water systems (DWS) can be harmful in various ways: risk for human health or can be responsible for corrosion processes. As DWSs are checked usually only on standard cultivation techniques, some bacterial groups can remain in the hidden diversity even if they can be responsible for any losses in the system. The aim of the present study was to check the water quality with molecular techniques (taxon specific PCR), with microscopic cell counts (after DAPI staining with epifluorescent microscopy) and cultivation at two Hungarian water supply system, in Jánoshida and Szigetvár. Water samples were collected from 4 sites in Jánoshida and Szigetvár to assess the microbiological state of the well water before treatment, after GAC adsorbent, after breakpoint chlorination and the tap water after the whole treatment process.

To conduct taxon-specific PCR, we filtered at least 1 litre of water from each sample through DNA filters, isolated DNA with PowerSoil DNA Isolation Kit then performed *Pseudomonas* spp., *Pseudomonas aeruginosa*, *Legionella* spp., *Legionella pneumophila*, (as pathogenes) *Stenotrophomonas maltophilia*, *E.coli*/coliform, *Acinetobacter baumannii*, *Bacteroides fragilis* (as faecal indicators) SRB, *Acidithiobacillus ferrooxidans* and *Geobacillus stearothermophilus* (indicator bacteria for corrosion) taxonspecific PCR reactions. We have found *Pseudomonas* spp. and *Stenotrophomonas maltophilia* in each sample of Jánoshida, and most of Szigetvár, it is not surprising as they are ubiquiter bacteria, widely distributed in aquatic ecosystems. Faecal indicator bacteria were not present by cultivation but from one tap water sample they could be detected by taxonspecific PCR, most probably they are originating from the old tap itself. *Acidithiobacillus ferrooxidans* was present in many samples but *Geobacillus* could not be detected. The microscopic cell counts differed in the samples: breakpoint chlorination destroyed most bacteria but at the GAC adsorbent their number increased at least 2 magnitude.

INFLUENCE OF MANGANESE(II) ION UPTAKE ON CITRIC ACID PRODUCTION IN *ASPERGILLUS NIGER*

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Citric acid (CA; 2-hydroxypropane-1,2,3-tricarboxylic acid) is one of the most important bio-products, widely used in the food, chemical and pharmaceutical industry. The estimated 2.1 million tons of citric acid manufactured annually is mostly produced by fermentation using the filamentous fungus *Aspergillus niger*. The presence of manganese ion (Mn^{2+}) in the growth medium in concentrations >5 ppb seriously decreases CA yields (Yp/s), therefore creating a Mn^{2+} limited environment in the fermentor is a prerequisite. Understanding manganese ion metabolism in *A. niger* is critical to improve citric acid production. By homology to the characterized *S. cerevisiae* manganese ion transporters a gene (NRRL3_07789) was identified in *A. niger* and named *mntA*. We constructed both deletant (ΔMntA) and overexpressed (OEMntA) mutant strains; their genotype was verified by Northern blot analysis. Specific Mn^{2+} uptake rate of the deletion strain dropped to almost zero at 10, 100 and 1,000 ppb concentrations, while that of the overexpression strain significantly increased at the two lower (but not at the highest) concentrations. Under citric acid producing conditions both the reference and the deletion mutant strains displayed molar citric acid yields over 80%, but maximal biomass of the mutant decreased by a third. In contrast, molar yield of the OE cultures dropped to 17% of that of the reference strain, while biomass formation doubled.
The overexpression strain displayed a particularly filamentous morphology characterized by branched mycelial clumps as opposed to the pellet-dominated cultures of the reference and deletion strains. Our results demonstrate that MntA is important in the transport, and hence metabolism of Mn^{2+} ions; and is highly relevant to citric acid overflow *A. niger*.

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BEYOND *CANDIDA ALBICANS*: VIRULENCE AND PATHOGENESIS OF AN EMERGING FUNGAL PATHOGEN, *CANDIDA PARAPSILOSIS*

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Patients with suppressed immunity are at highest risk for hospital-acquired infections. Among these, invasive candidiasis is the most prevalent systemic fungal nosocomial infection. Over recent decades, the combined prevalence of non-albicans *Candida* species outranked *C. albicans* infections at several geographical regions worldwide, highlighting the need to understand their pathobiology in order to develop effective treatment and to prevent future outbreaks. *C. parapsilosis* is the second or third most frequently isolated *Candida* species from patients. Besides being highly prevalent, its biology differs markedly from *C. albicans*, which may be associated with *C. parapsilosis*' increased incidence. Differences in virulence, regulatory and antifungal drug resistance mechanisms and the patient groups at risk indicate that conclusions drawn from *C. albicans* pathobiology cannot be simply extrapolated to *C. parapsilosis*. Such species-specific characteristics may also influence their recognition and elimination by the host and the efficacy of antifungal drugs. Due to the availability of high-throughput, state-of-the-art experimental tools and molecular genetic methods adapted to *C. parapsilosis*, genome and transcriptome studies are now available that greatly contribute to our understanding of what makes this species a threat. In this talk, I will summarize ten years of findings

on *C. parapsilosis* pathogenesis, including the species' genetic properties, transcriptome studies, host responses and molecular mechanisms of virulence.

EPIDEMIOLOGY AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF THE MORGANELLACEAE FAMILY IN URINARY TRACT INFECTIONS IN INPATIENTS AND OUTPATIENTS BETWEEN 2008 - 2017: A RETROSPECTIVE AND COMPARATIVE STUDY

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Members of the Morganellaceae family (namely *Proteus*, *Providencia* and *Morganella*) are peritrichous Gram-negative rods, which, in addition to being strong urease producers, possess virulence factors crucial for the pathogenesis of UTIs (IgA protease, haemolysin, cytotoxins and fimbriae). They are intrinsically resistant to various classes of antibiotics (tetracyclines, nitrofurantoin, colistin) and they frequently produce AmpC-β-lactamases, therefore making the management of these infections difficult. Complicated UTIs caused by the species are associated with several complications (stone formation, pyelonephritis), recurrence and prolonged antibiotic treatment. Urinary tract infections caused by Morganellaceae were identified retrospectively by reviewing the computerized records of the Department of Clinical Microbiology, Albert Szent-Györgyi Clinical Centre (tertiary-care teaching hospital). The data screening included samples taken at inpatient departments and outpatient clinics over a 10-year period (2008 - 2017).

Statistical analyses were performed using IBM SPSS Statistics Software 24.0. Morganellaceae members were more frequently isolated in case of inpatients (7.20 ± 1.74% vs. 5.00 ± 0.88% out of 1,392 and 1,058 positive urine cultures, respectively; $p = 0.003$). 69.38% of isolates originated from catheter-specimen urine in the inpatient group. *Proteus mirabilis* was the most frequently isolated species (inpatients: 81.54 ± 2.76%; outpatients: 82.49 ± 4.76%) of the group. In a small proportion of cases (7.28% for inpatients, 8.33% for outpatients; $p > 0.05$), co-infection occurred with another pathogen of the urinary tract. The ratio of resistant strains in the inpatient group were significantly higher to co-trimoxazole (62.03 ± 7.74% vs. 46.32 ± 11.85%; $p = 0.003$), ciprofloxacin (47.58 ± 10.39% vs. 23.27 ± 10.62%; $p < 0.0001$) and ceftriaxone (36.19 ± 10.83% vs. 21.10 ± 11.10%; $p = 0.007$), but not in case of gentamicin (16.31 ± 4.03% vs. 14.55 ± 3.67%; $p > 0.05$). No meropenem-resistant isolates were recovered. Fosfomycin susceptibility testing was performed in 14.49% of isolates overall, 56.91% isolates were susceptible. The high incidence of strains resistant to ceftriaxone and ciprofloxacin (>40% of isolates) has been consistent since 2010, while for co-trimoxazole (>60% of isolates) since 2011. Isolates originating from outpatient departments showed similar growing trends in the survey period. The knowledge of local resistance patters should influence the choice of antimicrobial therapy in these infections. The marked (5.7-times higher) increase in the isolation of *Morganella* and *Providencia* spp. since 2013 should also be noted.

EPIDEMIOLOGY AND RESISTANCE TRENDS OF *STENOTROPHOMONAS MALTOPHILIA* ISOLATED FROM LOWER RESPIRATORY TRACT SPECIMENS: A RETROSPECTIVE SINGLE CENTER SURVEY (2008 - 2017)

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Stenotrophomonas maltophilia is a non-fermenting Gram-negative bacillus, commonly associated with severe pulmonary infections in cystic fibrosis patients and nosocomial outbreaks and an increasing number of community-acquired pneumonia (CAP). The treatment of choice in *S. maltophilia* infections is trimethoprim-sulfamethoxazole (co-trimoxazole), but the emergence of resistant strains (MSTM) is a grave concern, as there are not many therapeutic alternatives (due to the intrinsic resistance of this pathogen to several classes of antibiotics), in addition, EUCAST has only published breakpoint criteria for this drug. *S. maltophilia* isolates were identified retrospectively by reviewing the computerized microbiology records of the Department of Clinical Microbiology, University of Szeged. The data screening included lower respiratory samples (e.g., sputum, tracheal secretions, broncho-alveolar lavage, miniBAL, thoracocentesis) from patients admitted to the Albert Szent-Györgyi Clinical Centre (Szeged, Hungary) between 1st of January 2008 and 31st of December 2017, who had clinical signs or symptoms of infection. The identification of isolated bacteria was carried out using VITEK 2 (bioMérieux) and MALDI-TOF MS (Bruker Daltonics). The majority (75.82%) of patients were below 5 or over 50 years of age, the male-to-female ratio was 1.77. 89.64% of isolates originated from inpatient departments (including the Perinatal, Paediatric and Adult Intensive Care Units [47.49%], Department of Internal Medicine [27.29%] and Department of Otorhinolaryngology [8.11%], however, the number of positive samples from outpatient departments has increased considerably since 2013. 12.09% of isolates were resistant to co-trimoxazole and 5.87% of isolates were also resistant to fluoroquinolones and colistin, based on non-species-specific breakpoints. Based on our local data, *S. maltophilia* infections are most commonly associated with certain age groups (infants-toddlers and the elderly) and patients with severe underlying conditions (malignant diseases, congenital abnormalities).

As the incidence of community-acquired *S. maltophilia* infections is increasing, physicians working in the outpatient setting need to consider this pathogen as a potential aetiology. Institutional therapeutic protocols are required for the treatment of co-trimoxazole-resistant infections.

METAL-BASED ANTIMICROBIAL STRATEGIES: AN *IN VITRO* STUDY ON THE EFFICACY OF HYDRAZONE-BASED TRANSITION METAL COMPLEXES

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The emergence of multidrug resistant pathogens presents an urgent need for new classes of antimicrobial agents. Metal ions play central roles in the structural organization of microorganisms and in the biological functions of various enzymes. Metal based-antimicrobials have potential for being sustainable solutions for the treatment of infectious diseases. Pyridazine-based metal complexes have received substantial attention in various fields of basic and clinical pharmacology. The aim of our study was the evaluation of the antimicrobial activity of novel hydrazone-type Schiff-base ligands with N-donor atoms and their coordination complexes with transition metals [Co(III)/Co(II), Ni(II), Cu(II), Zn(II)]. The antibacterial activity of 10 tested compounds against reference strains of Gram-positive and Gram-negative bacteria was evaluated using broth microdilution method, according to EUCAST standards. A MIC reduction assay was performed to quantify the effects of these complexes on the minimum inhibitory concentrations of reference antibiotics against *Staphylococcus aureus* and *Escherichia coli*. The potency of the tested compounds as quorum sensing-inhibitors was evaluated using the sensor strain *Chromobacterium violaceum* CV026 and the N-acyl homoserine lactone producer *Enterobacter cloacae* 31298. The anti-HSV-2 activity of the tested compounds was evaluated using MTT assay on Vero cells. The cytotoxicity of the metal complexes was assessed on murine (NIH/3T3) and human (MRC-5) fibroblast cell lines using MTT method. The hydrazone-type ligands and their metal complexes did not present antibacterial activity against Gram-negative bacteria, while the Co(III)-containing complex showed promising antimicrobial properties against Gram-positive pathogens. The tested compounds reduced the MICs of tetracycline and ciprofloxacin by 2 - 7-fold. The metal complexes had no relevant quorum sensing inhibitory activity. The complexes containing Cu(II) and Zn(II) showed moderate selectivity in the preliminary antiviral assay. Barring a few exemptions, the compounds showed no signs of toxicity on the tested cell lines. The compounds had no effects on Gram-negative bacteria, which may be attributed to their inability to pass through their complex cell wall. Although their mechanism of action is unclear at this time, hydrazone-based transition metal complexes presented promising properties as antibiotic adjuvants and antiviral agents. The further elucidation of their biological activities using alternative *in vitro* models is warranted.

IDENTIFICATION OF ENDOPHYTIC FUNGI ISOLATED FROM AGRICULTURAL AND NON-AGRICULTURAL PLANTS OF NORTHERN KAZAKHSTAN

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Fungal endophytes consist of various fungi, which colonize plant tissues without causing symptoms. Endophytes have a crucial role in plant health, as they can defend plants from abiotic and biotic conditions and improve the growth and yields. These endophytic fungi can be used as biocontrol agents for sustainable agricultural development, hence, to study the diversity and different members of the community is important and can lead to applied investigations. In the present study, we aimed

to isolate and identify root colonizing endophytic fungi of gramineous plants in agricultural and non-agricultural areas of the Akmola region, Northern Kazakhstan. Root samples of plants were collected from agricultural and non-agricultural areas in Akmola region, Northern Kazakhstan. The roots of various species of Poaceae were sampled: wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), wild barley (*Hordeum jubatum*), oat (*Avena sativa*) and *Stipa capillata*. After surface-sterilization with hydrogen peroxide and 70% ethanol, the root sections were placed on potato-dextrose agar plates (PDA). The colonies of fungi growing out of the roots were transferred to new plates. For molecular identification, DNA was isolated using CTAB method and ITS (Internal transcribed spacer) of the nrDNA was amplified and sequenced. In the case of *Fusarium* isolates, the TEF1 region (translation elongation factor 1 alpha) was also sequenced. Altogether 266 isolates were collected from different plants: barley (70), wheat (132), oat (19), *Stipa* (23) and wild barley (22). The endophytic fungi isolated represented several fungal orders dominated by ascomycetes and less lineages were represented by basidiomycetes and mucoromycetes. The majority of the endophytes belongs to the orders Hypocreales (Sordariomycetes) and Pleosporales (Dothideomycetes), and the most common taxa were *Fusarium* spp., *Alternaria* spp., *Microdochium bolleyi*, *Periconia macrospinosa*, *Ophiosphaerella* sp., *Bipolaris* sp. and *Chaetomium* sp. Nine hypothetical "novel lineages" were found, which could be identified only on genus or higher level. These strains were isolated from cultivated barley and wheat, as well as from wild barley and *Stipa* in the steppe and forest-steppe zone of Northern Kazakhstan. Nine Fusarium species were identified based on the ITS+TEF1 sequences. To conclude, the significant number of endophytes showed the variety of isolates represented by different fungal orders isolated from agricultural and non-agricultural sites of Northern Kazakhstan. These are the first results on fungal root endophytes of the region, which species might have potential role in plant survival and production in Northern Kazakhstan.

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INVESTIGATION OF THE MYCOBIOTA OF GRAPEVINE TRUNKS AFFECTED BY TRUNK DISEASES

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Grapevine trunk diseases (GTD) are currently considered as one of the most relevant challenges for the viticulture. These destructive fungal diseases cause in vineyards several damages every year, and they are of rapidly growing concern in all wine producing countries. Since banning arsenites in 2003 in the European Union, there is no effective control technique against GTDs. Biological disease control is an approach that has proven succesful in combating several plant diseases. *Clonostachys rosea* is a soilborn ascomycete known for its mycoparasitic and antagonistic abilities against a wide range of plant pathogens including *Alternaria* spp., *Botrytis cinerea*, *Fusarium culmorum*. However it's capability to control GTDs haven't been investigated in details yet. The possible use of *C. rosea* as a biocontrol agent against GTDs was examined in the present study. During 2018, endophytic fungi were isolated from a total of 100 grafted grapevines. In addition to the several plant pathogen fungi five *C. rosea* strains were isolated. With these isolates confrontation test were performed with 6 GTD related pathogenic fungi: *Cadophora*, *Phaeomoniella*, *Botryosphaeria*, *Phomopsis*, *Eutypa* and *Phaeoacremonium* spp. Mycoparasitism was examined both macro-, and microscopically, and it was the most effective against *Botryosphaeria* spp. The phenomenon of antibiosis was also observed. The greatest inhibiton of growth was measured in case of *Phaeomoniella* spp. After these tests the most

promising *C. rosea* isolate (100C/1) was selected for further investigation of the antibiotic effect. Agar diffusion tests were carried out with a *Phaeomoniella* spp. to characterize the active antifungal compound in the fermentation broth of 100C/1 strain. The results showed that the molecule wich is responsible for the antibiotic effect was not proteinlike, soluble in chloroform, and thermosensitive, possibly some kind of secondary metabolite. The chloroform extract of 100C/1 was also examined by thin layer chromatography, and several different compounds were detected. In addition, we also examined the *C. rosea* isolates in other technical aspects, like the endophytic growth, the sporulation rate, and the ability of the growth in soil. The effectiveness of *C. rosea* isolates to produce elicitors was also tested by the measurmement of salycilic acid accumulation in leaves treated with the water extracts of soils, colonized by the tested fungi. Our results showed that *C. rosea* could be a potent inhibitor against GTDs and its effectiveness is based on several modes of action. All isolates showed mycoprasitism and antibiosis against different GTD related pathogens and one isolate suggested to produce water soluble elicitors, which stimulate the immune system of grapevine.

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COMBINATORIAL STRESS RESPONSES IN FUNGI

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Studying stress responses of fungi using transcriptomic approaches let us obtain an overall view on the behavior of secondary metabolite gene clusters. In most of these studies, the production of secondary metabolites is difficult to be detected due to technical problems. We adapted a simple method for rapid testing of the secondary metabolite spectra of stress treated cultures. This method was used to record how different stresses (menadione, terc-butyl hydroperoxide and diamide induced oxidative stresses as well as Congo Red, NaCl and $CdCl_2$ stresses) affected the secondary metabolism of an *Aspergillus nidulans ΔatfA* mutant and its parental strain.
The mutant was more sensitive to menadione, terc-butyl hydroperoxide, diamide and NaCl stress and it was more tolerant to Congo Red stress than the parental strain. Multidimensional scaling of the data suggested that: 1) Secondary metabolite production of stress treated and untreated cultures highly depended on the presence of the AtfA bZip transcription factor. 2) The secondary metabolite spectra of NaCl and Congo Red treated cultures in case of both strains as well as of menadione treated cultures in case of the control strain differed markedly from those recorded under the other stresses. 3) Secondary metabolite production of cultures did not show strong correlation with the transcription data of the secondary metabolite cluster genes.

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UTILIZATION OF CARBON AND NITROGEN SOURCES BY NITROGEN FIXING ROOT NODULE SYMBIONTS OF GRAIN LEGUMES

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The globally emerging demand for protein nutrition has triggered a significant increase in the production of grain legumes like soybeen (*Glycine max* L.), chickpea (*Cicer arietinum* L.) and pea (*Pisum sativum* L.). Agricultral world production of soybean has reached around 339 million tons in the 2017 - 2018 season, the highest quantity ever reached so far. In Hungary only GMO free soybeen cultivars are sawn, with the disadvantage of variable crop yields. Effective grain legume cultivation requires seed inoculation with nitrogen fixing rhizobium symbionts, providing proper levels of nitrogen uptake for the plants. Efficient seed inoculation happens by using high (min. 10^9/mL) cell counts of viable symbionts in the seed coating process (10^5 - 10^6 cells/seed). The aim of our experiments was to create fermentation media that provides the desired high cell count (min. 10^9/mL) in a faster growth procedure than observed with the broadly used yeast extract mannitol (YEM) medium [1]. Soybeen symbionts used for the experiments were *Bradyrhizobium japonicum*, and *B. diazoefficiens*, pea symbiont was *Rhizobium leguminosarium* bv. *viciae* whereas chickpea symbiont was *Mezorhizobium ciceri*. The medium optimalization experiments fine-tuned the symbionts carbon and nitrogen sources for each species, respectively.
Results from these studies indicated that xylose, acetate, glutamic acid may serve as a potential alternative carbon sources to the certain species. Studies on the nitrogen sources included besides the complex nitrogen sources (yeast extract, corn steep liquor (CLS), peptone and soya peptone) also amino acids, organic nitrogen derivatives and inorganic ammonium salts. The application of L-histidine has shown a significant improvement for *B. diazoefficiens*, L-alanin for *Mezorhizobium ciceri* growth speed and fermentation stability compared to complex nitrogen sources.

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MAJOR CHANGES IN THE TAXONOMY OF VIRUSES

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Thanks to the novel molecular techniques and growing interest towards viruses of exotic animals and the whole environment, the number of identified or predicted viruses is on a rapid increase. Nowadays, the full genomic sequence can be obtained even if the isolation of a certain virus fails. A recent decision of the International Committee on Taxonomy of Viruses (ICTV) allows for the approval and official classification of such new viral sequences (under certain circumstances). According to the new rule, a novel virus species can be created even for “sequence-only” viruses if they are adequately different from members of the earlier established species. Presently, already 5,560 virus species are officially approved (there was only 3,704 in 2016). It is notable, that any scientist who discovers a new virus is free to submit a proposal to ICTV for a new virus species. The forms, simplified recently, can be downloaded from the ICTV home page. However, the future of species naming may cause serious debates. ICTV is getting close to adopt the Linnaean nomenclature, i.e. binomial names for virus species similar to those used for animals and other forms of life. These would start with the genus name followed by a single specific name (e.g. *Mastadenovirus hominis*). An undecided but vigorously disputed question is about the use of

Latinized or English names. Regardless of nomenclature, the demarcation criteria for most of the virus species should be redefined. By pair-wise sequence identity testing, the Sequence Demarcation Tool could (usually) provide a way to distinguish virus species.
Finally, the introduction of taxa higher than the "order" has also happened. Ten new taxon levels have been introduced besides the five levels that had existed earlier (with the "order" being the highest). Provisionally, only one realm, with the name of Riboviria for all RNA viruses that use cognate RNA-dependent RNA polymerases (RdRPs) for replication, has been approved. Presently, it contains one phylum Negarnaviricota for the (-)RNA viruses (Class Baltimore V). The real challenge will be to create a similar high level taxonomy for DNA viruses, which might have undergone numerous recombination events and do not possess a ubiquitous common gene.

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OUTER MEMBRANE VESICLE FORMATION IN GRAM-NEGATIVE BACTERIA AS MULTIPLE STRESS RESPONSE MECHANISM LEADING TO HYDROPHOBIC CELL SURFACES AND BIOFILM FORMATION

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

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WHOLE-GENOME SEQUENCING OF *BURKHOLDERIA PSEUDOMALLEI* ISOLATE IN HUNGARY

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Melioidosis is an emerging infectious disease caused by a free living soil dwelling bacterium *Burkholderia pseudomallei*. The disease is endemic to most parts of Southeast Asia, northern Australia, and Brazil, mostly present is soil and water. Infections may occur through inhalation, skin abrasions or ingestion of contaminated soil or water. In Europe only imported cases have been described. Melioidosis can be difficult to diagnose due to its varying clinical manifestations and and the organism is often misidentified by methods used routinely in clinical laboratories. The real global burden of melioidosis is still unknown; predictions suggest that the agent can cause 165,000 case annually worldwide. Melioidosis can cause a wide range of symptoms with the most serious form of disease, septic shock, resulting in fatality of up to 95% of untreated cases. Neurological cases occur rarely with the prevalence of 3 - 4% and the fatality rate is high without prompt and adequate therapy. The bacterium is commonly resistant to a wide range of available antibiotics. In 2017, our laboratory started to implement the whole-genome sequencing method using Illumina platform and set up a reliable custom-made bioinformatic analysis pipeline for highly dangerous bacterial pathogens. Here, we present the complete genome sequence of the first imported *Burkholderia pseudomallei* case in Hungary. The strain marked as 584/OEK was isolated in 2008 from a 30 years old male patient with encephalomyelitis who travelled to India to work and contracted the disease. The prodrome symptoms included weakness, fever, headache and facial-neuralgia developed three weeks after his arrival back to Hungary. The strain was isolated from blood culture. After the adequate therapy the patient fully recovered in spite of his serious symptoms.

The aim of this study was to investigate the virulence potential of this isolate. Whole-genome sequencing was performed using MiSeq2000 Illumina platform. Structural gene prediction and functional annotation were performed. The final assembly consisted of two chromosomes. The total genome size was 7.2 Mb with 67.9% GC content, the number of coding sequences were 6,937. As an indication of antibiotic resistance OXA57 beta-lactamase was present. The whole-genome sequence was used to extract sequences for MLST analysis. We found that the strain is belonging to a novel ST that had not been previously recorded. The allelic profile of ST1643 was 1-12-6-4-1-8-87 (http://bpseudomallei.mlst.net). For further virulence profile identification, custom-made virulence databases were set up using the main virulence related genes. Laboratory work was performed at the National Public Health Center, National Biosafety Laboratory, Hungary. To the best of our knowledge our laboratory performed the first whole-genome sequencing and comparative analysis of the highly pathogenic Burkholderia pseudomallei in Hungary.

PafC: THE THIRD SMALL, CYSTEINE-RICH, CATIONIC ANTIFUNGAL PROTEIN FROM *PENICILLIUM CHRYSOGENUM* EFFECTIVELY INHIBITS THE GROWTH OF *CANDIDA ALBICANS*

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Fungal diseases affect over one billion people worldwide, whereby *Candida* species represent the most common opportunistic human pathogens and are known to have intrinsic resistance or a high potential for resistance development to licensed antifungal agents. This therapeutic limitation urges the development of new antifungal compounds for the treatment of *Candida* infections. Small, cysteine-rich, cationic antimicrobial proteins (AMPs) are secreted by numerous filamentous Ascomycetes and have been shown to inhibit the growth of several fungal pathogens, rendering them potential candidates for new antifungal drug development. *Penicillium chrysogenum* is quite unique in its ability to produce three different AMPs: PafA, PafB and PafC, which possess growth inhibitory activity against *Candida albicans*.The aim of the present study was to analyze the antifungal mode of action of PafC against *C. albicans*. PafC is an orthologue of the *Penicillium brevicompactum* "bubble" protein, which has been found in fungal exudates of surface cultures. PafC is similar to PafA and PafB in overall structural properties but shares an amino acid identity of only 14% and 17%, respectively. This phylogenetically classifies the protein more distant from the group of PafA and PafB. To study the antifungal activity, recombinant PafC was produced in a *P. chrysogenum*-based expression system and purified from the culture supernatant by cation-exchange chromatography. Broth microdilution assays in diluted potato dextrose medium were applied to determine the minimal inhibitory concentration (MIC) of 2.5 μM against *C. albicans*. Localization studies with fluorophore-labelled protein (PafC-BODIPY) revealed that PafC is taken up by *C. albicans* and induces cell death which was visualized by propidium iodide (PI) co-staining. Scanning electron microscopy indicated that PafC exposed *Candida* cells suffered from severe changes in the morphology of the outer cell envelope, pore formation and leakage of the cell content.
These effects were PafC concentration dependent and more prominent with longer treatment indicating that PafC is able to disrupt the cell membrane. We also tested the cell-killing efficacy of PafC in a time-course experiment with increasing protein concentrations by fluorescence-activated cell sorter (FACS) analysis. The determined number of PI positive cells suggested that PafC kills the majority of *Candida* cells at 2 x MIC within a short time of exposure.

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GLOBAL DISTRIBUTION AND GENETIC IDENTIFICATION OF FOWL ADENOVIRUSES DETECTED OVER A 15 YEARS PERIOD

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Adenoviruses are infectious agents in poultry. Adenoviruses of chickens that comprise species Fowl adenovirus (FAdV) A to E belong to the genus *Aviadenovirus*. The three major syndromes caused by FAdVs include inclusion body hepatitis (IBH), hepatitis hydropericardium syndrome and gizzard erosion, but other symptoms, like respiratory disease may also occur in association with FAdV infections. Records on the geographic distribution of FAdV species and serotypes are not available.

In addition, linking disease associations to particular FAdV species and serotypes is a relevant scientific and diagnostic issue. During the diagnostic activity conducted between 2003 and 2018 at the Scientific Support and Investigation Directorate, Ceva-Phylaxia (Budapest, Hungary) FAdVs were regularly detected in domestic chicken (*Gallus g. domesticus*). Here we summarized the available clinical, epidemiological and genetic data to provide a comprehensive picture on the global distribution of fowl adenovirus species and the disease associations of different serotypes. The specimens collected from broiler, layer and breeder flocks (n = 145 submissions) originated from 37 countries of 4 continents. Typically, frozen organ samples or organ prints on FTA® Card were submitted for diagnosis. Sample processing for adenoviruses included molecular detection and virus isolation. In particular, partial DNA polymerase gene-specific PCR assay was used to detect adenovirus infection and sequencing and phylogenetic analysis was carried out to determine FAdV species and serotype. From frozen organ specimens FAdV strains were isolated by using LMH cell line. Over the past 15 years adenovirus sequences were obtained from 280 specimens.
All sequences could be classified into the genus *Aviadenovirus*. FAdV serotypes (and species) were represented as follows: 37 sequences were FAdV-1 (species A), 95 were FAdV-2 (D), 5 were FAdV-4 (C), 12 were FAdV-5 (B) and 131 were FAdV-8 (E). Sequence analysis of a 272 bp fragment of the viral DNA polymerase revealed the greatest genetic distance among FAdV-4 (8.1%) strains, followed by FAdV-2 (7.9%), FAdV-1 (4.9%), FAdV-8 (4.4%) and FAdV-5 (2.6%) isolates. The most frequent syndromes in the cases were IBH and gizzard lesions. Data presented here could help better understand molecular epidemiology and evolution of FAdV infections in chickens which could lead to improving syndrome-specific strategies in disease control and prevention.

COMPARISON OF ANTIBIOTIC RESISTANCE AND VIRULENCE OF BLOODSTREAM MRSA AND MSSA ISOLATES FROM THE SEMMELWEIS UNIVERSITY, BUDAPEST

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Staphylococcus aureus is a clinically important pathogen causing bloodstream infections (BSI) in hospitalized patients all over the world. Frequent antibiotic resistance, toxin and adhesin production of the bacterium results in significant morbidity and mortality in BSI patients. However, not all *S. aureus* isolates are the same: antibiotic resistance and virulence of methicillin resistant (MRSA) and methicillin sensitive (MSSA) *S.aureus* differs significantly, contributing to the outcome of *S. aureus* bacteraemia. In this study, all 153 MRSA blood culture isolates, recovered in our laboratory during a 6-year period were analysed and were matched and compared to 153 MSSA BSI strains, collected during the same period. The isolates originated from several Semmelweis University clinics in Budapest, Hungary. The antibiotic susceptibility of the isolates to 13 drugs was determined according to the EUCAST guidelines. Genetic profiles for the presence of several different cytotoxin genes, sugerantigens and adhesins were examined. MRSA strains were significantly more resistant to ciprofloxacin, erythromycin, clindamycin, tobramycin, amikacin and gentamicin, compared to MSSA isolates. Doxycycline was the only drug with higher resistance rate in MSSAs. Most of the isolates (including both MRSA and MSSA) were sensitive to TMP-SMX and rifampin.
All isolates were sensitive to glycopeptides and linezolid, however, average vancomycin MIC values of MRSAs seem to creep higher during the tested years. Antibiotic resistance increased with the age

of patients. Most of the MRSAs carried 7 virulence genes, while MSSAs had 4.9 virulence genes on average. The most prevalent virulence genes were *spa*, *hla* and *ica*. Panton-Valentine leucocidin was found in 2.3%, tst in 1.3% of the isolates. Four virulence genes, *cna*, *sea*, *ica* and *hlb* were significantly more frequent in MRSA, while *tst*, *eta* and *hlgv* were significantly more prevalent in MSSA. Genotyping by PFGE divided the MRSA isolates into 3 large groups at 85% similarity level, while MSSAs were highly diverse. SCCmec typing of the MRSA strains revealed the dominance of SCCmec type IV (66.7%), SCCmec types II and I were responsible for 23.5% and 9.2% of the MRSAs, respectively. MLST typing of representative isolates have shown that the majority of our MRSAs belong to ST22-MRSA-IV (EMRSA-15). ST5-MRSA-II (New-York - Japan) clone and ST1-MRSA-I were also found. Mortality was significantly higher in MRSA BSI (39.9%) compared to the MSSA group (30.7%). Female gender, older age and infection with SCCmec IV isolate was associated with higher mortality rate. In conclusion, MRSA and MSSA isolates differ significantly in their antibiotic resistance, virulence and associated mortality.

OPTIMAL ENVIRONMENTAL CONDITIONS FOR BETTER ANTIMICROBIAL CAPACITY OF THE PULCHERRIMIN PRODUCING *METSCHNIKOWIA* STRAINS

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Certain species within the genera *Metschnikowia* have strong antifungal properties because they are able to produce a red pigment (pulcherriminic acid) and consequently they have potential for use in the biocontrol of fungi. The presence of *Metschnikowia* yeast-based biocontrol product 'Shemer WDG' (*M. fructicola*) in the marketplace demonstrates the commercial feasibility of this approach in plant disease control. One of the essential criteria for the use of microorganisms in bioprotection is continuous and stable maintenance of their antagonistic effects. Variability and problems in production of biocontrol agents can be at least partially overcome by finding the optimal experimental conditions and combining antagonistic yeasts with other alternative methods. For example, stimulating production of the antimicrobial agents with nutrients, optimizing environmental factors to achieve an effective yeast biomass or manipulating yeast physiology to increase their antimicrobial capacity. Therefore, our aim was to determine how different environmental factors affect antagonistic ability of the *Metschnikowia* yeasts. We measured the pigment production of *Metschnikowia* cells under different environmental circumstances, because correlation between the size of the pigmented area around the yeast colony and the boundary of its inhibition zone has proved. We managed to prove that different environmental and nutritional factors, such as temperature and oxygen requirements, optimum pH, osmotolerance, carbon sources, nitrogen sources, vitamins etc. can seriously influence the biocontrol capacity of the yeasts. According to our results the most intensive pigment formation was observed on complete media at low pH and high temperature. Furthermore, our data suggest that the largest pigmented and inhibitory zone were formed on the medium containing sucrose. These results correlated with the specific growth rate of the yeast cells on different carbon sources. Since, copper compounds, such as copper sulfate have extensive employment in agriculture we also tested its effect on cell division and antagonistic capacity of the *Metschnikowia* strains. Our data suggested that the fungicide copper sulfate could decrease the size of the inhibitory zone around the *Metschnikowia* isolates. Taken together, these data

can be very useful in the improvement of biocontrol capacity of yeasts and all these factors must be considered in a possible field application of yeast strains.

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TAILORING HOST CELL SPECIFIC DELIVERY AND BIOAVAILABILITY OF ANTIMYCOBACTERIAL COMPOUNDS

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Tuberculosis (TB) remains a major public health threat; the causative agent *Mycobacterium tuberculosis* (MTB) kills approximately two million people each year and is thought to latently infect one third of the world's population. One of the most remarkable features of MTB is its ability to remain dormant within an individual for decades before reactivating. Their non-replicating state make latent mycobacteria resistant to most of the drugs currently employed. Nontuberculous (atypical) mycobacteria (NTM) are increasingly recognized as causative agents of various opportunistic human infections most often originating from contaminated water supplies, and their incidence is expected to rise. Their treatment is complicated with limited efficacy, e.g., due to the high levels of natural and acquired resistance. Among them *M. abscessus* is the most resistant. Mycobacteria as intracellular pathogens, are uniquely adapted and equipped to survive within host cells (mainly macrophages, dendritic and epithelial cells etc.); therefore their elimination could be more efficient with host cell directed delivery targeting host cell surface molecules and receptors (lectins, scavenger, tuftsin, fucosyl receptors etc.). Host cell specific drug delivery systems as peptide based bioconjugates and nanocarriers can be successfully implemented for improving the efficacy of chemotherapy. Isoniazid is a first-line antitubercular drug used for the treatment and prophylaxis of TB. While Isoniazid is active against extracellular Mtb in the sub-micromolar range, the drug is not effective against the intracellular form even at 1,000-times higher concentration. Therefore, Isoniazid is a suitable model compound to study host-directed drug delivery and drug susceptibility of intracellular MTB. To improve cell penetration ability and intracellular killing efficacy of Isoniazid, and antimicrobial peptide (AMP) based carriers were employed. AMPs can the phospholipid membrane and/or cell wall and provoke a broad spectrum of antimicrobial activity against bacteria, viruses, and fungi. Despite the fact that bacteria are frequently exposed to AMPs, manifestation of AMP resistance is low therefore an AMP-drug conjugates can act by multiple mode of antibacterial action without developing significant resistance. Nevertheless, internalization rate of the covalently attached drug to the host cells can be dramatically improved by AMP conjugation. In this project, representative AMPs were employed as drug carriers and after careful *in vitro* evaluation a promising antibacterial drug conjugate with potent penetrating ability, antibacterial effect and suitable selectivity was tested *in vivo* in MTB infected animal model.

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MYCOBACTERIUM TUBERCULOSIS RELATED T-CELL EPITOPE PEPTIDE-BASED VACCINE CANDIDATES

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In this study, promiscuous T-cell epitopes from different proteins expressed by *Mycobacterium tuberculosis* (Rv1886c, Rv0341, Rv3873) were selected based on previously reported antigenic properties. To induce a more potent immune response, epitope peptides were conjugated to a Tuftsin carrier in branched chain arrangement. The trivalent conjugate showed higher tendency to fold and increased internalization to professional antigen presenting cells. Cellular uptake was further improved by the incorporation of a palmitoyl group to the conjugate. Immunization of CB6F1 mice with the conjugates resulted in significantly higher T-cell proliferation with prominent expression of IFN-γ, IL-2 and IL-10 cytokines, compare to free epitopes. To enhance bioavailability and vaccine efficacy the multi-epitope conjugate was encapsulated to poly(D,L-lactic-co-glycolic acid) (PLGA) nanoparticles. As adjuvant, trehalose-6,6-dibehenate (TDB) was used, which is a synthetic analogue of trehalose-6,6-dimycolate, the most studied immunostimulatory cell wall component of *Mycobacterium tuberculosis*. Characterization (DLS, SEM analysis) of PLGA constructs revealed that the size of the nanoparticles was between 100 - 120 nm and the encapsulation efficacy of the multiepitope conjugate was 80%. The injection site clearance, which was followed by MRI, was significantly slower in the case of PLGA encapsulated conjugate compared to the free conjugate. Vaccine efficacy of the compounds was evaluated in a murine model of tuberculosis. In histologic sections prepared from the organs of un-vaccinated control animals, rod-shaped acid-fast bacteria were observed within small groups of epitheloid macrophages. This indicates, that the used infection method was successful in modelling experimental tuberculosis. When mice were immunized with the nanoencapsulated constructs, significantly lower number of bacteria were enumerated compare to the un-vaccinated control group. Finally, the results highlight the importance of appropriate formulation of epitope peptides which allow developing epitope-based vaccine candidates against tuberculosis.

APPLICATION OF GENETIC FINGERPRINTING AND A NEW, RAPID MULTIPLEX PCR SHOWS THAT CLINICAL *SACCHAROMYCES* ISOLATES FREQUENTLY ORIGINATE FROM PROBIOTIC SUPPLEMENTS

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Saccharomyces cerevisiae is among the most widely used microbial species in the food industry and it is increasingly applied as a promising probiotic agent. However, the species has also been reported

as an opportunistic pathogen. An increasing number of infections originating from probiotic use are reported worldwide. These probiotic yeasts are marketed as *Saccharomyces 'boulardii'*, but in fact are a subtype of *S. cerevisiae*. Reliably linking infectious cases to probiotic products requires unequivocal intraspecific genotyping, however, such techniques are often time-consuming and difficult to implement in routine diagnostics. This leads to a widespread lack of genetic data regarding the origin of *Saccharomyces* infections. We propose a quick and reliable PCR-based protocol for the identification of probiotic-derived infections and we compare it to other established typing techniques. By applying our proposed method to the clinical yeast collection of the Clinics of the University of Debrecen we show that probiotic origin is common.
The new multiplex method enables rapid and unequivocal identification of probiotic yeast infections. This method can be applied for the identification of yeast infection sources, helping decisions on probiotic use and the safety assessment of probiotic products.

FUNGAL ROOT ENDOPHYTES OF THE DOMINANT GRASS *STIPA KRYLOVII* IN MONGOLIAN STEPPE REGION

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Plants form symbioses with various microorganisms through their roots, including endophytic fungi. Endophytic fungi are characterized as microorganisms found within internal tissues of living plants without any immediate, overtly negative effects. Fungal root endophytes are important members of the plant microbiota and may have various effect on plants survival under different stress conditions. Dark septate endophytes (DSE) are widespread form-group of endophytic fungi mostly belonging to Ascomycota, and these root colonizers are common under abiotic stress conditions. However, the function of fungal root endophytes in ecosystems is still unclear. Our information on their communities of Asian grasslands is even more limited, and only few studies have been focused on the root endophytes of plants in the Mongolian steppe. The present study was carried out to isolate, taxonomically characterize endophytic fungi from the roots of *Stipa krylovii*, a common gramineous plant species of semiarid grasslands in Mongolia. We also aimed to 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, partial translation elongation factor 1-α (TEF) gene, large subunit (LSU) and in few cases small subunit (LSU) region of rDNA were 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. Altogether, we gained 135 isolates. Based on their ITS region identification 110 isolates belonged to Ascomycota and 25 isolates belonged to Basidiomycota. Further DNA regions of 61 representative isolates were also sequenced. Majority of the isolates caused no negative effect to the leek. Most of the isolates could be identified on species or genus level, 19 new lineages, representing novel taxa are surely present among these endophytes.
We found numerous DSE clades, which have been detected previously in roots of gramineous hosts in semiarid grasslands of the Great Hungarian Plain and from prairies of the United States, too.

Common and dominant pleosporalean lineages of grasslands, such as *Periconia macrospinosa* and *Darksidea* species were also found in the Mongolian steppe. Our results confirm that already known dominant members of root endophytic fungal communities are present in the Mongolian steppe.

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THE EFFECT OF PLANT EXTRACTS AND ZINC OXIDE ON INTESTINAL MICROBIOTA OF PIGLETS

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The gut microbiota plays important role in maintaining the health of the host animal. Beneficial microbes are mainly lactic acid bacteria (LAB) which can contribute to the improvement of immune system, pathogens suppression and production of beneficial compounds. Escherichia coli and other members of Enterobacteriaceae (commonly called “coliforms”) are part of the normal intestinal microbiota of humans and animals. They can be harmless or beneficial symbionts, but many of them are known as pathogens or disease causing bacteria. Disease-causing coliforms represent a risk in livestock breeding, especially in the case of swine. Antibiotics and other antimicrobial agents has been used as feed supplements to preserve the health of farm animals for decades. In 2006, the risks associated with use of antimicrobial agents led to the prohibition of antibiotics as feed additives in the European Union. In case of swine, zinc oxide is very efficient against post-weaning diarrhoea, although increased use of it can cause the emergence of multi-resistant *E. coli* strains as well as environmental hazards. In 2017 the European Medicines Agency decided to ban the use of zinc. However, removal of these substances from animal feeding increases the pathogen pressure in livestock. Thus, pig breeders need to find new solutions against post-weaning diarrhoea, such as the use of pre/probiotics or herbal extracts. In this study three different mixtures of herbal extracts and zinc oxide (as a control) were used as feed additives. Treated and control groups consisted of 10 - 10 animals. Stool samples were collected four times during an approximately 80 day long period: on the 10^{th} day after the birth of the piglets, before and two weeks after the weaning and at the end of the pig breeding. Changes in the total number of coliforms (potential pathogens) and lactic acid bacteria (beneficial microbes) were determined in case of treated and control animals. The amount (CFU/g faces) of bacteria was evaluated on semi-selective and differential agar plates: De Man, Rogosa and Shapre (MRS) and Eosin Methylene Blue (EMB) media were used in case of LAB and coliforms, respectively. There was only a slight difference in the amount of LAB between the stool samples of treated and control animals. The amount of coliform bacteria has decreased continuously. Our results suggest that different plant extracts may prove to be suitable alternatives to prevent infection caused by coliforms without disturbing the beneficial microbes of the intestinal microbiome.

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TYPING OF HUNGARIAN FOWL ADENOVIRUS STRAINS REVEALS A POSSIBLE NEW GENOTYPE

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Recently, we examined the diversity of fowl adenovirus (FAdV) types occurring in Hungary. From diseased chicken flocks in Eastern Hungary, 29 FAdV strains were isolated between 2011 and 2015. We performed molecular typing of the isolates based on their partial hexon sequences. The results showed that representatives from every FAdV species from A to E are present in Hungary, but compared to the findings from our previous survey, a lower number of different FAdV types were detected. Inclusion body hepatitis was always associated with FAdV-2 or -8b, gizzard erosion was caused in almost every case by FAdV-1. Numerous strains belonging to species FAdV-B were found. The complete genome sequence of a candidate new genotype strain, showing the highest divergence from the reference FAdV-5, was determined using next-generation sequencing. In order to provide results compatible with the serology-based type classification, multiple genomic regions, including the major antigenic determinants, of the new isolate (strain 40440-M/2015) were compared to their counterparts in the prototype FAdV-5 (strain 340) from species FAdV-B, at both nucleotide and amino acid sequence levels. In different comparative analyses, the two strains were always found to have a larger divergence between each other than any two of the most closely related FAdV serotypes. This new emerging FAdV genotype is already present in Hungary and Austria, though its exact pathological role requires further investigations. The introduction of a novel FAdV (geno)type for the classification of these strains is further supported.

PURIFICATION AND IDENTIFICATION OF EFFECTOR PROTEINS OF THE FUNGAL PATHOGEN *EUTYPA LATA* WHICH INTERNALIZED BY THE CELLS OF THE HOST *VITIS VINIFERA*

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Eutypa lata is the main causal agent of the grapevine trunk disease eutypa dieback. The pathogen colonize the woody tissues of the plant and cause the development of necrosis in the infected wood. Alongside with the damaging of the colonized area, the fungus secrete effector molecules which can reach the green parts of the plant and cause further symptoms. These effectors have diverse chemical nature, secondary metabolites, polysaccharides, and proteins were suggested to be involved in the development of symptoms. Related secondary metabolites (e.g. eutypine, eutypinol) are well characterized, however very little is known about the effector proteins. Experiments were done only with total, or glycosylated extracellular protein fractions, which revealed that these proteins damage the mitochondrial functions of the host cell. The aim of the present study was the isolation and identification of *E. lata* secreted proteins which internalized by the host cell, and possibly responsibe for the damage of mitochondria. One *E. lata* isolate was cultivated in 200 mL of liquid minimal medium for one week at 25°C, with 180 rpm shaking. The fermentation broth was filtered, subjected to ammonium sulfate precipitation and diafiltration using 5 kDa cut-off column. A portion of the

concentrated and purified proteins was labelled with the fluorophore dansyl chloride. Another protion was labelled with biotin for subsequent affinity purification. Fluorescently labelled proteins were used for the treatment of onion epidermis and the localization of these proteins examined by fluorescence mycroscopy. The fluorescent sygnal can be detected not only in the apoplast, but also in the cyoplasm rich regions of onion cells, indicating that some proteins were internalized by the plant cells. Biotinylated *E. lata* extracellular proteins were used for the treatment of young leaves of *V. vinifera*. A leaf was placed in the protein solution with the petiole, and incubated in the fume hood to promote respiration and water uptake. Protein solution was prevented from evaporation by the applyacation of a top layer of mineral oil. After 5 hour incubation, the treated and untreated grapevine leaves were cut to 1 × 1 cm pieces and subjeted to three rounds of vacuum filtration/centrifugation to remove apoplastic fluid alongside with uninternalized fungal proteins. Total intracellular proteins were extracted from the leaves, subjected to avidin affinity chromatography and the eluates were analysed by polyacrylamide gel electrophoresis. Bands which are characteristic to the treated leaf were detected. The remaining purified proteins were analysed by shotgun proteomics, which resulted in the identification of 7 fungal proteins. Four of these are predicted to be involved in mitochondrial functions or the maintenance of energy balance of the cells.

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MANGANESE(II) IONS IN THE GROWTH MEDIUM: MEANS TO OVERCOME AN ARCH-ENEMY OF THE *ASPERGILLUS NIGER* CITRIC ACID FERMENTATION

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Citric acid (CA) is a major bulk product of fermentation biotechnology, extensively used in the food, chemical and pharmaceutical sectors. The estimated 2.1 million tons of CA manufactured globally and annually is produced using the filamentous fungus *Aspergillus niger*. CA overflow requires a combination of several unusual nutrient conditions such as excessive concentrations of carbon source, H^+ and dissolved oxygen and suboptimal concentrations of phosphate, which synergistically influence the yield of CA. The deficiency of manganese(II) ions (Mn^{2+}) in the growth medium is particularly critical: concentrations >5 ppb already seriously decreases CA yield. It is therefore crucial to create a heavily Mn^{2+} limited environment in the bioreactor or to prevent / limit Mn^{2+} ion uptake by the fungus. In this lecture I will summarize how Mn^{2+} ions can get into the growth medium during an *A. niger* CA fermentation, and how to limit their detrimental effects on the yield.

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MOLECULAR CHARACTERIZATION OF DSRNA GENOMES OF VIRUSES ISOLATED FROM *UMBELOPSIS* ISOLATES

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Presence of mycoviruses can be confirmed by the detection of double stranded RNA (dsRNA) elements in fungal isolates. Mycoviruses can be responsible for the effect of hypo- or hypervirulence, however most of the fungal viruses are asymptomatic in their hosts and their presence often remains unexplored. Although mycovirus-harbouring is common among fungi, our knowledge about them is still very limited, especially in Mucoromycota fungi. Our aim was to detect and characterise dsRNA fragments in *Umbelopsis* isolates with different molecular techniques as well as to detect virus-like particles (VLP) purified from the strains. CF-11 cellulose chromatography was used to purify dsRNA elements from total nucleic acid extracts. The isolation and cloning of DNA fragments from dsRNAs were obtained by the FLAC technique. Northern blotting was used to identify the RNA molecules containing the virus genes. VLPs were purified by ultracentrifugation and analysed with transmission electron microscopy. In *Umbelopsis ramanniana* NRRL 1296 strain, four clear dsRNA fragments (2.8 – 5.3 kb) could be observed. BLAST searches revealed that the determined virus sequences from both strains share high amino-acid sequence identities with viruses of Totiviridae family. The detected viruses have two ORFs encoding a putative capsid protein (CP) region and an RNA dependent RNA polymerase (RdRp). The Northern blotting analysis revealed, that the CP and RdRp genes are located on the two highest sized dsRNA fragments.
Transmission electron microscopy revealed the presence of at least two different sizes isometric virus particles in the purified extracts of *U. ramanniana* strain.

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GENOME ANALYSIS OF *ANSER ANSER* POLYOMAVIRUS 1 IN HUNGARY

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Anser anser polyomavirus 1 (AaPyV1) is the etiological agent of hemorrhagic nephritis and enteritis of domestic geese. The disease may accompany with high morbidity and mortality rate of young goslings triggering economical losses for the keepers. The virus has been identified in Muscovy and mule ducks also without any typical clinical signs. Here we describe the genome sequence of 21 AaPyV1 of geese from several Hungarian goose farms, collected in 2007 - 2015. Furthermore, we sequenced the complete genome of a sample from the first observation of HNEG in 1969. Phylogenetic evolutionary analysis has been also performed with the available AaPyV1 sequences. We pursue the inspection of other AaPyV1 strains and potential reservoirs, fulfil evolutionary and phylogenetic analysis, and examine expression profiles and the virus-host interaction.

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HUMAN POLYOMAVIRUS 10: DNA PREVALENCE IN RESPIRATORY SAMPLES AND SEROPREVALENCE

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Members of the Polyomaviridae family are in growing numbers, during the last 12 years complete genome sequences of 12 new, human polyomavirus were discovered. Human polyomavirus 10 (HPyV10) - as Malawi polyomavirus - was described from faecal sample in 2012. Although prevalence studies have been started, the number of studies published is limited, as the number of samples tested for the presence of viral DNA. The virus was detected in stool and respiratory samples. Since the seroprevalence data from two scientific articles available showed high rate of infection in the population, spreading via respiratory route is suggested. If the virus is able to establish persistent infection, secondary lymphoid tissues might be the site for it. One of our aims was to study the DNA prevalence of HPyV10 in respiratory samples. Nucleic acid was isolated from adenoids (n = 100), tonsils (n = 100) and throat swab samples (n = 150). Quantitative, real-time PCR was optimized, and then the presence of the viral DNA in the samples was studied. The viral DNA has not been detected in any samples. In order to perform seroprevalence study, viral antigen is required: VP1 capsid protein of the virus can be used to detect IgG antibody from serum samples. The VP1 coding sequence was codon optimized, then synthesized with recognition sequences of restriction endonucleases and sequence of 6XHis tag. The insert was cloned into pTriEx™-4 Neo vector, the plasmid was amplified and sequenced. Expression of VP1 protein induced by IPTG was performed in Origami™ B(DE3) bacterium. Purification of polyhistidine-tagged protein was carried out using Protino® Ni-TED column, then concentration was performed by Amicon® Ultra-4 centrifugal filter. The quality and purity of the protein was checked by Western-blot and Coomassie Brilliant Blue staining. Quantity of protein was determined using BCA Protein Assay. An indirect ELISA method has been developed to detect IgG antibodies against VP1 protein of HPyV10.

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ROLE FOR SPLICEOSOMAL TWIN INTRONS IN TWO MODES OF ALTERNATIVE SPLICING

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In the primary transcript of nuclear genes, coding “exons” usually alternate with non-coding “introns”. The latter are precisely excised by the U2 spliceosome to create the ORF that translates into the correct peptide. Spliceosomal twin introns (“stwintrons”) are complex intervening sequences where an “internal” U2 intron interrupts one of the canonical splicing motifs of an “external” U2 intron (viz. 5’-donor; 3’-acceptor; motif around branch-point A) and consequently, are removed by two consecutive splicing reactions. Originally, alternative splicing was presented as a means to increase protein diversity but more often it yields “dysfunctional” RNAs (not encoding the correct peptide), which are rapidly degradated by nonsense-mediated mRNA decay.

We investigated functional relations between bona fide stwintrons, and extant exon skipping and intron retention events. Public DNA databases, simple bio-informatics, RT-PCR. A donor-disrupted stwintron in a ubiquitous gene occurs broadly in the Pezizomycotina subphylum. The stwintron is crucially involved in "skipping" the exon behind it in certain species, like *Aspergillus niger*, by using alternative 3'-splice sites for its internal intron. A branch-point motif-interrupted stwintron was found in *Aspergillus nidulans*. Orthologue genes in related species specify a standard intron at the same position as the internal intron of the *A. nidulans* stwintron. Excision of the new external intron removes the AUG, implying that it must be retained to deliver a protein.

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A SPLICEOSOMAL TWIN INTRON (STWINTRON) PARTICIPATES IN BOTH EXON SKIPPING AND EVOLUTIONARY EXON LOSS

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In the primary transcript of nuclear genes, coding "exons" usually alternate with non-coding "introns". These latter have to be excised to form the functional mRNA, in the large majority of cases, by the major U2 spliceosome. Spliceosomal twin introns ("stwintrons") are complex intervening sequences (IS) 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 branch point A), i.e., the external intron can only be removed after excision of the internal intron [1]. To study spliceosomal introns, we can use fungal stwintrons as model systems. Thereto, we created an informatics tool to search for stwintrons in *Aspergillus nidulans in silico* [2]. Here, we describe a [D5,6] stwintron in the gene at locus AN5404 (encoding a reticulon-like protein) where the internal intron is situated within the donor element of the external intron, between the 5th and the 6th nt [5'-GUAAG|U]. This stwintron is old: it is present in 9 classes of Pezizomycotina as the first IS after the AUG. In certain taxa, the second IS is also a [D5,6].

In the specific context of this gene, the ubiquitous stwintron could be involved in alternative splicing by "skipping" the exon behind it in certain species, like *Aspergillus niger*, where it never results in frameshift. In most species of Onygenales, however, inclusion of that exon II does not lead to a full-length ORF. We have shown in *Malbranchea cinnamomea*, that the stwintron and the next U2 intron need to be excised first to create a new functional U2 intron in the transcript. It thus takes 4 consecutive splicing reactions to remove the whole IS.

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MICROBIAL DEGRADATION OF NAPHTHALENE BY BACTERIAL STRAINS ISOLATED FROM SOIL AND GROUNDWATER SAMPLES CONTAMINATED BY POLYCYCLIC AROMATIC HYDROCARBONS

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Polycyclic Aromatic Hydrocarbons (PAHs) are widely distributed environmental contaminants originating from natural as well as anthropogenic sources with toxic, mutagenic and carcinogenic properties. The bioremediation of these pollutants depends on several environmental conditions (pH, temperature, oxygen, accessibility of nutrients), chemical structure of the PAHs and composition of the microbial community of the soil or water. In this study nearly 250 bacterial strains were isolated from water and soil samples contaminated by PAHs and BTEX (benzene, ethylbenzene, toluene and xylene) compounds. The sole carbon and energy sources for the microorganisms were naphthalene, gasoline or cholesterol and agarose or gelrite was used for solidifying the culture media. Cultivation was carried out under aerobic conditions. All isolates were identified by 16S rRNA gene sequence comparisons and the closest relatives of the bacterial strains were denominated. Representatives of the most abundant groups were tested for naphthalene degradation in microcosms. Cultures were established in 50 mL modified Bushnell Haas Broth (BHB) with 5 ppm naphthalene concentration in crimp capped serum bottles. To determine the naphthalene concentration a fast and simple solid phase micro-extraction (SPME) method was applied followed by Gas Chromatography Flame Ionization Detection (GC-FID) measurement using a capillary column. The protocol was developed by the researchers of the Department of Analytical Chemistry. All isolates were tested for surfactant producing activity by using a drop collapsing test. Above all, antagonism tests were applied to find inhibitory effects of bacteria toward each other. Most of the isolated strains belong to Gram-negative *Pseudomonas* species. Furthermore, other different species with ability to degrade naphthalene and gasoline as a sole carbon source, such as *Sphingobium* spp., *Achromobacter* spp., *Stenotrophomonas* spp., *Rhodococcus* spp., *Ensifer* spp., *Frigidibacter* spp., *Micrococcus* spp. have been isolated. Considering our results, six relevant naphthalene degrading strains (*Pseudomonas* spp., *Micrococcus* spp. and *Sphingobium* spp.) were found. Each strain had high degradation ability determined at 92 - 96% over a period of 1 week. Concerning the results of biosurfactant production, two bacterial strains showed positive reaction (*Pseudomonas* spp., and *Lelliottia* spp.). In the antagonism tests some species of the genus Pseudomonas and Ensifer showed more strong inhibitory effect toward other tested bacterial strains than *Rhodococcus* and *Micrococcus*. Other tested groups of bacteria did not show any antagonistic interaction. The results helped us to select bacteria which might be used to compose site specific consortia for better degradation of PAHs during on-site bioremediation process.

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EFFECT OF TRACE ELEMENTS IN IRRIGATION WATER ON SOIL MICROBIAL COMMUNITY CHANGE

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Diversity of the soil microbial community is an important indicator of the ecological status of the soil. In soils used for agricultural purposes, different treatments - including the composition of irrigation water - can change the structure of the microbial community. Toxic and essential microelements occur at varying concentrations in irrigation water. The aim of the NVKP_16-2016-0044 project is to investigate the effects of toxic arsenic and boron and essential selenium and iodine trace elements on the soil - water - plant system, including the microbial community of the soil. Various irrigation experiments were carried out in light-room and outdoor rhizobox with different layouts by MTA Institute for Soil Sciences and Agricultural Chemistry. Trace element solutions were added to irrigation water in different concentrations: arsenic 0.1 - 0.5 mg/L; boron in 0.1 - 0.7 mg/L; iodine 0.1 - 0.5 mg/L and selenium in 0.1 - 1.0 mg/L.
French beans and lettuce were used in the rhizobox experiments. In outdoor experiments, three various soil types (sandy soil, loamy soil and sandy-loam soil) and four different vegetables (french bean, tomato, cabbage and potato) were used, rhizoplan and bulk soil samples were collected at the harvest phase. 499 representative samples were chosen for molecular based community investigation from the total 1,687 soil samples. From the soil samples total community DNA was extracted, and the partial sequence of 16S rDNA was multiplied by nested PCR. Following the denaturing gradient gel electrophoresis, every sample received an unique community fingerprint pattern which is specific to the bacterial community of the sample. Based on this patterns it is possible to determine what is the effect of the soil types and the grown plants in the vessels for the microbial community of the soil compared to the initial condition or controls.

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STUDY ON PLANT GROWTH PROMOTION EFFECTS OF LEGUME SYMBIONTS

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Legume crops represent a relevant nutrient source in human and animal diets. The pea (*Pisum sativum* L.), soybean (*Glycine max* L.) and chickpea (*Cicer arietinum* L.) worldwide tend to conquer larger and larger sawing surface every year. For efficient growth rate and crop building these plants need symbiont rhizobacteria. The relevance of Rhizobiales in nitrogen fixation is well known, however it may be less obvious, that they perform other plant growth promoting (PGP) effects as well. This study describes several PGP features and abiotic stress tolerance levels of selected legume symbiont strains: *Bradyrhizobium japonicum*, *B. shewense*, *Ensifer sesbaniae*, *Mesorhizobium ciceri*, *Rhizobium leguminosarum* and three strains of *B. diazoefficiens*.
The experimental results indicate P mobilization effect for *M. ciceri*, K mobilization for *R. leguminosarum*, siderophore production for *B. japonicum*, *B. diazoefficiens* G10, *E. sesbaniae*, *M. ciceri*, and for *R. leguminosarum*. Plant hormone production tests has proven IAA production for *E. sesbaniae* and *R. leguminosarum*, while gibberellic acid production could not be detected for any of the selected strains. According to the cultivation results the studied strains have an optimal pH

between 4.5 - 6.5. Considerable salt stress tolerance has been exerted by *R. leguminosarum*, and all species has proven to be moderately tolerant to osmotic stress conditions.

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STUDY ON A GENE DELETION MUTANT ENCODING A TRANSCRIPTON FACTOR REGULATING A SECONDARY METABOLITE GENE CLUSTER

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Whole genome sequencing and annotation projects of filamentous fungi in the last decade revealed numerous secondary metabolite gene clusters being silent under normal laboratory conditions. These gene clusters can be activated under environmental including oxidative stress and armour fungi with effective chemicals in competitive habitats. These chemicals can be exploited as bioactive agents like antibiotics, antimycotics, immunsuppresants for medical purposes. For example in the filamentous fungus model organism *Aspergillus nidulans* a non-ribosomal peptide synthase gene cluster including the synthase (ANID_07884) and its transcription factor (ANID_07872) activated under menadione sodium bisulfite treatment. In this study we generated the gene deletion mutant of ANID_07872 [1] and characterized the mutant phenotypically. The ΔANID_07872 gene deletion strain possessed higher sensitivity to oxidative stress inducing agents like diamide, tert-butyl hydroperoxide (tBOOH) compared to the control strain. Surprisingly the menadion sodium bisulfite sensitivity of the mutant and control strain was comparable. Secondary metabolite production of strains was also monitored in *Aspergillus* minimal medium supplemented with 2% maltose and 1% mycological peptone at 25°C. A red colour UV active band from the mycelia of the mutant was detected using thin layer chromatography which was absent in the control strain [2]. Determination of this chemical is in progress and hopefully new metabolite will be found with bioactive properties.

Acknowledgements: Financed by EFOP-3.6.1–16–2016-00022, K112181, and K119494 projects.

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DETERMINATE THE SOIL BIOLOGICAL ACTIVITY OF DRÁVA FLOODPLAIN BY FLUORESCEIN DIACETATE (FDA)

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Soil enzymes play a key role in the energy transfer through decomposition of soil organic matter and nutrient cycling. These enzymes catalyse many vital reactions necessary for the life processes of soil microorganisms and helps in stabilization of soil structure. Microorganisms are the primary source of soil enzymes. Soil enzymes respond also to any changes in soil management practices and environmental conditions. They are used as indicators for soil microbial status, soil physical-chemical

conditions, and for the influence of soil treatments or climatic factors on soil fertility. Understanding the possible roles of different soil enzymes in maintaining soil health can help in the soil health and fertility management, particularly in agricultural ecosystems. The fluorescein diacetate (FDA) hydrolysis assay is suitable for measuring the total catabolic enzyme activity of microbial populations in an environmental sample. Both free hydrolysed colourless fluorescein diacetate and membrane bound enzymes, release a coloured end product fluorescein which can be measured by spectrophotometry. Our aim was to determine natural background values of soil microbiota and dispersion of potentially toxic elements in the soil of the Drava floodplain by FDA. Samples were collected from the alluvial plains and river terraces of the whole part along the Hungarian river section. The FDA activity of the soil samples was determined by the modified SCHNÜRER and ROSSWALL method [1]. Altogether, 66 samples were collected from floodplain sediments and soils. The FDA value ranged between 0.649 and 14.89 [TPF] with the median of 2.49 [TPF]. Significantly higher FDA activity was found in the top soils compared to the bottom soil samples, however, at sample locations where soils are redisturbed by intensive agricultural activity, the top soils have low FDA values similar to the overall low values in the bottom soils.
Similar separation of the measured FDA values is not apparent between the alluvial plain and river terrace areas. In two bottom soil samples, significantly higher FDA activity was determined in the bottom soils than in the top soil samples, which is readily explained by their location: both of the samples are located in the actively flooded alluvial plain and in ox-bows, characterized by dense forest vegetation. It is concluded that determination of FDA activity in hydromorphic soils and sediments provides a potential soil quality indicator that shows strong correlation with microbial biomass, the organic matter content with soil depth and land cover.

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GROWTH MODULATING EFFECT OF *HEDERA HELIX* EXTRACT ON BACTERIA

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Chlamydia pneumoniae is an obligate intracellular Gram-negative bacterium which belongs to the Chlamydiaceae family. *C. pneumoniae* while responsible approximately for 10% of the community-acquired pneumonia cases, is also a common cause of sinusitis, bronchitis, exacerbation of chronic bronchitis and pharyngitis. *Hedera helix* L. is an evergreen climbing plant belonging to the Araliaceae family. *Hedera helix* is part of the European Pharmacopoeia as a traditional medicinal plant. The active substances are triterpenoid saponins and can be found in leaves. There are two forms: juvenile leave and adult leave. The extracts have anti-inflammatory, anti-microbial, antitumor, and mucolytic activities. In Hungary, *Hedera helix* is available as over the counter (OTC) medication. In this study our aim was to investigate the possible anti-microbial activity of the drug on human respiratory diseases associated pathogens. To examine the triterpenoid saponins compound quantity in the extracts HPLC-DAD analysis was used. 10 µl sample was injected into the column. Phosphoric acid/acetonitrile solution was used as a mobile phase at 25°C. The anti-microbial effect of the two-fold dilution extracts on *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas*

aeruginosa and the cytotoxic effect of the extracts on A549 cell line was tested with MTT test. The cell or bacterial growth was determined by measuring the optical density. In 96-wells plate A549 cells were infected with *C. pneumoniae* and treated with two-fold dilution *Hedera helix* extracts and after 48h post-infection the medium was changed to sucrose phosphate glutamate (SPG). After two freeze-thaw cycle the replication of the bacteria was examined with qPCR using *C. pneumoniae* specific OmcB primer. Alfa-hederin was identified as a main triterpenoid saponin. We found that the juvenile leaves contain 2.396 ± 0.0015 mg/g and the adult leaves 1.044 ± 0.0005 mg/g of alfa-hederin. Moreover, the extracts do not influence the replication of *S. aureus*, *K. pneumoniae* and *P. aeruginosa* at 125 mg/mL concentration. The juvenile form at 1.95 mg/mL and the adult form at 3.90 mg/mL doesn't have any effect on A549 replication, thus we chose this concentration for further investigation. Unexpectedly, the extracts increased the *C. pneumoniae* replication.
The control group Ct_{mean} was 26.470 SD = 0.427, juvenile form Ct_{mean} = 24.813 SD = 0.801 p = 0.004, adult form Ct_{mean} = 25.668 SD = 0.568 p = 0.03. The data implies that the juvenile form increases the *C. pneumoniae* replication by 315% while the adult form by 174%. Considering the effect of *Hedera* extracts on *C. pneumoniae* replication in A549 cell, it would be worth to examine further on other cells line and other pathogens.

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ITACONIC ACID PRODUCTION BY *ASPERGILLUS TERREUS* FROM D-XYLOSE AND XYLITOL

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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. IA is mainly produced from molasses or starch, but research over the efficient utilization of non-food, lignocellulosic plant biomass is soaring. D-xylose is the most abundant pentose in the lignocellulose complex. Importantly, D-glucose, D-xylose and L-arabinose interfere with each other's uptake and metabolism, hence the metabolism of these sugars in the absence of the others must be understood first. The first enzymatic steps of D-xylose catabolism could lead to cofactor imbalance. In principle this could be avoided by employing xylitol, the polyol of xylose and a common by-product from ethanol manufacturing as sole carbon source for IA fermentation. The aim of this study was to evaluate this option in terms of fermentation performance To this end, fermentations with D-xylose as well as xylitol liquid were performed at three different initial concentrations (10 g/L, 50 g/L, 110 g/L), by employing *A. terreus* NRRL 1960, a high IA producer strain. Low xylitol concentrations (10 and 50 g/L) resulted poor molar yield (0.04 ± 0.01 and 0.43 ± 0.01 respectively) compared to D-xylose (0.24 ± 0.01 and 0.61 ± 0.02), whilst no significant ($p < 0.05$) difference was found in the molar yields at the highest initial concentration (0.48 ± 0.02 vs. 0.55 ± 0.04). On xylitol a lag phase lasting for 2 days was observed, although the consumption rate has accelerated afterwards (0.58 g/L h average consumption rate versus 0.66 g/L h at 110 g/L initial carbon source concentration).
Xylitol as sole carbon source results in lower IA molar yieldsthan D-xylose at lower initial concentrations, whereas yields are similar at higher (e.g., 110 g/L) initial concentrations We are currently testing even higher initial xylitol and D-xylose concentrations.

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DEVELOPMENT OF HEAT KILLED *LACTOBACILLUS* CONTAINING IMMUNOBIOTICS TO ATTENUATE CHEMOTHERAPY INDUCED SYSTEMIC INFLAMMATORY RESPONSE SYNDROME

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Chemotherapy is the most effective tool to overcome different cancerous diseases. However administration of cytostatic entailing several side effects which not only deteriorate life quality but facing serious new challenges both the patients and physician. These are gastrointestinal symptoms such as vomiting, diarrhoea, losing weight (cachexia), heart failure, renal failure and liver damage. Depending the severity of these symptoms the therapy has to be interrupted or dosing of medications must be decreased which are a considerably restrictive factor in the success of cancer therapy. The background of these side effects is multi causal, the systemic inflammation emerges during chemotherapy can be regarded as the most contributory factor. This is supported by *in vivo* models in which increasing serum concentration of inflammatory cytokines IL-6, IL-1β, TNF-α have been detected even after a single dose of 5-fluorouracil. Increased gut mucus permeability after cytostatic treatment facilitates increased translocation of bacterial endotoxins (LPS) into the blood. Endotoxemia causes the activation of Toll receptor family (TLR) especially TLR4 which promotes gene transcription of pro-inflammatory cytokines. Eradicating gut microbiota by antibiotic treatments or blocking TLR4 signal transduction pathway in animals significantly attenuated inflammatory responses and drastically increased survival. These evidences strongly suggest that effective anti-inflammatory therapy may help to increase the tolerance of chemotherapy, would promote the efficacy of the treatment and improve life quality of patients. Application of probiotic bacteria proved to be a promising choice in the treatment of inflammatory diseases of gastrointestinal tract such as inflammatory bowel disease, Crohn disease, and in dysbiosis after antibiotic treatment. These immunomodulatory properties of certain lactobacillus strains have a key role in protection against endotoxin shock or endotoxin related organ damage have been reported in recent years. Our main goal is to develop a dietary supplement product containing heat killed lactobacillus strains to attenuate inflammation related adverse effect of cytostatic treatment. Several heat killed lactobacillus strains will be tested in order to determine their anti-inflammatory properties in animals being treated by cytostatic and/or LPS. The following parameters will be monitored: serum LPS, and pro-inflammatory cytokines (IL-6, TNF-α) from cytostatic, LPS and lactobacillus treated animals. Anti-inflammatory cytokine IL-10 will be determined also. Organ damage and function (liver and kidney) will be determined also, via determination of serum aspartate-amino transferase (AST), urea, creatinine. These laboratory parameters will be supplemented by macroscopic examination of liver and kidney.

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EFFECT OF LACTIC ACID BACTERIUM AND YEAST STRAINS ON AFLATOXIN B1 PRODUCTION OF *ASPERGILLUS FLAVUS*

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Aflatoxins are the most dangerous naturally occuring genotoxins among mycotoxins. Aflatoxin (AFB1) is produced by *Aspergillus flavus* and *A. parasiticus* strains, and in Hungary mainly corn and corn silo are affected/poisoned/contaminated. Based on investigations focusing on interactions between toxin production of aspergilli and environmental factors it is evidenced, that elevating carbon dioxide concentration, dryness and temperature over 30°C are provoking the expression of genes responsible for AFB1 toxin synthesis. Compared to this there are much less information on the biological background of AFB1 toxin production. In this research we added new aspects to the toxin production of *A. flavus* and besides abiotic factors, biotic factors were investigated. Our object, *A. flavus* zt40 was isolated from a Hungarian maize field and it had significantly high toxin production ability. We focused on the question, how the final toxin production and the expression of toxin synthesis genes were influenced, when the strain was co-cultured with lactobacilli and yeast strains isolated from silo. The *A. flavus* zt40 strain was grown on rice substrate at 30°C for 21 days. The cultures were infected on the 12th day with the mold inhibiting *Lactobacillus salivarius* SK29 strain, and 12 yeasts silo isolates belonging to *Candida*, *Debaryomyces*, *Saccharomyces*, *Rhodotorula*, and *Trichosporon* genera. Changes in expression level of *cypA* (*aflU*), *aflQ* (*ordA*), *omtA* (*aflP*), *aflM* (*ver1*) genes were monitored by qRT-PCR on the 12th, 16th and 21st days. Additionally, the AFB1 toxin level was determined on the 21st day by HPLC. The *L. salivarius* treatment dramatically (with 75%) suppressed the AFB1 production, which correlated well with the significantly low expression level of *aflU* (*cypA*) and *aflQ* (*ordA*) genes. Interestingly, three yeast strains (NM030, NM057 and NM058) significantly increased the AFB1 production. Out of these NM058 had the strongest activity increasing the AFB1 level with 37% (3.50 vs. 4.81 µg/g).

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LEAD RESISTANCE OF SOIL BORNE BACTERIA AND FUNGI

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Human activities and not appropriate industrial waste management can result in toxic metal accumulation in the environment. Lead is a well-known pollutant of soil, water and air and tends to accumulate especially in soil due to its low solubility. Lead belongs to the toxic metals however several microbes can resist high levels of lead. Lead resistant microbes are potential candidates for bioremediation purposes. Aim of the study was to isolate bacteria and fungi from soil samples and test their lead resistance. Soil samples were collected from different locations in Budapest. Bacterial and fungal strains were isolated using R2A and RBC media, respectively. Cell morphology as well as growth temperature requirement of isolates were determined. In case of bacteria KOH test for Gram

reaction, catalase and oxidase tests were performed. Pre-screening for lead tolerance was made on media containing 50 and 100 mg/L lead(II) nitrate. Resistant bacteria and fungi were further investigated up to 1,000 mg/L lead(II) nitrate concentration. Altogether 46 bacterial and 31 fungal isolates were collected from six soil samples. Most of the bacteria had rod shaped cell morphology. Strains were evenly distributed according to Gram reaction however catalase and oxidase positive bacteria were predominating. In case of fungi half of the strains had branched, brush-like conidiophores with globose or ellipsoid conidia. Maximum growths were obtained at 25 - 30°C and 20 - 25 - 30°C for bacterial and fungal isolates, respectively. In case of bacteria few strains showed good growth at 37°C too. Bacterial isolates proved to be resistant to lead, all of the strains could grow during pre-screening on media containing 100 mg/L lead(II) nitrate. At higher concentration half of the strains failed to grow however 20 of the isolates could resist even 1,000 mg/L lead(II) nitrate. In case of resistant bacteria the growth was usually decreased except for five bacteria. Amongst the fungal isolates seven sensitive strains could be identified (MIC values: 50 or 100 mg/L lead (II) nitrate) however the other strains showed very good tolerance. Calculated tolerance index was above 83% for 1,000 mg/L lead(II) nitrate. Analysis of soil borne bacteria and fungi revealed potential isolates which could be used in bioremediation of lead contaminated resources.

SCALE-UP OF CHLORINATED SHORT-CHAIN HYDROCARBON AND POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) DEGRADING MICROBIAL CONSORTIUM

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Due to the careless industrial applications, large amount of chlorinated hydrocarbons and PAHs penetrated to soils and groundwater causing serious environmental damages. These hazardous components endanger both wildlife and human population, because of their acute and chronic toxicity. The largest problems are the contamination of drinking water sources (short chain carbohydrates) and soils (PAH, BTEX), thus the remediation of the contaminated areas are crucial. Numerous bioremediation techniques exist to remediate contaminated soils. In situ biological remediation is an efficient and economical method to cleanse contaminated soils. Site-specific microbial consortia were selected for both the biodegradation of chlorinated hydrocarbons and PAH molecules. Two basically different methods were developed for the anaerobic and aerobic biodegradation of short-chain chlorinated hydrocarbons and PAH molecules, respectively, by the researchers of ELTE. A novel site-specific in situ bioaugmentation method was developed, using site-specific autochthon anaerobic microbial community with enzyme systems capable to dechlorinate short-chain chlorinated hydrocarbons. The microorganisms were isolated from contaminated area's groundwater. Dechlorinating microorganisms such as *Dehalococcoides* sp. are responsible for the dechlorination of trichloroethylene (TCE) to ethene. To enrich dechlorinating bacteria in cultures, optimal chlorinated hydrocarbon concentration and suitable electron donor are required to be present throughout the scale-up process. For this purpose, TCE as electron acceptor and H_2 as electron donor were added to the medium. Scale up was carried out in 500 mL, 10 L and 100 L volumes. When TCE was dechlorinated by the organohalide respiring consortia, another portion TCE was added to enhance the growth of the microbial community. The concentration of TCE, intermediates (cis-dichloroethylene, vinyl chloride) and end product (ethene) were monitored in the headspace using gas

chromatography measurement. The presence of the organohalide respiring genus *Dehalococcoides* was detected based on taxon-specific 16S rRNA gene. With this method organohalide-respiring microbial communities were successfully enriched at laboratory-scale and in industrial fermenters. The microbiological degradation of PAH molecules is mainly effected by aerobic bacteria.
Soil-specific isolates from PAH contaminated sites have been isolated by researchers of ELTE that are responsible for PAH degradation. It was expedient to avoid using PAH molecules during the scale up process but maintenance of the PAH degradation capacity was crucial. Therefore, we tried to add substitute or inductor molecules (cholesterol, salicylic acid, phthalic acid, etc.) while continuously monitoring the PAH degrading activity of the produced culture.

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VALIDATION OF THE PASPALITREM GENE CLUSTER OF *CLAVICEPS PASPALI* BY *AGROBACTERIUM TUMEFACIENS* MEDIATED GENE REPLACEMENT APPROACH

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Claviceps paspali is an industrially and ecologically important hypocrealean fungus. In the pharmaceutical industry it has been used for the production of ergot alkaloids for decades. However, in its natural habitat in association with *Paspalum* spp., this fungus causes significant losses in livestock due to its production of tremorgenic indole-diterpene (IDT) mycotoxins. Despite its pharmaceutical and ecological importance, no practical tools for the genetic manipulation of this fungus has been published prior to our work. We adapted *Agrobacterium tumefaciens* mediated transformation (ATMT) to *C. paspali* to establish a facile and robust genetic transformation method suitable for the creation of stable homokaryotic transformants. To test the utility of this system, we replaced the *idtCBGF* genes in the putative paspalitrem biosynthetic gene cluster of *C. paspali* with the hygromycin phosphotransferase gene. The resulting knockout strains were unable to produce IDT mycotoxins, validating the putative function of the paspalitrem gene cluster in *C. paspali*. The method is also suitable for the generation of industrial ergot alkaloid producer *C. paspali* strains that are devoid of the production of harmful IDT mycotoxins.

STUDIES ON TODAY'S PROBLEMATIC MICROBES - THE ROLE OF A MICROBIOLOGICAL LABORATORY

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Nowadays infections are caused even more frequently by multiresistant bacteria or microbes with special virulence properties. The microbiological laboratory has a key role in the swift and thorough

examination of pathogenic microbes. In our studies, different Gram-positive and Gram-negative bacteria isolated from invasive clinical samples were examined. Significant differences between MSSA and MRSA strains were characterized by investigations on antimicrobial susceptibility and virulence factors. The ability of *Staphylococcus haemolyticus* for acquiring resistance and its potential pathogenicity in immune-compromised patients confirm the significance of species-level identification of coagulase-negative staphylococci and the importance of investigating epidemiology. The first VIM-4 metallo-β-lactamase producing *K. pneumoniae* and *K. oxytoca* strains in Hungary was characterized. The molecular epidemiology of infections caused by ESBL-producing *K. pneumoniae* and *K. oxytoca* in a perinatal intensive care unit during a five-year period was investigated. Emergence of VIM-4- and SHV-12-producing *Enterobacter cloacae* in a neonatal intensive care unit was underlined. Major changes in population structure of multidrug-resistant *Acinetobacter baumannii* between 2010 - 2017 were observed at institutional and at national level. Resistance to carbapenem in multidrug-resistant *Pseudomonas aeruginosa* strains was studied with phenotypic and genotypic methods. Possible antibacterial agent combination effect against *Stenotrophomonas maltophilia* were analysed in in vitro studies. Colistin resistance among blood culture isolates were checked and the first mcr-1-positive *Escherichia coli* strain found in Hungary. Microbiological laboratories have to play important role in the prevention and elimination of nosocomial infections. It is crucial to be aware of the pathogen properly in case of every nosocomial infection. Moreover, microbiologists may be the first detecting outbreak by observing typical microbes and their characteristic resistance patterns on the wards and is able to help in the establishment of empirical therapeutical strategy on a certain ward. After the emergence of a special resistance mechanism the putative reasons of its appearance should be revealed as the first step by proper examination of microbe(s) as well as the determination of genetic background. That may provide information whether further dissemination should be expected. Results can be built in the routine diagnostic work and infection control.

EXAMINATION OF BIOFILM FORMATION ABILITY OF PLANT GROWTH PROMOTING RHIZOBACTERIA FOR USE IN AGRICULTURE

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In order to increase the efficiency of soil bioinoculants in the sustainable agricultural environment, mapping of the multi-level soil microbial community is highly relevant. The biofilm-forming and qourum-sensing molecule (OSM) producing mechanisms allow long-term survival of soil-inoculated bacteria and ensure protection from the repression effect of the endemic microflora. Biofilm decreases susceptibility for the extreme conditions of the rhizosphere, such as UV radiation, antimicrobials, dehydration and osmotic shock, phagocytic predators, extreme pH values, temperature, salinity and nutrient deficiency. The introduction of biofilm-forming bacteria to agricultural practice promotes the beneficent activity of plant growth promoting rhizobacteria (PGPR) and mycorrhiza inoculants. Biofilm-producing bacteria colonize plant roots and mycorrhiza hyphae to help plants to reduce abiotic and biotic stress, nutrients uptake and growth. The role of soil

microorganisms in the formation of biofilms may be essential for the proper functioning rhizosphere. Bacterial communities may solve challenges that a single cell or a sole species would not be able to overcome. The indispensable condition of the community organization is communication. The quorum sensing (QS) mechanism allows bacterial populations to synchronize their metabolism. Biofilm-producing microbial cells - algae, fungi, bacteria - exist in self-constructed extracellular polymeric substances (EPS) matrix. The EPS biosynthesis is also a QS dependent process.
The aim of our work was the selection of biofilm-forming PGP rhizobacteria which can promote the plant's abiotic and biotic stress tolerance thus increasing the yield of crops. Quorum sensing molecule (QSM) production was tested by mutant biosensor strains - *Chromobacterium violaceum* CV026, *Agrobacterium tumefaciens* NT1 - on agar plate and detected with color reaction. The exopolysaccharide production was deduced from the colony morphology test. Biofilm formation was tested by stained with crystal violet in 6 different media - N, TSB, BHI, King B, MRS, Szach II - in microtiter plates. Nearly 140 PGP strains were examined from our culture collection among them there are biofilm producers from the genera *Azospirillum*, *Bacillus*, *Gordonia*, *Enterobacter*, *Pseudomonas*, *Paenibacillus*, *Klebsiella*, *Sphingobacter*, *Variovorax*, *Luteibacter* and *Serratia*. 94 strains showed some QSM-producing activity from examined strains. The exopolysaccharide production was clearly visible in agar plate in case of 28 strains belonging to 9 genera. There are probably 16 strains which capable C4 - C14 long carbon chain QSM-mediated biofilm formation and exopolysaccharide production belonging to the genera *Pseudomonas*, *Serratia*, *Enterobacter*, *Luteibacter*, *Azospirillum*, *Paenibacillus* and *Vanrija* (soil yeast).

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ANALYSIS OF MICROBIOME OF *VITIS VINIFERA* CV FURMINT FROM DIFFERENT VINEYARDS IN HUNGARIAN WINE REGIONS - DETECTION OF ENDOPHYTIC FUNGI

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Terrestrial plants generally have a large endophytic community. Endophytes are organisms that spend at least one period of their lives inside the plant but do not cause any pathological symptoms. In previous studies, it has been shown that grapevine has a great fungal endophyte community that may affect growth, yield, resistance. Our aim is to describe the endophytic structure in *Vitis vinifera* cv Furmint focusing on filamentous fungi. We took samples from different vineyards around Eger and Tokaj wine regions where Furmint is one of the main cultivated varieties. Sampling took place at different times of the year collecting different plant parts at distinct ontogenetic stages. After collection, we divided the samples for three different experiments: microscopy, endophyte isolation and for DNA based community analysis by next generation sequencing. To prepare our samples for microscopic analysis we cleared the plant parts then stained them with a fluorescent dye Wheat Germ Agglutinin (WGA)-AlexaFluor488. For isolation we placed our surface sterilized samples on PDA media then after hyphae growth we separated different looking isolates to smaller dishes individually. From these individual isolated fungi, we extracted DNA and amplified the internal transcribed spacer (ITS) region then sent them to sequencing. We were able to detect fungi in the plant samples at all sampling times and regions with this microscopic preparation method. Results of the molecular

identification has shown a great variety in the fungal community within the plant tissues. Thus far we saw fungi in the plant samples and could detect the microbes that are most common in grapevines such as *Botrytis cinerea* and *Alternaria alternata*.
Our ultimate goal is to broaden our knowledge in the community structure of grapevine and to further our understanding of the effect this microbial community has on the plants.

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REVEALING THE DIFFERENT ADAPTATION MECHANISMS AND GENETIC VARIATIONS OF THE CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS USING NEXT GENERATION SEQUENCING

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Crimean-Congo hemorrhagic fever orthonairovirus (CCHFV) can cause an acute, often severe viral hemorrhagic fever in humans with 30% fatality rate among hospitalized patients. The CCHFV is a member of the *Nairovirus* genus, *Bunyaviridae* family. The negative, single stranded RNA (-ssRNA) genome with 19.2 kb in length consists of three circular segments, the small (S), the medium (M) and the large (L). The genome encodes the nucleoprotein (S segment), the glycoprotein precursor complex (M segment) and the RNA-dependent RNA-polymerase (L segment). RNA viruses have higher mutation rates due to the lack of proof-reading activity of the L polymerase enzyme that can result in enhanced genetic diversity and the emergence of minor viral quasispecies. In different segments of the CCHFV, the mutation rate was shown to alter based on the nature of the host cell, where the replication occurs (0 - 10^{-3} mutation/segment/year). In laboratory settings for propagation, the virus is often serial passaged on different susceptible cell cultures or inoculated into suckling mice that may lead to genetic variations throughout the whole genome. However, no information is available about the main sites that are more likely to vary and compose a new subcluster of quasispecies within the virus population. The main aim of our study is to introduce RNA virus whole genome sequencing as a new tool for virus identification and characterization into the applied molecular methods of the National Biosafety Laboratory and to determine genetic variations resulted by cell and tissue adaptation mechanisms. Analyses of the CCHFV genome was performed from the supernatant of different cell cultures and the brain tissue of suckling mice. As part of the study, a sequence-independent amplification of the viral genome was also established and optimized. Whole-genome sequencing was performed using the MiSeq2000 Illumina platform. Based on the bioinformatic analysis, the full genome sequences were determined (at a consensus level) and single nucleotide variations (SNVs) with allele frequency >0.05% were identified in each segments. Mutation frequency was also calculated. To estimate the selection pressure in different cell cultures, the ratio of non-synonymous and synonymous mutations were calculated for the functional domains. Our findings may help better understand the adaptation and underlying mechanisms of the CCHF virus to different cell cultures and help find better models for virus propagation and isolation.

DEVELOPMENT OF MICROBIOLOGICAL SOIL INOCULANT TO IMPROVE SOIL WATER MANAGEMENT AND SOIL STRUCTURE ON HUMUS SANDY AND CALCAREOUS CHERNOZEM SOILS

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An emerging problem of the intensive agricultural cultivation is soil degradation. As a consequence, soil water management and water retaining capacity declines, soil aggregate decomposition accelerates, triggering wind and water erosion. The microbial activity decimates with excess chemical input and consequently the nutrient mobilization capacity and abiotic stress resilience decreases. Soil reconstitution is a rather difficult and complex issue, where the otherwise „alternative" biological approach is the only way to go, since conventional methods (organic matter supply, mineral colloids or liming) are considerably expensive and have variable results. The trendline of the Europian Union Soil Thematic Strategy is the development of reliable technologies in order to control soil degradation. The aim of presented research was to develop a microbiological soil inoculant that reliably improves the stability of soil aggregates, therefore improves the structure and water management of soils. Moreover, the inoculant enables an enhanced nutrient uptake for the plants through various plant growth promotion abilities that helps diminishing the chemical footprint of agricultural production by lessening the demand for fertilizers.

Experiments were carried out on two deteriorated soil types: a humus sandy soil and a degraded, dusty calcaroeus chernozem at various scales with dicot and monocot crops. Several rhizobacteria were tested in numerous strain combinations (e.g. *Kocuria rosea*, *Pseudomonas chlororaphis*, *Pseudomonas jessenii*, *Paenibacillus peoriae*, *Azospirillum irakense*, *Paenibacillus jamiale*, *Bacillus simplex*, *Luteibacter rhizovicinus*) in order to maximize the soil remediation and the crop yield improvement capacity. The study has been going on for several years to be able to exclude weather effects and achieve a general, reliable outcome of improvements.

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MICROBIAL COMMUNITY COMPOSITIONS IN THE RHIZOSPHERE OF MAIZE IN A LONG-TERM FIELD EXPERIMENT OF DIFFERENT AGRICULTURAL PRACTICES

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The value of long-term field experiments is increasing as the agriculture is facing challenges of serving the growing market, being profitable, but also being sustainable at the same time. These experiments provide valuable information regarding the widespread effects of different agricultural practices on the soil microbial communities that are recognized as a major contributor to the multifunctionality of soil ecosystems. Microorganisms participate in the transformations of organic carbon compounds, water management, soil structure formation processes and soil fertility, and show complex interactions with plants. The long-term agricultural field experiments established in 1950s in Martonvásár (HU) aimed to analyze the impact of different cropping systems and fertilization

regimes on the crop yield, however, microbiological examinations have not been conducted so far. In this study the rhizosphere microbiome composition was monitored in two crop sequence systems (maize monoculture and maize-winter wheat diculture), using three treatments (unfertilized, NPK, and NPK supplemented with farmyard manure) and four replications in a split-plot design during the growing season. Altogether 30 rhizosphere and 5 soil samples were collected and subjected to next-generation bacterial and archaeal 16S rRNA gene amplicon sequencing. The results of the sequencing were evaluated according to the 5 crop phenological development stages.
Based on the sequencing data, the community characteristics of microbiome developed in response to cropping systems and fertilization practices have been identified.

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SIZE MATTERS: CHARACTERIZATION OF VIRAL AND HOST TRANSCRIPT ISOFORMS DURING ACMNPV INFECTION USING LONG-READ SEQUENCING

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The *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is an insect virus belonging to the *Baculoviridae* family. Its 134 kbp long double-stranded circular DNA genome encompasses 150 tightly packed open reading frames. Gene expression of the AcMNPV happens in three phases: early, late and very late. Short-read sequencing techniques were previously used to elucidate the structure, function and expression characteristics of the AcMNPV and host transcriptome during viral infection. These techniques can not tackle the overlapping nature of the viral transcriptome, providing limited information about the transcript isoforms. We used long-read sequencing of the AcMNPV and Sf9 RNAs to discover novel length and splice isoforms, and to characterize viral and host gene expression during infection. Additionally we analyze the epitranscriptome using direct RNA sequencing. The Sf9 cell line was inoculated with AcMNPV and incubated for nine post infection time points. Total RNA was isolated from the samples, followed by poly(A) selection for MinION and PacBio libraries or ribodepletion for the cap-selected MinION library. The libraries were sequenced using the Oxford Nanopore Technologies' MinION and the Pacific Biosciences' Sequel system. Previously sequenced direct RNA data was retrieved for the epitranscritome analysis. We used the LoRTIA software suit and ti.py to annotate and characterize transcript isoforms, and Tombo for modification detection. In this work we increased the number of know transcript isoforms for both the AcMNPV and the Sf9 cell line. We characterized the expression dynamics of the viral isoforms, and demonstrated the acting of the viral RNA polymerase (RNAP) on the host genome. Furthermore, we detected nucleotide modifications and A - to - I RNA editing on the viral RNAs. The AcMNPV is widely used in insect protein expression systems and as a biopesticide.
Our work shed light on the intricate transcriptional landscape of the virus, consisting of many length and splice isoforms, and extensive overlapping. We characterized viral gene expression in nine time points following infection using long-read sequencing, and discovered A - to - I editing on one of the viral transcripts. By analysing direct RNA sequencing data we discovered RNA modifications. The analysis of the Sf9 cell line's transcriptome revealed novel RNAs and RNA isoforms, as well as host RNAs possibly transcribed by the viral RNAP.

CYANIDE-RESISTANT ALTERNATIVE OXIDASE CONTRIBUTES TO ITACONIC ACID OVERFLOW ON D-XYLOSE IN *ASPERGILLUS TERREUS*

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Itaconic acid (IA) is a five-carbon dicarboxylic acid with an unsaturated alkene bond, produced by the filamentous Ascomycete *Aspergillus terreus*. Itaconic acid is a frequently used building block for resins, acrylic polymers, super-absorbents, and anti-scaling agents. Its fermentation technology requires high concentration of rapidly metabolizable carbon source and suboptimal concentration of other nutrients in the growth medium, as well as high dissolved oxygen tension (DOT). These conditions will lead to the so-called "overflow metabolism", a term that describes the seemingly wasteful strategy of some fungi including *A. terreus* to incompletely oxidize their carbon source. A key component in this mechanism is the operation of the non-phosphorylating, cyanide-resistant alternative oxidase (AOX), which is located on the matrix side of the inner mitochondrial membrane and which uncouples co-factor regeneration from ATP synthesis.

Reduced ATP formation rate would in turn relieve carbon assimilatory pathways from ATP inhibition, thereby allowing overflow metabolism to proceed. D-xylose is a major component of plant hemicellulose and a potential growth substrate for industrial fermentations. In this study we tested the hypothesis that the operation of AOX on D-xylose as a sole carbon source is as important to achieve high IA yields as on D-glucose. We could demonstrate that increased DOT and/or suboptimal levels of phosphate ions in the growth medium positively correlated with the levels of the alternative oxidase transcript and with an increase in alternative respiratory activity. Ultimately, both adjustments led to higher IA yields. It was concluded that the mechanism through which AOX facilitates IA overflow on D-xylose appears similar to that on D-glucose.

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COMBINATORIAL SYNTHETIC MICROBIOLOGY OF UNNATURAL NATURAL PRODUCT FUNGAL POLYKETIDES

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Synthetic microbiology is an applied scientific discipline that aims to construct microbial cellular factories for the efficient, economical and scalable production of biologically active molecules, including small molecule secondary metabolite natural products (NPs). Combinatorial synthetic microbiology (CSM) ventures to create novel metabolic pathways that incorporate non-cognate combinations of biosynthetic enzymes and/or engineered enzymes with altered chemo-, regio-, or stereoselectivity, and produce unnatural NPs (uNPs). CSM may be diversity-oriented when it targets the biosynthesis of a variety of uNP scaffolds, or may yield focused libraries of a common scaffold

"decorated" with the help of various non-cognate tailoring enzymes. To develop CSM, my group exploits fungal polyketide biosynthetic pathways. Polyketides are one of the largest families of small molecule natural products with an astonishing structural variety that have provided numerous lead compounds and chemical probes for drug discovery. The biosynthesis of polyketide scaffolds in fungi employs multi-domain iterative polyketide synthase enzymes (iPKS) that follow a complex and currently not well understood biosynthetic program to create structural diversity. Mature polyketides are then produced by the action of various "tailoring" enzymes that modify the polyketide scaffold. In this presentation, I will review our recent successes in developing CSM for the biosynthetic production of uNPs based on fungal polyketides. We have recapitulated the production of model benzenediol lactone and azaphilone polyketides in the "domesticated" host *Saccharomyces cerevisiae*, and used hybrid iPKSs for the diversity-oriented biosynthesis of novel uNP scaffolds. We co-opted enzymes from fungal xenobiotic catabolism to "sugarcoat" (glycosylate) these uNPs as well as other drug-like small molecules. We engineered O-methyltransferase enzymes by active site remodeling to generate novel tailored uNPs, and investigated the interplay of the engineered regioselectivity of these recombinant enzymes with their substrate promiscuity.
Diversity-oriented or focused CSM to produce uNPs will broaden the medicinally relevant chemical space, and provide valuable entry points for drug discovery and development.

EXTRAORDINARY INCREASE IN THE NUMBER OF WEST NILE VIRUS CASES AND FIRST CONFIRMED HUMAN USUTU VIRUS INFECTION IN HUNGARY, 2018

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In Hungary, the first neuroinvasive human West Nile virus (WNV) infection was reported in 2004. Usutu virus (USUV) was first detected in a blackbird in 2005 and since then the continuous circulation of the virus was recorded in Hungary; however there has been no laboratory evidence for human infections until now. The objective was to improve the diagnostic capacity by comparing the efficiency of viral RNA detection in different sample types and to investigate samples for the presence of USUV, from patients with clinical suspicion of acute WNV infection. Indirect-immunofluorescence and ELISA tests for detection of WNV, USUV and Tick-borne encephalitis virus (TBEV) antibodies were performed in serum and CSF samples. Serological results were confirmed by virus neutralization. Molecular testing included nested and real-time RT-PCR assays for both WNV and USUV detection, followed by Sanger-sequencing and lineage determination. During the 2018 WNV transmission season a large number of human WNV infections were reported in Hungary. Until the end of 2018, altogether 215 locally-acquired and 10 imported human WNV infections were notified. The number of human WNV infections reported this year exceeded the cumulative number of cases since 2003. Serological cross-reactions between WNV and USUV were experienced in high proportion of samples tested. Besides urine, the use of whole blood samples for molecular diagnostics instead of sera improved the efficiency of viral RNA detection. The first autochthonous human USUV infection was confirmed in a patient with aseptic meningitis, without

any known immunosuppression or other co-morbidities. Our findings are raising awareness for the importance of thoroughly performed serological tests combined with molecular assays to differentiate WNV and USUV infections and to avoid misdiagnosis of human USUV cases.
Targeted investigations of neurological cases in other, USUV-affected countries might reveal USUV in the aetiology of further human cases.

DEVELOPMENT OF A PLASMID FREE CRISPR/CAS9 SYSTEM FOR THE GENETIC MODIFICATION OF OPPORTUNISTIC PATHOGENIC MUCOROMYCOTINA SPECIES

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Most fungal species, which can cause human infections belong to the genera *Candida* and *Aspergillus*, but in the recent years, the number of infections caused by Mucoromycotina species (e.g., *Rhizopus oryzae*, *Lichtheimia corymbifera* and *Mucor circinelloides*) has significantly increased. The properties and potential virulence factors that make the Mucoromycotina species enable to cause disease (mucormycosis) in patients, are not known. Potential virulence factor genes may play role in fungal dimorphism, proteolytic enzyme production, iron uptake, and production of cell surface proteins. Genetic manipulation of Mucoromycotina species, based on homologous recombination still can be a great challenge. In this study, a plasmid free CRISPR/Cas9 system was used for the genetic manipulation of *Mucor*, *Lichtheimia* and *Rhizopus* species. PEG-mediated protoplast transformation was used to introduce the Cas9 enzyme and the synthesized gene-specific gRNA with or without a deletion cassette into the fungus. In case of *Lichtheimia* and *Rhizopus*, the plasmid free CRISPR/Cas9 system was successfully used to disrupt the *pyrG* gene by NHEJ.
Our results suggested that the disruption of the *pyrG* gene has no effect on the growth of fungal strains. These strains and the developed methods will be suitable for further transformation procedure in the future. Based on the potential virulence factor genes (*cotH*, *svf*, *cyp51*, *hsbA*), a mutant library has been constructed from *M. circinelloides*. We have started to analyse the function of these genes in the pathogenesis and azole resistance of *Mucor*.

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DEVELOPMENT OF A NEW RNA-SEQ ANALYSIS PIPELINE FOR DETECTING ALLELE-SPECIFIC GENE EXPRESSION IN *CANDIDA ALBICANS*

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Candida albicans is a diploid opportunistic pathogenic yeast. Without having sexual reproduction and thus recombination, the *Candida* chromosome pairs evolve independently. The traditional RNA-

seq analysis pipelines such as the Tophat/Cufflinks cannot differentiate between the expression of the allele A and B of a certain gene. To analyze genome-wide transcriptional changes caused by different stresses we have developed a new pipeline. First, we mapped the reads into the genome A and B genes separately without allowing mismatches. In the next step, we picked up the reads, which mapped into those places where there were differences between the sequence of genome A and B. Finally, we counted these reads at all genes and continued with the usual statistical analyses.
In this way we found that among the *Candida*'s 6,300 genes, the expression of more than 100 genes were significantly different between chromosome A or B.

MONITORING THE EFFECT OF A RECENTLY DEVELOPED BIOAUGMENTATION AGENT ON FIELD CONTAMINATED BY SHORT-CHAIN CHLORINATED HYDROCARBONS

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Short-chain chlorinated aliphatic hydrocarbons (CAHs) (e.g.: tetrachloroethene - PCE, trichloroethene - TCE) are one of the most serious pollutants. Bioaugmentation, applying cultures containing *Dehalococcoides* sp. capable of complete reductive dechlorination, provides a possible technique to remediate CAHs contamination. Our aim was to develop an efficient treatment strategy including the development of a new bioaugmentation culture capable of complete dechlorination. Our goal was to perform pilot tests to verify the efficiency of our culture. Dechlorinating consortia were isolated from contaminated sites and were enriched in microcosm experiments at bench-scale and at industrial-scale (in 100 L fermenter). To monitor the effectiveness of the dechlorination in laboratory and field experiments polyphasic approach was applied using gas chromatography, water chemistry measurement, terminal restriction fragment length polymorphism, next generation sequencing and fluorescence in situ hybridization techniques. Organohalide respiring microbial communities were successfully enriched at laboratory- and industrial-scale. Thereafter, pilot-scale test was conducted at dosage of the consortia for five times, and the continuously application of biostimulation agent during the 29 months' period. Complete dechlorination of TCE to ethene was observed in laboratory and field experiments which were linked to three ribotypes of *Dehalococcoides* sp. and to genera *Sulfurospirillum*. Due to the applied bioremediation technique total CAHs contamination was decreased by up to 90% while cell counts increased and microbial community altered which was dominated by anaerobic fermenting Bacteria and methanogenic Archaea originated from bioaugmentation and biostimulation agents. We concluded that organohalide respiring microorganisms originated from the bioaugmentation agent and was enhanced by biostimulation indicating the effectiveness of the developed inoculum and bioremediation approach.

MANGANESE ION LEACHING DURING *ASPERGILLUS NIGER* CITRIC ACID FERMENTATION

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Citric acid (2-hydroxypropane-1,2,3-tricarboxylicacid) is one of the most prominent bulk products in biotechnology, employing the filamentous fungus *Aspergillus niger*. High-yield citrate production will only occur when Mn(II) ions are present in extremely low (<5 ppb) concentrations in the growth medium. Such concentrations are easily introduced by other nutrients or by the water, thus Mn(II) must be removed from the feedstock prior to sterilization, contributing significantly to the overall costs of the process. Citric acid fermentations take place in agitated and aerated bioreactors (fermenters). Although bench-scale fermenters are typically made of glass, several parts (aeration system, sparger, sampling tube, etc.) are made of stainless steel. Larger-scale fermenters are stainless steel-made. In fermentation biotechnology, type 316L stainless steel is the most frequently used alloy, which apart from the main metals (Fe and Cr) contains several other elements such as nickel, molybdenum, and manganese. In this study we investigated whether the conditions necessary for citric acid overproduction may facilitate the leaching of manganese ions from the stainless steel, and whether this phenomenon influences citric acid yield. We identified two stages of Mn(II) release in the upstream part of citric acid fermentation. First, manganese concentration in the medium increases by 1 - 20 μg/L during sterilization of the vessel (121°C), independently of the method used (autoclave or in situ). Secondly, low medium pH typical for well-growing *Aspergillus niger* cultures also contributes to the increase of Mn(II) concentration in the medium. This effect is negligible (<0.2 μg/L) over pH 2, but intensifies below this threshold level (3 - 30 μg/L), significantly decreasing citric acid yield. Several methods to counteract Mn(II) release was tested. First, frequent electropolishing of the metal parts to heal surface corrosion helps even in case of older equipments. Secondly, treatment of the medium with potassium ferrocyanide results in Mn(II)-complex formation and concomitant reduction of Mn(II) concentration, but it may also react with other essential metals and thus inhibit fungal growth. Thirdly, independent sterilization of the vessel and the growth medium is an effective method but obviously difficult to apply at larger scale. Finally, keeping medium pH over 2.1 in the first 3 days of citric acid fermentation prevents inhibition of citric acid biosynthesis at the later stages of the fermentation.
From this latter result we concluded that sensitivity of *A. niger* citric acid biosynthesis to Mn(II) is more pronounced in the early stages of growth and diminishes as the fermentation progresses. We are currently investigating the biology behind this phenomenon.

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EFFECT OF FERMENTATION OF MANGO JUICE BY SOME LACTIC ACID BACTERIA ON THE ANTIOXIDANT ACTIVITY AND PHENOLIC COMPOUNDS

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Mango contain numerous nutrients and sugars which may serve as a suitable medium to cultivate probiotic lactic acid bacteria to enhance the health benefits of the food product. Furthermore, the juice is a rich source of phenolic compounds which are catechins, quercetin, anthocyanins, gallic and ellagic acids. Lactic acid bacteria have been reported to improve bioactivity of polyphenols by

hydrolysing them into the simpler compounds during fermentation. Three probiotic strains, including *Lactobacillus acidophilus* 150, *L. plantarum* 299V, and *L. casei* 01 were used to ferment mango juice for 16 hours at 37°C. Evaluation of the effect of lactic acid bacteria on the growth, antioxidant activity and phenolic compounds in fermented mango juice was carried out. The microbial population was significantly increased by the three applied bacteria from 10^6 at initial time to around 10^9 CFU/mL during 16 h of the process. *L. casei* 01 strain exhibited the fastest growth and reduction of pH in the mango juice comparing to the other strains. Folin-Ciocalteu method revealed that total phenolic content was significantly decreased by 8 - 13% in the mango juice after fermentation. The fermentation of juice by *L. plantarum* 299V did not affect antioxidant activity, and the value remained 3.99 mM $FeSO_4$/mL. However, the trend of drop in ferric ion reducing antioxidant power was observed in the cases of the *L. acidophilus* 150 and *L. casei* 01 strains.
These findings suggested that the mango juice can be considered as a good medium for the growth of *Lactobacillus* sp. strains. The concentration of phenolic compounds and antioxidant activity decreased in fermented juices exception of *L. plantarum* 299V strain.

ZOONOTIC BORNA DISEASE VIRUS 1 SPILL-OVER INFECTIONS LEADING TO FATAL HUMAN ENCEPHALITIS AND ISOLATION OF THE FIRST HUMAN VIRUS STRAIN

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Borna disease virus 1 (BoDV-1; family *Bornaviridae*) is endemic in bicolored white-toothed shrews (*Crocidura leucodon*) in parts of Germany, Austria, Switzerland and Liechtenstein. Following spill over transmission to erroneous mammalian hosts, particularly horses and sheep, the virus causes Borna disease, a mostly fatal neurologic disease. Human BoDV-1 infections have been a matter of controversial debates for almost 30 years. Recently, the first unequivocally confirmed human BoDV-1 infections were reported from four patients, including three recipients of organ transplants from the same donor. All four patients developed severe encephalitis resembling Borna disease, which was fatal in three cases. We retrospectively investigated human encephalitis cases for BoDV-1 infection to assess the incidence of human BoDV-1 infections and collect further data on the course of infection and disease and on its epidemiology. More than 50 archived brain biopsies from encephalitis cases collected in Bavaria from 1995 to 2018 were screened for the presence of BoDV-1 RNA by qRT-PCR. Positive results were confirmed by high throughput sequencing and by detection of BoDV-1 - reactive antibodies in serum and cerebrospinal fluid using indirect immunofluorescence. BoDV-1 RNA and bornavirus-reactive antibodies were detected in seven retrospectively analyzed patients. Strikingly, they made up for the majority of fatal encephalitic cases of unclear causality in the sample collection, while none of the surviving patients tested positive. Five BoDV-1 - positive patients were of inconspicuous health until their fatal disease, whereas two were severely immunocompromised due to organ transplantation several months earlier. The typical clinical presentation was headache, fever and confusion followed by deep coma and death. BoDV-1 sequences from the patients differed from each other.
Their similarity to animal sequences from the respective patient´s home regions suggested independent spill-over transmissions from the local wild animal reservoir. A BoDV-1 strain was isolated from a recent human encephalitis case in 2019 and propagated in cell culture. We confirmed

that BoDV-1 can act as a severe zoonotic human pathogen in both immunocompromised and apparently healthy persons. Despite a rather low incidence, it may make up for a considerable proportion of fatal encephalitis cases of unclear causality in endemic areas.

INFLUENCE OF NON-*SACCHAROMYCES* YEAST CULTURES ON THE YEAST AND LACTIC ACID BACTERIA POPULATION DURING PREFERMENTATIVE COLD MACERATION OF RED GRAPES

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Pre-fermentative cold maceration during red wine making is a relatively new technology, which elevates the extraction of the grape skin-related volatile and coloring agents. Employing non-*Saccharomyces* yeasts (NSC) in fermentation is a new and popular trend in oenology too. The application of NSC strains is also suggested during prefermentative treatments, especially for protection of the grape from oxidation and unwanted microbiological growth. An interesting but not fully understood question is, how these NSC cultures could influence the complex microbiome of fermenting wine. The aim of this study was to follow the changes of indigenous yeast and bacteria populations of inoculated red grapes. For inoculation of red grapes three non-*Saccharomyces* starters were used as protective cultures: *Pichia kluyveri*, *Lachancea thermotolerans* (syn. *Kluyveromyces thermotolerans*) and *Torulaspora delbrueckii*, in comparison with a non-inoculated control. The 72-hour cold maceration was carried out at 8°C. After maceration the treatments were fermented with a *S. cerevisiae* starter, then a spontaneous malolactic fermentation is occurred. The dynamics of indigenous yeast and lactic acid bacteria population were monitored by taking samples at different fermentation stages and plating of samples on selective agar. The presumed *Saccharomyces* yeasts were identified by *Saccharomyces*-specific PCR method. The fermented young wines were chemically analyzed as well. The results clearly showed that non-*Saccharomyces* yeasts could dominate during cold maceration, but the ratio of non-*Saccharomyces* and *Saccharomyces* (SC) yeast decreased during the fermentation. There were a slight difference between the NCS : SC ratio of the experimental wines at the third day of fermentation, but at the end of fermentation only SC yeasts could be detected. These results suggest that the alcoholic fermentation was not influenced considerably by the non-*Saccharomyces* cultures. The indigenous LAB biota was generally suppressed by the protective cultures with two order of magnitude during the cold treatment and ca. one order of magnitude difference was found compared to the control. After seven days of alcoholic fermentation the LAB population decreased considerably but the treatment effect has been demolished. Later the spontaneous malolactic fermentation was not influenced by the various protective cultures. It can be argued that the fermentation and population dynamics of experimental wines did not differ considerably from the control.

The applied NSC starters did not show any significant antagonistic effect to neither the yeasts nor the bacteria in the complex microbiome. Moreover, the basic analytical profile of the finished wines were not significantly different. From oenological point of view these results would be a useful confirmation to employ non-*Saccharomyces* starters for bioprotection of the grape must before fermentation, without adverse effects on the later vinification steps.

CHANGE IN METABOLIC FOOTPRINT OF SOME WINE-RELATED YEASTS INDUCED BY EXTREME INITIAL SUGAR CONTENT

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There is a current trend in the wine industry to use selected non-*Saccharomyces* yeasts as starter cultures. *Starmerella bacillaris* (syn. *Candida zemplinina*) is a promising starter candidate, this species is particularly connected with sweet, bortytized wine fermentation, but it is frequently isolated from other sources. From oenological aspect it has several valuable properties. The major metabolite pattern plays a significant role in forming the final wine quality, while a moderate nitrogen demand could help to avoid a problematic fermentation. A relevant question is how the originally described character of different wine yeasts can shift according to the varying 'stress' parameters of the alcoholic fermentation, e.g. higher initial sugar concentration. Our aim was to evaluate the changes in some major metabolites and in the nitrogen utilization induced by extremely high initial sugar concentration, in case of the two most important wine yeasts *Saccharomyces cerevisiae*, *Saccharomyces uvarum* and *S. bacillaris*. The metabolites analyzed were chosen on the basis of their oenological importance: ethanol, glycerol, volatile acidity, malic acid, succinic acid, YAN and proline; the stress was put on the effect of high sugar concentration on these parameters. Three selected strains of *S. bacillaris*, *S. uvarum* and six of *S. cerevisiae* were investigated. In the latter species two groups of strains: robust and moderate fermenters were distinguished. The chemically defined grape-juice medium was completed with 220 and 320 g/L of sugars, to represent a fully matured and an over ripen state. Canonical discriminant analysis was used to assess the sugar-induced changes in the metabolite pattern as a complex footprint. The concept was, whether the differences in the given metabolites due to the extreme sugar level could make an obvious discrimination among the performances and among the species groups.
With three significant functions the species groups were clearly distinct on the different sugar levels. Function 1 is ethanol itself, it was not grouping with either factors, Function 2 represented the positively sugar-dependent factors: volatile acidity and proline utilization, while Function 3 meant the negatively sugar-dependent factors: consumed YAN, succinic acid and malic acid consumption. However, the two *S. cerevisiae* sub-categories were blurred together, the *S. uvarum* group was closer to the overall *S. cerevisiae* performance than the *S. bacillaris* group. Based on the distance between the group centroids in the discriminant function matrix, *S. bacillaris* performance changed the most, while the *Saccharomyces* groups were similar in this aspect. The results suggest that *S. bacillaris* possesses an excellent adaptation mechanism to extreme sugar concentration.

COMPARISON OF THREE FOREST STANDS BY THEIR SOIL CATABOLIC ACTIVITY PROFILES

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The biological activity of soil has major effect on its fertility, influencing the composition of its vegetation. Nevertheless, forests modify the microclimatic characteristics of their environment and through a special nutrient cycling, they are associated with a typical microbial composition of forest

soils. Soil samples were collected from a 10-year-old black locust, a newly reforested (2 years old) and a middle aged (80-year-old) oak forest stands in order to compare the catabolic activity of their soils. The sampled forest stands were located close to each other (within 1 km), their soil types were quite similar (residual-carbonate chernozem and leached chernozem). The soil samples were collected from two depths (0 - 10 cm and 10 - 40 cm) in three repetitions and 5 times between April and October in 2018. Microrespiration analysis (MicrorespTM) was carried out on the soil samples with 23 different compounds (carbohydrates, amino acids, carboxylic acids) in order to identify the main carbon sources being utilized by the soil microbial communities. Additionally the main soil physical and chemical parameters texture (sand %, silt %, clay %), total C%, TOC%, TIC%, total N%, NO_3^- - N, AL - P_2O_5, AL - K_2O and AL - Na) were measured. For the identification of forest stands specific catabolic activity characteristics one-way ANOVA and discriminant function analysis were carried out on the data derived from substrate utilization patterns. According to our results soil depth has a significant effect on carbon source utilization, independently from forest type and sampling times. In 5 months' average DL-malic acid, D-glucose and D-fructose were the most utilized carbon sources by the forest stands, furthermore the upper soil layer of the black locust stand showed the highest microbial activity. The young oak forest showed the lowest carbon compound utilization in case of all compound groups, although significant difference was found only in the case of carboxyl acids. Except of organic carbon content (TOC%) and phosphorus (AL - P_2O_5) content the soil physical and soil chemical parameters showed significant differences among the forest types. The highest amount of nitrogen compounds (including nitrates) was detected under the black locust stand.

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GENERATION AND CHARACTERIZATION OF AN OVEREXPRESSION STRAIN COLLECTION IN *CANDIDA PARAPSILOSIS*, HUNTING FOR VIRULENCE FACTORS

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Candida parapsilosis is a commensal of the human skin, however also recognized as an opportunistic fungal pathogen. It is particularly associated with low birth weight neonatal and nosocomial infections claiming for extensive survey of the virulence factors of *C. parapsilosis*. When conventional gene deletion methods are not applicable, the artificial overproduction of a given gene might refer to its function. In this study we aimed to create and characterize an overexpression (OE) mutant strain collection involving ORFs that - according to our preliminary experiments and literature data - are thought to be involved in the pathogenesis of the fungus. Transcriptional analysis of *C. parapsilosis* co-incubated with THP-1 human monocytes was performed in order to identify potential targets for overexpression. Differentially expressed fungal kinases, transcriptional factors and additional ortholog genes of *C. albicans* associated with virulence were sentenced to overexpression. We successfully generated 37 BAR-coded OE mutant strains by using Gateway cloning method. Transformants were validated by colony-PCR, Southern-blot analysis and qRT-PCR. The fitness of the verified mutants were monitored *in vitro* at various temperatures and in the

presence of different stressors that mimic the conditions of what the fungus exposed to when interacting with the host.
Growth disabilities were detected in the presence of SDS, Calcofluor White and Congo Red in comparison with the control. Morphology and pseudohyphae forming capacity of the mutants were recorded and quantified by using microscopy and flow cytometry. Overexpression of given genes ended up in altering the biofilm forming capacity as 3 mutants developed less while 8 mutants performed more biofilm compared to the control. According to our results, several mutants from our OE collection showed a phenotype different from the control under conditions that are thought to impact the pathogenicity of this species. In order to strengthen this hypothesis we further aim to perform virulence studies including both *in vitro* and *in vivo* infection models.

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ROAD FROM INFECTION TO LONG-TERM SHEDDING: EBOLA VIRUS ISOLATION FROM HUMAN BODILY FLUIDS

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To estimate and mitigate the risk of transmission of the Ebola virus disease (EVD) from patients or survivors is essential to understand the dynamics of the newly rediscovered Ebola virus persistence and shedding in the diverse body compartments and fluids. The most sensitive diagnostic tool for Ebola-virus is the RT-PCR, however it does not provide appropriate information about contagiousness. In the frame of the EVIDENT (Ebola Virus Disease - correlates of protection, determinants of outcome, and clinical management) project different clinical samples from five EVD patients were sequentially collected from selected Guinean patients as well as control samples (from Favipiravir antiviral drug treated and non-treated patients) for the purpose of virus isolation then shipped to the National Biosafety Laboratory at the National Public Health Center, Hungary. The samples were tested for bacterial contamination and were cultivated on VeroE6 cell culture. Individual samples were selected for further virus isolation studies using suckling mice (BALB/C and C57BL/6 populations) and SCID mice. In total 206 samples were selected for virus isolation among which 124 samples arised from patient with Favipiravir treatment and 82 samples (control) with no Favipiravir treatment. All the samples were tested for bacterial and fungal contamination by classical culturing methods. Some of the identified contaminants showed a wide range and high level of antibiotic resistance spectra. The samples were categorized based upon Ct values, as a predictive value for the abundance of infective virus in the body fluids. In categories with low Ct values (high virus copy number) the ratio of the samples from which Ebola virus was successfully isolated was between 37 - 44%. In case of the samples with high Ct values (lower copy number) this ratio was only 0 - 9%. When comparing the results of the *in vitro* isolation and *in vivo* animal isolation we found that the animal model was more sensitive.
Only 18% of the samples could be isolated on VeroE6 cell cultures, while virus replication was observed in case of 30% of the SCID mice and 26% of the suckling mice groups. Most of the isolates originate from blood or plasma, but we could isolate the Ebola virus from other bodily fluids, such as placenta, mother milk, umbilical cord or amniotic fluid samples. Virus isolation is a crucial tool to

obtain reliable data on the infectiveness of the body fluids of sick patients and survivors to mitigate the risk of transmission from EVD survivors to the community.

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A NEW POSSIBILITY TO UNLOCK THE INNOVATION POTENTIAL TO RISK GROUP 4 PATHOGENS RESEARCH: ROLE OF THE EUROPEAN RESEARCH INFRASTRUCTURE ON HIGHLY PATHOGENIC AGENTS (ERINHA) AND THE NATIONAL BIOSAFETY LABORATORY

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The climate changing, globalization, migration and the increasing tourism play important role in the rise, emergence and reemergence of Risk Group 4 (RG4) pathogens. Therefore, these pathogens are prioritized for research and development on the WHO Blueprint list. Appropriate prevention and more effective response to the threats caused by the RG4 pathogens demand a coordinated global action involving multiple research capacities and expertise. The European Research Infrastructure on Highly Pathogenic Agents (ERINHA) as a pan-European distributed Research Infrastructure reinforces the European capacities to study RG4 pathogens, enhances the coordination of Biosafety Level 4 (BSL-4) and complementary facilities and supports cutting-edge research in the field of pathogenesis of the human/animal diseases caused by the most dangerous microorganisms. The National Biosafety Laboratory (NBL) at the National Public Health Center is the national and also the Central and Eastern European node of ERINHA. ERINHA (including NBL) with their shared capacity can provide trans-national access to high-containment facilities for development of own or collaborative research and innovation programs including those ambitious projects that cannot be performed in the framework of just one laboratory. ERINHA's research approaches tend from discovery to prevention: providing diagnostic capabilities (provision of new diagnostics for field studies, whole genome sequencing and bioinformatic analysis), development of immunological tools for identification of correlate protection discovery, studies of viral pathogenesis, vaccine target discovery using animal models (small animal and non-human primates), new antivirals and drug carrier discovery, testing existing therapeutics against emerging agents and GLP testing of intervention manufacturers. ERINHA can provide training for the potential users of the infrastructure to conduct experiments under BSL4 conditions and access to pathogen specimens. In the frame of an EU funded project (ERINHA-Advance) Trans-National access will be to offered to some external users - based on project selection - as a coordinated and free-of-charge access to the whole spectrum of ERINHA facilities with the ultimate goal of testing and improving provided services. Access programs will focus on the development of innovative animal models for highly infectious agents and innovative therapeutic approach for RG4 pathogens.
The ERINHA as a new model for shared research capacities for highly pathogenic agents will contribute to the health security of citizens in Europe and beyond.

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DEGREE OF HYDROLYSIS OF PROTEINS USED AS NITROGEN SOURCES INFLUENCE LACTOSE ASSIMILATION AND GROWTH OF LACTIC ACID BACTERIA

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Whey is the main by-product of cheese making. Over 200 million tonnes are produced annually worldwide, thus its environmentally conscious and cost-effective processing is of scientific and industrial interest. Our approach is to apply lactic acid bacteria (LAB) for producing a whey-based fermented drink. Although lactose content of deproteinized whey (DPW) can be metabolized by LAB, it is necessary to supplement DPW with a well utilized nitrogen source. In this experimental work we investigated the effect of various organic nitrogen sources (whey protein, soy and casein peptones) on the growth and fermentation vigour of LAB and aimed to find the connection between lactose and protein utilization. We carried out lab-scale fermentations with five different LAB strains (*Lactobacillus casei* PB8, *L. helveticus* PB9, *L. acidophilus* N2, *L. plantarum* 2108 and *Lactococcus cremoris* B1) in culture media consisting of 1% lactose and 0.25% organic nitrogen source. We measured the growth of strains, acidification, amino acid and lactose content of the fermentation broth over a period of 48 hours. Our results indicated that soy and casein peptones highly supported the growth of *Lactobacillus* strains, while the growth and viability of *Lc. cremoris* B1 was poor. Uptake of the amino acid content of peptones was fast but there was a considerable difference in the maximum of amino acid utilization relative to the growth phase of *Lactobacillus* strains. When whey protein was added as a nitrogen source, the amino acid uptake was intensive during the exponential phase of growth. When peptones were added to the culture media, significant amino acid utilization was only measured in the stationery phase of growth. Peptones were more favorable than whey protein regarding lactose uptake as well. LAB strains utilized approximately 20 - 25% lactose throughout fermentation whereas when whey protein was added, only *L. acidophilus* N2 could decrease lactose concentration by 10%.

As the main conclusion protein hydrolysates (peptones) supported the growth and lactose metabolism of LAB strains more than whey protein. Moreover we observed that although LAB strains had a good growth in culture media supplemented with whey protein and utilized its amino acid content at the initial phase of the fermentation, they did not metabolize lactose at all. This indicates that LAB strains prefer utilization of amino acids as carbon sources instead of lactose.

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PESTICIDE TOLERANCE AND NUTRIENT MOBILISATION OF LEGUME SYMBIONT AND HELPER BACTERIA

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Legumes, mainly beans and soybeans gain increasing importance in the agricultural production due to the national Common Agricultural Policy Agro-Environment Programs (CAP-AEP) in Hungary.

These crops require symbiont bacterial species for nitrogen fixation and - accordingly - proper development and yield. Plant protection and fertilization using mainly chemicals, however, cannot be neglected in the production process, therefore the seed inoculating bacterial strains face a harsh selective environment. The emerging trend of sustainable agriculture offers a wide palette of rhizosphere microbes involved in alternative plant growth promotion (PGP) as soil inoculants. Legumes need an elevated level of phosphorous and iron for efficient nitrogen fixation, therefore PGP bacteria mobilizing macro- and microelements are often recruited for this purpose (Biofil technology: http://terragro.hu/img/gallery/downloads/BioFil_Sz%C3%B3ja_haszn_HUN.pdf). Accordingly, PGP and symbiont strain selection for use in integrated crop production requires thorough evaluation of nutrient mobilization capacity and pesticide-tolerance. This work concentrates on fungicide (Vitavax, Rancona), insecticide (Force) and herbicide (Gladiator) tolerance of legume symbionts (*Rhizobium leguminosarum*, *Bradyrhizobium diazoefficiens*) as well as PGP bacteria (*Bacillus* spp., *Pseudomonas* spp., *Paenibacillus* spp., *Azospirillum* spp., *Arthrobacter* spp., *Kocuria* spp.) that may be used for macro- and microelement mobilization in soil inoculation of legume seedbed. PGP abilities of the selected bacterial species were also tested *in vitro*.

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CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM BULK MILK FROM TWO DAIRY FARMS IN HAJDÚ-BIHAR COUNTY, HUNGARY

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Staphylococcus aureus is one of the most important microorganisms that can cause mastitis in cows in dairy farms. Therefore its economic importance in the dairy industry is not negligible and its public health significance is considerable too. The aim of our study was to examine the occurrence of *S. aureus* in bulk milk from two different dairy farms in Hajdú-Bihar County, Hungary, which are identical in their keeping and milking technology, and after identifying the *S. aureus* strains isolated from the samples by the latex agglutination test, their characteristics (tellurite reducing ability, lecithinase activity, hemolysin producing ability) and their antibiotic resistance were determined. Based on the results, *S. aureus* occurred in all bulk milk samples collected from the two dairy farms, and the mean *S. aureus* counts were 3.54 ± 0.21 lg CFU/mL in Farm "A" and 3.22 ± 0.20 lg CFU/mL in Farm "B". There was significant difference ($P < 0.05$) between the mean *S. aureus* counts. In all the samples, the *S. aureus* count exceeded the limit (M = 2.70 lg CFU/mL) set in the regulation of the Hungarian Ministry of Health 4/1998. (XI. 11.). A total of 24 *S. aureus* isolates (17 from Farm "A" and 7 from Farm "B") were chosen from the samples for further investigations. All strains had tellurite reducing ability and lecithinase activity. Among the 17 strains isolated from bulk milk from Farm "A", 1 strain showed β hemolysis, 2 strains showed weak α and β hemolysis and 14 strains showed α and β hemolysis on blood agar. In the case of Farm "B", all the 7 isolated strains showed β hemolysis. In antibiotic resistance testing, it was found that all strains were susceptible to cefoxitin, chloramphenicol, clindamycin, erythromycin, gentamicin, penicillin, tetracycline and trimethoprim/sulphamethoxazole in the case of Farm "A". Furthermore, it was found that all the

strains isolated from bulk milk from Farm "B" were susceptible to all antibiotics, except for penicillin. The results show differences in the characteristics of *S. aureus* strains isolated from bulk milk from different dairy farms. Furthermore, differences can be observed between the characteristics of strains that can be isolated from the same farm. The information gathered during the analysis of the characteristics of *S. aureus* strains isolated from bulk milk of dairy farms contributes to a better understanding of the *S. aureus* strains which can occur in bulk milk of dairy farms, and thus more effective protection could be provided against them.

THE SURPRISING EFFECT OF THE DOMESTICATION-DRIVEN GENOME EVOLUTION OF *S. CEREVISIAE* ON ITS POTENTIAL TO COLONIZE AND INFECT US

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The status and origin of *Saccharomyces cerevisiae* as a member of the human microbiome, or as an emerging pathogen is still not fully understood. In this study we conducted a detailed genetic characterization of clinical *S. cerevisiae* isolates from Hungarian clinics from dozens of patients, along with commercial probiotic and baking strains from the same geographic region. The results were supplemented by re-evaluating previously described human yeast isolates from three continents. We show that the majority of human isolates worldwide are derived from commercial strains that evolve inside the host. Our results show that the domestication history of commercial yeasts has profound and previously unrecognized effects on microevolution and evolvability upon human colonization. While baking-derived clinical isolates undergo genome reduction and rearrangements, generating heterogeneity through an apparently clonal mode of reproduction, probiotic derived clinical yeasts and indigeneous nations' gut isolates are less prone to genome structure variation.
Our observations highlight that the pathogenic potential of *S. cerevisiae* has been completely altered by domestication and directed strain improvement in the last decades.

INVOLVEMENT OF AtfA AND MnSOD HOMOLGUES FROM *FUSARIUM VERTICILLIOIDES* IN OXIDATIVE STRESS RESPONSES, SEXUAL REPRODUCTION, AND SECONDARY METABOLITE PRODUCTION

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The aims of present study was to construct and characterize the Δ*fvatfA* and Δ*fvmnSOD* gene deletion strains of the maize pathogen fungus *Fusarium verticillioides*. *FvatfA* encodes a bZIP-type transcription factor with considerable homology to *Aspergillus nidulans atfA*, which plays a pivotal role in the orchestration of the oxidative stress defense system of the fungus. *FvmnSOD* putatively codes for the mitochondrial MnSOD of *F. verticillioides*, which is a likely key player in the elimination of harmful superoxide anion radicals. After the successful deletion of the studied genes we also genetically complemented the mutants. We performed a wide spectrum of various physiological and virulence assays to map the biological functions of the selected genes. As a result, FvatfA was found to be a key player in the regulation of the defense against oxidative and cell wall integrity stress, the regulation of sexual reproduction as well as fumonisin and carotene productions, the down-regulation of bikaverin biosynthesis, the maintenance of growth on Czapek-Dox medium and also in the promotion of invasive growth on tomato fruits. On the other hand, FvmnSOD was indispensible in the protection against superoxide stress and apoptotic cell death elicited by the *Penicillium chrysogenum* antifungal protein PAF. Further experiments are needed to see whether or not FvatfA transcription factor regulates FvmnSOD expression under superoxide stress.

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HIDDEN COMPLEXITY OF THE VARICELLA ZOSTER VIRUS TRANSCRIPTOME REVEALED BY LONG-READ SEQUENCING

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Varicella zoster virus (VZV) is member of the *Alphaherpesvirinae* subfamily. VZV is the causative agent of chickenpox (varicella), affecting mainly children, and shingles (zoster) affecting adults. The initial infection is followed by a life-long persistency in the dorsal root ganglia. The virus can be reactivated, causing secondary infection, resulting in painful skin rashes with blisters. Compulsory vaccination has been planned to introduce from the year 2019 in Hungary. Former short-read sequencing and traditional Northern-blot analyses revealed some length and splice isoforms of VZV transcripts; however, these methods are not optimal for resolving complex genomic regions expressing overlapping transcripts. In our study we used the Oxford Nanopore Technologies' MinION long-read sequencing platform (ONT MinION) to annotate previously known and novel transcriptional start and end site isoforms, novel polycistronic transcripts and novel splice isoforms of the VZV. Our results show the full length transcripts of VZV first time. We identified 114 novel transcripts, including mRNAs, non-coding RNAs, polycistronic RNAs and complex transcripts, as well as 10 novel spliced transcripts and 25 novel transcription start site isoforms and transcription end site isoforms. A novel class of transcripts among Herpes- and other viruses, the near to replication origin RNAs (nroRNAs) are described as well. These transcripts are encoded by the genomic region located close to the viral replication origin. Additionally, our results show that the ORF63 (the immediate early transcriptional regulator, ICP22 homolog) exhibits a complex structural variation

encompassing the splice sites of the recently identified VZV latency transcripts (VLT), forming a variety of long, non-coding RNAs. Another result of our experiments is the detection of RNA editing in novel non-coding RNA molecules, which seems to be a rare phenomenon among Herpesviruses.

EXAMINATION OF EXOPOLYSACCHARIDE (EPS) PRODUCTION CAPACITY OF SOIL MICROORGANISM STRAINS AND SEPARATION OF PRODUCED EPS BY SEC-HPLC

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Organic material content of cropfields may be significantly decreased by intensive cultivation, therefore it may have negativ effect on physical and biological parameters and structural conditions of the soils. Applying polysaccharide producers in BIOFIL® soil inoculants, which have even ability of plant-growth promotion, we can achive double impact: positive effect on soil structure, simultaneously providing beneficial substances - like nitrogen, phosphorus, potassium, siderophores and some plant hormones - for the crop plants. The structural unit of soil is the soil aggregate, which in addition to the inorganic fraction, is composed of organic plant residues and microbial biomass materials. Soil aggregate stability is based on the adhesion of microbial exopolysaccharide (EPS), binding the bacterial cell to solid surface, forming biofilm and thus supporting soil aggregate bacterial communities. In addition, high water retention ability of EPS prevents soil bacteria from dessication. Accordingly, it is a high priority task to study the EPS production ability of BIOFIL® strains. Thetefore, the aim of our work was to develop a fast and simple method for screening in order to test the EPS production capability. Polysaccharide monomers were supplied by high concetration of sucrose in the culture medium. Separation of polysaccharides was carried out by molecular weight exclusion cromatography (SEC-HPLC). After preparation of EPS by isopropanol precipitation 25 of the examined 88 strains were able to produce exopolysaccharide at varying quantity levels. According to the molecular weights detected, the examined strains may produce dextran, xantan, curdlan and succinoglucan. *Pseudomonas* sp., *Ensifer* sp. and *Microbacterium* sp. strains produced dextran, curdlan and xanthan EPSs (molecules below 5,000 kDa). Beside a large polysaccharide molecule (beyond 5,000,000 kDa) smaller, xanthan, curdlan and succinoglycan were gained from *Paenibacillus* sp. strains. A soil yeast strain from the *Cryptococcus* genus, included in this study, also produced a large exopolysaccharide (beyond 5,000,000 kDa) and two xanthan molecules sizing 30,000 and 2,300 kDa, respectively.

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ANALYSIS OF HIGH-RISK HPV E7 ONCOPROTEIN INTERACTION WITH CYTOPLASMIC PROTEIN TYROSINE PHOSPHATASES

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Human papillomavirus (HPV) infection of the genital mucosa is the most common sexually transmitted disease, but only a minor part of infections leads to tumour - most often to cervical cancer in women. The combined effect of HPV E6 and E7 oncoproteins is essential for the development and progression of HPV-associated tumours. These viral proteins are able to interact with a number of host cell regulatory proteins. Protein phosphorylation - which is regulated by kinase and phosphatase enzymes - is a fundamental mechanism for determining the activity of cell signaling pathways. Recent extensive studies on protein interaction, have suggested association between HPV E7 protein and some cytoplasmic protein tyrosine phosphatases - PTPN14 and PTPN21. In our experiments we aimed to characterize these relationships. In the first part of our experiments, we investigated the effect of HPV E7 in HPV negative (HEK293, C33a) and in HPV positive cervix carcinoma derived cell lines (HeLa, CaSki) for the expression of PTPN14 and PTPN21 phosphatase proteins. A mammalian expression vector encoding by the high-risk HPV-16 E7 protein was then transfected into C33a or HEK293 cells and we investigated the expression of phosphatase proteins by Western blot. In HPV positive cell lines, we investigated the effect of E7 ablation by siRNA on the expression of the phosphatase proteins. The interaction between HPV-16 E7 and phosphatases was verified by pull-down and Western blot. We have shown that high-risk HPV E7 oncoprotein can interact with both phosphatases and significantly affect their protein expression. In the presence of HPV-16 E7, PTPN14 protein expression was significantly reduced, whereas the level of PTPN21 protein increased. Thus, our results suggest that high-risk HPV E7 oncoproteins interact with both PTPN14 and PTPN21 phosphatase and adversely affect their protein expression. We would like to further investigate these interactions and their effect on various signaling pathways, since these phosphatases can alter the structure of the actin cytoskeleton, affect cell adhesion, and proliferation. Thus, they can play a key role in both the life cycle of the virus and in the malignant transformation of the host cell.

SUBSTANCE, ENERGY, EVOLUTION - THE LIFE OF OUR EARTH. WHAT IS THE SCIENTIFIC BASE OF THE SUSTAINABLE DEVELOPMENT

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The Universe is endless and timeless substance and energy flow with motion of celestial systems, bodies and cosmic powder on the basis of the low of Nature/God. In spite of the fact that men are not able to imagine and to know the total Universe the author deals with general philosophical questions and the life of our Earth which can be divided into two basic periods: true abiotic physical life and biological life. These periods are the result of the atomic, chemical and biological evolution. Biological life begun when the first living unit appeared. The effect of human beings and farm animals on the environment of our Planet is presented and the question of the Sustainable Development is discussed.

RECALLING OUR EARLY TIME DATA ON THE FAECAL EXCRETION OF ENTERIC BACTERIA AND THE PROTECTIVE ROLE OF GUT

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By the recent days implication of the so called "living flora" preparations the authors have felt worth to recall their own experimental results and observations from the 60th and 70th of the last century. Mice and healthy volunteers consuming different bacterial extracts, killed or living enteric bacteria, and patients with acute *Salmonella* gastroenteritis were studied. It was observed that neither the consumption of high dose Boivin extract/endotoxin nor a great amount of heat killed bacteria caused any enteral or general symptom in men. When high dose non-pathogenic living bacteria were consumed by the volunteers the ingested germs disappeared from their faces after some days excretion without eliciting any symptoms. However, when infant specific Enteropathogenic *Escherichia coli* (EPEC) strains were administered to adults in very high doses signs of diarrhoea could be observed. On the basis of the data collected in cases of infected mice or patients it can be stated that there is a connection between the pathogenic invasive property of the strain and the length of its presence in the faecal samples. Finally the authors raise the question what kind of effect can be expected from the preventive or therapeutic probiotic preparations.

STAPHYLOCOCUS AUREUS CARRIAGE IN COMPANION ANIMALS AND THEIR OWNERS

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Staphylococcus aureus is a major human pathogen which can cause life threatening infections and is often multiresistant. In addition to diseased or asymptomatic persons, the source of infection can be the non-living environment and several animal species, as well. According to the literature, *S. aureus* can spread from pets to owners and vica versa. Our aim was to conduct a survey in Budapest to establish staphylococcal carriage among owners and their pets. As part of this study we wanted to further explore whether companion animals carry *S. aureus* and hence pose any risk to their owners. Cotton swab samples were taken from skin and nose of owners, pets and veterinary staff at different animal hospitals. 103 humans and 82 animals were screened. After selective culturing, the species identification and detection of resistance or virulence genes were done by PCR. Antibiotic susceptibility was tested according the EUCAST guidelines. PFGE was used to determine clonality of isolates deriving from owners and their pets. In total, 52 *S. aureus* (39 from humans 13 from pets) were found. Only one isolate proved to be methicillin resistant (MRSA), otherwise they were mainly sensitive to antibiotics; higher resistance rates were observed to macrolides and beta-lactams. Toxic shock syndrome toxin gene was found in a surprisingly high proportion. PFGE could reveal genetic identity between human and animal isolates in two cases. Carriage rates for MSSA and MRSA found in this study were similar to those published in the literature.
We could verify the direct transmission of *S. aureus* between humans and their pets. The toxin producing strains can lead to even more severe infections. Our findings seem to strengthen the theory that pets can serve as reservoirs for human *S. aureus* infections.

COMPARISON OF BIOLOGICAL EFFECTS OF STERIGMATOCYSTIN AND AFLATOXIN ON BIOMONITORING SYSTEMS

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Aflatoxin B1 (AFB1) is the most potent natural carcinogen, thus one of the most harmful mycotoxins known. It has also been shown to be teratogenic and to induce immunosuppression. *A. nidulans*, *A. versicolor*, and *A. creber* are apparently unable to biotransform STC into o-methylsterigmatocystin, the direct precursor of aflatoxin B1 (AFB1). Consequently, substrates colonized by these fungi can contain high amounts of STC. While there is abundant data on the biological effects of AFB1, STC is a not well characterized mycotoxin. Despite the structure of these toxins is very similar AFB1 is a much more harmful toxin than STC, according to the published data on their biological effects. It has been suggested that sterigmatocystin is about 1/10 as potent mutagenic as aflatoxin B1 measured by Ames test. In this research, the biological effects of STC and AFB1 were examined in two different biomonitoring systems. SOS-Chromotest, based on *E. coli* PQ37, makes use of S9 rat liver homogenate for producing genotoxic epoxide derivatives from AFB1 and STC. Equal concentrations of the toxins were measured for genotoxicity in intact form and after metabolic activation. The SOS-inducing potency of these toxins was almost the same: AFB1 (2.15), STC (0.22) AFB1-epoxide (99) and activated STC (96), respectively. For the second biomonitoring system Zebrafish embryo was chosen, because it is widely used as a model in toxicological testing, including embriotoxicology, since its development is very similar to embryogenesis in higher vertebrates. In the newly developed test, S9-bioactivated aflatoxin B1 and sterigmatocystin were microinjected into newly fertilized zebrafish eggs. Mortality, sublethal effects, and DNA strand breaks were registered on the 5th day of the treatment. Generally, when comparing the treatments, activated sterigmatocystin caused the highest mortality and DNA strand breaks in all injected volumes. Sterigmatocystin + S9 treatment caused twice as many breaks (330.6437 ± (16.07319) DNA_sb µg/mg protein) as activated aflatoxin B1 (154.71417 ± (2.91667) DNA_sb µg/mg protein) in the largest injected volume. The representative development dysfunctions were: moderately bent body, not well defined olfactory region, and irregular shaped lower and upper jaws. The scarce information available suggests that AFB1 is a more potent genotoxin than STC. Our findings contradict this assumption, as in the *E. coli* based SOS-Chromotest, the two toxins exert the same genotoxicities. Moreover, according to the newly developed zebrafish monitoring system, STC seemed even more toxic than AFB1. These results raise the demand for more complex biomonitoring systems for mycotoxin risk assessment.

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BACTERIAL CONTAMINATION OF BOTTLED WATER DISPENSERS IN HEALTH INSTITUTIONS

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Bottled water dispensers (BWDs) are present in a wide range of public institutions as an alternative source of drinking water. Previous researches targeting the microbial properties of drinking water derived from BWDs had contradictory results. The aim of this study has been to examine the quality of water provided by BWDs operating in healthcare facilities (hospitals, pharmacies, dental clinics) in Budapest, Hungary. In total 36 BWDs were sampled and for each a questionnaire was filled to assess the physical and chemical parameters, the bacterial contamination of the water and the usage habits of the appliances. Heterotrophic plate counts (HPC) at 22°C and at 37°C were determined according to the MSZ EN ISO 8199: 2005 and the confirmation of the presence of *Pseudomonas aeruginosa* was performed according to the MSZ EN ISO 16266: 2006 standards. All of the physical and chemical characteristics of the water from the examined dispensers have met the relevant national and European limit values. Whereas 51.4% of the HPC values at 22°C and 75.7% at 37°C were higher, than the limit values (10,000 and 2,000 colony forming units (CFU)/mL) recommended by a document about BWDs compiled for the European Commission by Water Coolers Europe. Based on the Hungarian guidance values used in practice for drinking water quality (500 CFU/mL and 100 CFU/mL respectively) 97.3% at 22°C and all of the samples at 37°C were objectionable. *P. aeruginosa* was detected in 11% of the samples indicating insufficient disinfection. HPC values showed positive correlation with the total organic carbon (22°C: $p = 0.0023$, $R = 0.5$; 37°C: $p = 0.0014$, $R = 0.52$) and negative correlation with the number of days remaining until the expiration date of the bottle (22°C: $p = 0.0295$, $R = -0.37$; 37°C: $p = 0.0458$, $R = -0.34$). There was a positive relationship between the results of HPC and the presence of *P. aeruginosa* (22°C: p = 6.3e-15; 37°C: p = 1.2e-14). According to the questionnaire, the month of sampling (22°C: $p = 0.0051$; 37°C: $p = 0.0004$), the type of the institution (22°C: $p = 0.002$; 37°C: $p = 0.0012$) and the frequency of the disinfection process (22°C: $p = 0.0159$; 37°C: $p = 0.0067$) affected the HPC values most significantly. The number of *P. aeruginosa* positively correlated with the concentration of NO_2^- ($p = 0.0016$, $R = 0.5$). HPC and *P. aeruginosa* tests are indicators of the biofilm reduction efficiency of the disinfection process. Bacteria and *P. aeruginosa* in BWDs may pose a health risk to sensitive subpopulations, especially to visitors of health institutions, with clientele including immunocompromised individuals. Minimization of biofilm growth, reduction of the stagnation time of water in the pipe network of the devices, optimization of organic carbon removal and disinfecting processes should be highly essential. The improvement of water quality provided by BWDs is cardinal, especially in health institutions.

INFECTION CONTROL AND RISK FACTORS FOR CARBAPENEMASE-PRODUCING ENTEROBACTERIACEAE. A 5 YEAR (2011 - 2016) CASE-CONTROL STUDY AT AN TERTIARY UNIVERSITY HOSPITAL

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Carbapenemase-producing enterobacteriaceae (CPE) are a major threat for severely ill patients admitted to intensive care units or for patients undergoing bone marrow transplantation. However, only limited data on the epidemiology and on evidence-based infection prevention and control measures are available. The aim of this study was to investigate the epidemiology of patients with CPE, characterizing the CPE isolates by their resistance mechanisms and genetic similarity, to explore risk factors for their acquisition, and to evaluate the effectiveness of the current CPE infection control measures. A retrospective case control study was performed using data from 2011 to 2016 in a 1,900-bed academic hospital in Central Europe. Clinical data of all patients admitted during this period and positive for carriage of CPE were analysed and compared to a matched control group (case-control ratio of 1 : 3). We performed univariate and multivariate statistical analysis to identify risk factors for CPE acquisition. All CPE isolates were genotyped using a standardized repetitive sequence-based PCR. Overall, 75 patients with carriage of carbapenem resistant enterobacteriaceae were included. Carbapenemase-encoding genes were detected in 77.3% (58/75) of carbapenem-resistant enterobacteriaceae. *BlaOXA-48* was found in 34.5% (20/58), *blaKPC* in 29.3% (17/58), *blaNDM* in 20.7% (12/58) and *blaVIM* in 8.6% (5/58) of the isolates. Multivariate analysis revealed 4 risk factors to be independent predictors of CPE carriage: the length of hospital admission >20 days until CPE isolation (AOR: 4.9, 95% CI: 1.4 - 15.5; $p<0.001$), hospital admission within the previous year (AOR: 22.3, 95% CI: 3.9 - 88.4; $p<0.001$), exposure to a healthcare facility in a country with high CPE prevalence 3 months before admission (AOR: 11.8, 95% CI: 2.2 - 63.2; $p<0.01$) and the use of antibiotics longer than 10 days (AOR: 5.2, 95% CI: 1.4 - 35.9; $p<0.05$). Attributable mortality of CPE was 53.3% (8/15). Epidemiological investigation and genotyping revealed confirmed that no outbreaks due to CPE occurred during this period.

Carbapenemases were the main cause of carbapenem resistance in enterobacteriaceae. Hospital admission >20 days, past hospital admission within a year, exposure to a healthcare facility in a country with high CPE prevalence 3 months before admission and antimicrobial treatment >10 days are independent risk factors for CPE carriage and should be used to trigger targeted screening. In this setting, a risk based targeted screening strategy together with strict application of standard infection control measures were effective for prevention of outbreaks.

POSSIBLE ANTIVIRAL EFFECT OF FLAVONOIDS AMONG THEM AMAZONIAN PLANT EXTRACTS ON HIV-1 INFECTED CELLS

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HIV infected cells *in vitro* express increased number of -SH groups. As we earlier demonstrated thyolated pyrimidine nucleotides modify –SH groups and inhibit the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), suggesting that these compounds may interfere with the function of the essential –SH groups of CD4 molecule as well as –SH groups in HIV viral envelope, resulting a certain antiviral effect.It was reported, that some flavonoids found in citrus fruits such as hesperetin (3',5,7-trihydroxy-4'methoxyflavonon), also modulates GAPDH enzyme expression. Other flavonoids extracted from Amazonian tropical plants such as *Phyllantus amarus*, *Dorstenia contrayeva*, *Calophyllum brasiliense* and others may also have inhibitory activity on HIV-1 infection *in vitro*.The

aim of this ongoing studies is to investigate the effect of flavonoids, first hesperetin on HIV-1 infected cell. Materials and methods On uninfected and HIV-1 infected HeLaCD4+β-gal monolayer cells acute and chronic cytotoxic effect and antiproliferative modification of cell cycle of hesperetin have been investigated *in vitro*. Hesperetin was added to the cells on 96 well plates in concentration ranged 10 - 300 μM in triplicate, for 24 hrs and 48 hrs respectively. Cytotoxic effect has been determined quantitatively *in vitro* by XTT based Toxicology Assay Kit (Sigma-Aldrich), which is a spectrophotometric measurement of cell viability based on mitochodrial dehydrogenase activity in living cell. Hesperetin in concentration of 10, 25, 50, 100, 150 μM concentration respectively had no effect on cell proliferation. However at 200 μM concentration cell proliferation was seriously affected, and morphological as well as biochemical signs of cellular cytotoxicity have been observed. Hesperetin in >200 μM dose used at 24 h and 48 h time periods induced cell death and modified cell cycle. This effect may be related to the modification of GAPD expression.
Based upon these pilot studies HIV-1 infectivity assays *in vitro* on HeLaCD4+β-gal cells (MAGI assay), and H9 human lymphoid cell line will be performed in the presence and absence of hesperetin in <200 μM, and other plant extract flavonoids.

NIGELLA SATIVA ESSENTIAL OIL AS POTENTIAL SOURCE OF ANTIMICROBIAL AGENTS AGAINST *STAPHYLOCOCCUS AUREUS*

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The rapid spread of bacterial antimicrobial resistance is a major concern in the treatment of infectious diseases, leading to the need of finding new antimicrobials or adjuvants. Based on preliminary studies *Nigella sativa* essential oil (EO) and its bioactive compounds (thymoquinone, carvacrol, and p-cymene) have a broad antimicrobial spectrum. For this reason, the objective of this study was to investigate the antimicrobial and resistance modifying activity of *N. sativa* EO, thymoquinone (Thq), carvacrol (Car), and p-cymene (P-cy) in reference and methicillin resistant *Staphylococcus aureus* strains. Minimum inhibitory concentrations (MICs) of EO and its bioactive compounds were determined on *S. aureus* strains. The combined effects of antibiotics (tetracycline and ciprofloxacin) and compounds were studied by MIC reduction assay. The antimicrobial efflux inhibiting activity of compounds was assessed by ethidium bromide accumulation assay. The relative expression of *mepA* efflux pump gene was evaluated by real-time reverse transcriptase quantitative polymerase chain reaction. The anti-biofilm activity of compounds was investigated by crystal violet stain, furthermore the membrane disrupting effect was studied by LIVE/DEAD BacLightTM Kit. Both strains of *S. aureus* were susceptible to *N. sativa* EO, Thq and Car. In the MIC reduction assay Thq strengthened the antimicrobial activity of antibiotics in MRSA strain. *N. sativa* EO and Car induced the increase of EtBr accumulated by both *S. aureus* strains. Thq significantly down-regulated the expression of *mepA* after 4 hours of exposure in the reference strain, while in the MRSA the same effect was observed for P-cy. *N. sativa* EO could reduce most effectively (40%) the development of bacterial biofilm of MRSA. Membrane integrity of ATCC strain was disrupted by Car and P-cy, while for the MRSA strain the membrane integrity was disrupted by each compound. *N. sativa* essential oil (EO) and its bioactive compounds could be applied alone or as adjuvants in the management of infections caused by sensitive and methicillin-resistant Staphylococci.

Based on the results it can be concluded that further investigations are required on the EO.

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

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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, Hungary since 2010. Microsatellite markers (SSRs) have been one of the most widely used markers for genotyping eukaryotes, because these markers 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 and foliar lesions and from fungal cultures was analyzed using 11 pre-selected SSR primers [1]. The all microsatellite loci was successfully detected in all *G. bidwellii* samples examined. Our observations are consistent with the existing data in the literature. Our results show that SSRs can be useful for characterisation of grape black rot at molecular level. In additional experiments we are going to involve further wine-countries of Hungary in our sampling. Furthermore we are going to use the QIAxcel Advanced (QIAGEN) fully automates sensitive, high-resolution capillary electrophoresis system and the high-resolution DNA melting analyis for more accurate genotyping results

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[1] Narduzzi-Wicht et al. (2014) Phytopathol Mediterr 53, 470.

MALDI-TOF MS IDENTIFICATION DATABASE COVERING THE COLLECTION HOLDINGS OF DSMZ

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The success of MALDI-TOF MS in identification of bacterial species is impressively documented in the scientific literature and this technique is challenging sequence-based identification methods in terms of speed and costs. The built-up of databases of commercial identification systems is mainly driven by the interests of financially strong target customers. Therefore commercial databases usually suffer from underrepresentation of taxa being merely of academic interest as e.g. extremophiles, environmental and marine isolates. On the other hand, the acquisition of strains by biological resource centres reflects research trends and taxonomic publication activities. Therefore the taxonomic coverage of a MALDI-TOF mass spectral database which represents the holdings of a service culture collection is expected to be of broader and more comprehensive coverage when compared to client-specific databases and to be beneficial for research topics with focus on environment and ecology. A current project at DSMZ is aimed at the generation of a large-scale customized database for automated identification of microorganisms by using the software package of the MALDI BioTyper (Bruker Daltonics). Spectra representing more than 1,000 genera to date have been generated, collected and curated in the course of internal quality control of the DSMZ culture collection during the last 15 years. The database will be used for enhancement of the DSMZ Identification Service for customers from academia and industry.
The selection of reference strains for building up a database as well as its regular quality control and continuous completion require responsible attention because most erroneous identifications are due to wrongly classified reference strains or insufficient coverage by identification databases. A database for identification of bacteria has to be based on type strains as “taxonomic marker entries” in order to provide a systematically correct name, e.g., for searching of scientific literature. As mass spectra may show strain-specific differences, a species should be represented not only by the type strain but also by additional authentic strains. Strains on which database entries are based have to be specified by catalogued collection numbers, have to be characterized polyphasically and their quality control should be regulated by a quality management system. Preferably such reference strains should be available to the scientific community for the purpose of comparison and for judgement of the reliability of identification hits. Approved methods for recording quality spectra, their re-calibration and transformation into database entries for automated identification are presented. Current volume and taxonomic coverage of the steadily growing MALDI-TOF mass spectral database at DSMZ are compared to those of the latest version of the MALDI BioTyper database.

FIRST DETECTION OF POLYOMAVIRUSES IN EUROPEAN BATS

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Bats are important reservoirs and transmitters of viruses and other pathogens. They can carry dangerous human and animal viruses (Ebola, rabies, SARS) and also less important pathogens. Our research focuses on detection, diversity analysis and phylogenetic study of bat polyomaviruses (PyVs). For the detection of unknown PyVs, we used nested PCR to amplify a fragment from the gene of the major capsid protein (viral protein 1, VP1). We examined 89 samples from 26 bat species. Thirteen samples (14.6%), representing 6 vesper bat species (Vespertilionidae) and two horseshoe bat species (Rhinolophidae) proved to be positive. The sequence analyses identified 9 novel sequences,

i.e., putative novel PyVs. Identical sequences were derived from individuals of the same bat species. This is the first demonstration of bat PyVs in Europe. Polyomaviruses show high diversity. According to our phylogenetic study, based on the partial amino acid sequences VP1, some of the newly detected PyVs are closely related to other bat PyVs, whereas some of them seemed to be closer to human and primate PyVs. These findings indicate that host switching events between bats and other mammalian hosts may occur. Because of their potential virus reservoir role, bats are very important subjects for virology research, thus further study of the European bats is expedient. For deeper examination of the novel PyVs, we determined the complete genome sequence of two PyVs, from a vesper bat (serotine bat, *Eptesicus serotinus*) and from a horseshoe bat (lesser horseshoe bat, *Rhinolophus hipposideros*), that seemed to be the most divergent from the previously known bat PyVs. The full-genome characterization of two additional PyVs (Daubenton's bat, Myotis daubentonii and Mediterranean horseshoe bat, *Rhinolophus euryale*) is in progress. Based on relevant taxonomic criteria, these four putative PyVs merit the establishment of four novel PyV species.

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COMPLETE GENOME OF A HISTORICAL *SHIGELLA DYSENTERIAE* SEROTYPE 1 STRAIN, AND COMPARATIVE STUDY OF ITS SHIGA TOXIN HARBORING PROPHAGE REGION

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Shigella dysenteriae was the first identified agent of bacillary dysentery, accounting for a historical outbreaks with high morbidity worldwide, and is still responsible for a considerable number of illnesses annually. The Shiga toxin (Stx) encoded by a defective prophage is the key virulence factor of *S. dysenteriae* type 1 (SD1) strains. In this study, we present the complete genome sequence of historical Hungarian SD1 strain, HNCMB 20080 isolated in 1954, in order to analyze its relation to other sequenced SD1 genomes, as well as to assess the diversity of their Stx-prophages harbored by the SD1 strains which have been sequenced up to date. The genome of HNCMB 20080 is 4,074,091 nt long and contains 4,496 ORFs. Comparative genomic analysis revealed a high degree of uniformity in the SD1 genomes, including the structure of Stx prophage (Spp) regions, which we found to fall in two groups termed SppI and SppII. Out of the 11 available complete SD1 genomes and 10 PacBio genome assemblies, 10 complete genomes and all the PacBio assemblies harboured SppI. Out of the complete genomes carrying SppI, 9 belonged to sequence type (ST) 146, including HNCMB 20080, and one belonged to ST260. The only SppII harboring strain, Sd1617 proved to be of untypeable ST according to the Achtman's scheme. Although in harmony with the literature the Stx1 prophage could not be induced from HNCMB20080, but unexpectedly, the Spp region's instability was observed after 10 and 20 days of consecutive passage in liquid cultures.

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PREVALENCE OF P2-LIKE PROPHAGE GENES IN CYTOLETHAL DISTENDING TOXIN (CDT) PRODUCING AND NON-PRODUCING *ESCHERICHIA COLI* STRAINS ISOLATED FROM HEALTHY CATTLE

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Cytolethal distending toxin (CDT) is a potent virulence factor produced by several Gram-negative pathogenic bacteria, including Shiga toxigenic *Escherichia coli* (STEC) and enterohemorrhagic *E. coli* (EHEC) strains. All the genetic variants (CDT-I to CDT-V) are associated with mobile genetic elements, mostly with prophages. Earlier we demonstrated variability of the P2-like prophage region carrying CDT-V in several CDT-producing atypical, *E. coli* O157 strains of bovine origin. This prompted our current study, in which we screened for the presence of characteristic P2-like phage genes among 27 freshly isolated CDT+ as well as 16 CDT-negative bovine *E. coli* strains. Seventeen PCR reactions targeted all the important structural and regulatory genes of the P2-like prophage. Additionally, two reactions detecting the regions directly adjacent to the cdt gene cluster, and three reactions for typing the regulatory gene C of the P2-like prophages were also used. We found a general tendency that P2-like genes are more frequent in CDT-positive strains, with the latter carrying 7.6 investigated regions on average, while the cdt-negative strains harbored only 3.5 regions on average. Thirty-nine out of 43 strains carried at least one P2-like prohage gene, and 8 prophage regions were exclusively associated with the presence of cdt genes.
Twenty strains carried at least one variant of the regulatory gene C, with 5 strains carrying two variants at the same time. In 5 out of the 27 CDT+ strains the direct association of the *cdt* gene cluster to the T/O integration hotspot of the P2-like prophage was shown. There were also three *cdt*+ strains that did not carry any P2-like prophage genes, suggesting an alternative genetic vector for the *cdt* gene cluster in bovine *E. coli* strains.

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INVESTIGATION OF URACIL-DNA REPAIR IN *STAPHYLOCOCCUS AUREUS*

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The core genome of the biomedically relevant *Staphylococcus aureus* bacterium lacks the usually essential deoxyuracil triphosphate pyrophosphatase (dUTPase) enzyme, which is responsible for preventing uracil incorporation into the genome. In the lack of dUTPase the elevated uracil content of the DNA may overload uracil-DNA repair, resulting in the increase of mutational rate and double strand breaks. Interestingly, the mobile genetic elements of the bacterium that encode antibiotic resistance and/or pathogenicity factors carry dUTPases, or the SaUGI (*S. aureus* Uracil-DNA Glycosylase Inhibitor) protein which protects from overloading uracil-DNA repair. Our aim is to

investigate I) how does *S. aureus* survive without the usually essential dUTPase enzyme II) whether the presence of mobile genetic elements integrated into the genome of the bacterium has any role in counterbalancing the lack of genomic dUTPase III) whether the mobile genetic elements themselves take any advantage of possessing the dUTPase and/or the SaUGI protein. For this purpose, we measured the uracil level and the deoxynucleoside-triphosphate (dNTP) pools of *S. aureus* strains with different genetic background (+/- mobile genetics). Besides, we also introduced the UNG inhibitor SaUGI to an *E. coli – S. aureus* shuttle vector, pRMC2 which allows for the directed and controlled expression of the protein in *S. aureus*. Although we found that all investigated *S. aureus* strains have an elevated uracil-DNA level, we neither could see a remarkable difference in the uracil content of the genomic DNA nor in the dNTP pools of the different strains. However, the directed expression of the SaUGI protein from the pRMC2 plasmid resulted in an even higher uracil-DNA level. These results together indicate that i) the SaUGI protein acts as a uracil DNA repair inhibitor *in vivo*; ii) the SaUGI protein is not expressed in the investigated strains from its integrated, resting mobile genetic element. Based on the present result we assume that *S. aureus* cannot take advantage directly from the dUTPase and the SauGI proteins carried by integrated mobile genetic elements. To answer our further research questions, further investigations are necessary.

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SYNERGISTIC ACTION OF PROTEIN PHOSPHATASE Z1 DELETION AND OXIDATIVE STRESS IN THE OPPORTUNISTIC PATHOGEN *CANDIDA ALBICANS*

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Candida albicans is an opportunistic human pathogen. It constitutes a common component of the normal flora, and has only mild or no symptoms in a heathy individual, but can cause life threatening infections in immunocompromised patients. Nowadays fluconazole, echinocandins, and as a last resort amphotericin B can be used to control candidemia. However, resistance against antifungals is steadily increasing, and the quest for new drug targets become a key question of the development of novel treatments. Since protein phosphorylation and dephosphorylation play a major role in cell signaling pathways and metabolism we selected protein phosphatase Z1 of *C. albicans* (CaPpz1) as a possible target. This fungus specific serine/threonine protein phosphatase has several important physiological roles such as the regulation of cation homeostasis, cell wall biosynthesis, morphological changes, oxidative stress response, and virulence of the pathogen. In the present work we investigated the consequences of CaPpz1 phosphatase deletion under oxidative stress induced by tert-butyl-hydoperoxide (tBOOH). First we confirmed that tBOOH reduced the growth rate and blocked the division of the fungal cells without a dramatic modification of their morphology, viability and vitality; both in the *cappz1* mutant (KO) and the genetically matching QMY23 (WT) strains. In these initial experiments we noted, that the combination of KO with tBOOH treatment reduced growth rate more efficiently than separately. To find a relationship between the global gene

expression and the above observation we carried out a transcriptome analysis by RNA sequencing. The quality of the sequence data was confirmed by PCA and Cluster analysis.
According to the numbers of genes affected and the size of the changes observed, we confirmed the synergism between the phosphatase deletion and oxidative stress. Based on gene ontology enrichment we selected 64 genes for further RT-qPCR validations. We confirmed that the expression of genes coding for cytosolic ribosomal proteins and cell surface proteins were downregulated by tBOOH, while the amounts of mRNAs associated with transport processes, oxidoreductase activity, and RNA processing were upregulated. All of these changes were enhanced in the KO strain. Our transcriptome data were confirmed by a single DNA chip hybridization experiment with one notable exception. The effects of KO alone were quite variable indicating that the relatively instable consequences of the CaPpz1 deletion are stabilized and elevated under oxidative stress.
From this results we can conclude that in the wild type *C. albicans* CaPpz1 protein plays a protective role against oxidative damage, and suggest that the specific inhibition of this phosphatase combined with mild oxidative treatment could be a feasible approach of antifungal therapy.

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MANGANESE SUPEROXIDE DISMUTASE IS INVOLVED IN OXIDATIVE STRESS DEFENSE, RESPIRATION AND APOPTOSIS PREVENTION IN *FUSARIUM VERTICILLIOIDES*

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Both prokaryotic and eukaryotic organisms sense and respond to various types of environmental challenges. One of these challenges is the generation of intracellular reactive oxygen species (ROS) under aerobic conditions. ROS production is a side-effect of mitochondrial respiration and its generation is stimulated by stress. To minimize the cellular damage caused by ROS, all aerobic organisms developed a complex antioxidant defense mechanism. Antioxidant defenses include a number of enzymes, such as catalases, superoxide dismutases and also the gluthatione system. The mitochondrial manganese superoxide dismutase (MnSOD) is responsible for the elimination of superoxide radicals and plays a pivotal role in the preservation of mitochondrial integrity and function. Deletion of the *mnSOD* gene in the baker's yeast, *Saccharomyces cerevisiae* resulted in significantly reduced growth under aerobic conditions; damage of mitochondrial DNA was also observed. The *ΔmnSOD* mutants of *Aspergillus nidulans* showed high sensitivity to menadione and increased sensitivity to apoptotic cell death elicited by the *Penicillium chrysogenum* antifungal protein (PAF). The present study is aimed at the functional characterization of FvmnSOD, the manganese superoxide dismutase of the maize pathogenic fungus, *Fusarium verticillioides*. Deletion of *fvmnSOD* resulted in increased sensitivity to 5 μM menadione on Czapek-Dox agar plates.
In addition, the *fvmnSOD* deficient mutants were more sensitive to the PAF-elicited apoptotic cell death, similarly to the same mutants of *A. nidulans*. The respiration rate of the *ΔfvmnSOD* strain were

higher than that of the wild-type strain. Deletion of *fvmnSOD* had no adverse effect on secondary metabolite production. The mutants showed no decline in sexual fertility and their invasion capability assessed on tomato fruits was not hampered either.

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THE BZIP-TYPE TRANSCRIPTION FACTOR, FVAtfA AFFECTS SECONDARY METABOLITE PRODUCTION AND INVASIVE GROWTH IN *FUSARIUM VERTICILLIOIDES*

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Successful adaptation to changes in either intracellular or extracellular conditions is a universal requirement for all living organisms. The maintenance of cell homeostasis is coupled to the delicate and coordinated regulation of a number of genes, which relies on the activity of various transcription factors including members of the basic leucine zipper (bZIP) transcription factor family. In the fission yeast, *Schizosaccharomyces pombe*, Atf1, a bZIP-type transcription factor regulates the expression of a number of genes involved in sexual sporulation and the adaptation to osmotic and oxidative stress. In *Aspergillus nidulans*, the orthologous transcription factor AtfA plays an important role in osmotic and oxidative stress defense. In *Fusarium graminearum*, deletion of the *fgatf1* gene resulted in higher sensitivity to osmotic stress accompanied with an elevated tolerance to oxidative stress. In addition, the Δ*fgatf1* mutant produced reduced amounts of deoxynivalenol (DON). The aims of this study were to construct and characterize Δ*fvatfA* deletion mutants as well as their restored strains complemented with the wild-type *atfA* gene in the maize pathogen fungus, *Fusarium verticillioides*. Deletion of *fvatfA* resulted in increased sensitivity to menadione, tert-butyl hydroperoxide, H_2O_2 and Congo Red on Czapek-Dox agar plates. Fumonisin production of the *ΔfvatfA* mutants decreased in comparison to the wild type parental strain and the restored strains. Moreover, the *ΔfvatfA* mutant produced reduced amounts of carotenes as compared to the wild type strain. Interestingly, the deletion mutant produced significantly higher amounts of bikaverin, a naphtoquinone pigment with antiprotozoal and antifungal activity. Colonization test on tomato fruits showed that the *fvatfA* gene plays an important role in the invasive growth of F. verticillioides in plant tissues. Deletion of the *fvatfA* gene resulted in female sterility as demonstrated in sexual crosses on carrot agar.

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ISOLATION OF NAPHTHLENE-DEGRADING AND BIOFILM PRODUCING BACTERIA

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous highly toxic and recalcitrant pollutants. They are released into the environment either from natural (e.g. volcano eruption) or anthropogenic (e.g. incomplete combustion) sources. Consequently, elimination of PAHs from the environment is a must. Still nowadays, bioremediation of PAH impacted ecosystems seems to be a sustainable approach. Bacteria able to use aerobically PAHs as carbon and energy source are widespread and have been widely described. However, little is known about PAH biodegradation under oxygen-limited or strictly anoxic conditions. Therefore, in this study we aimed at the aerobic, as well as oxygen-limited enrichment, identification and isolation of PAH-degrading bacteria. During the selective enrichment mineral salts solutions supplemented with naphthalene (1 g/L) as model PAH compound were used. Enrichment cultures with distinct O_2 concentrations (aerobic ~8 mg/L; oxygen-limited ≤0.5 mg/L) were inoculated with a bacterial biofilm sampled from a hydrocarbon contaminated groundwater. Enrichments were done for six consecutive weeks. Population dynamics of the initial biofilm community, which occurred due to the selective enrichment, was followed by terminal restriction fragment length polymorphism (T-RFLP). To determine the precise taxonomic composition of the initial biofilm and of the final aerobic and oxygen-limited enrichments Illumina 16S amplicon sequencing was used. Species level identified isolates were screened for their naphthalene degradation abilities, as well as for their biofilm producing capabilities. As per T-RFLP results no clear difference was observed between the enriched aerobic and oxygen-limited PAH-degrading bacterial communities. Next-generation sequencing supported this observation. By the end of the experiment both enrichments were overwhelmingly dominated by Gammaproteobacteria and at genus level by the facultative anaerobic *Pseudomonas* species. The majority of the obtained isolates also belonged to the genus *Pseudomonas*. Moreover, the obtained pseudomonads showed remarkable naphthalene degradation abilities, as well as prolific biofilm producing capabilities.
These isolates can be used in the future for bioremediation purposes in the development of biofilm based reactive biobarriers for decontamination of PAH-impacted groundwater.

COMPARATIVE ANALYSIS OF MOBILE RESISTOMES OF *ESCHERICHIA COLI* AND *SALMONELLA* INFANTIS FROM BROILERS

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Multiresistant strains of *E. coli* and *Salmonella* Infantis are frequently isolated from broiler chickens. We hypothesised that simultaneous carriage of both bacterial species allows an active intraspecies and intergeneric exchange of mobile resistance elements between *E. coli* and *S.* Infantis in the chicken population. Therefore, we characterized mobile resistomes and genomic diversity of *E. coli* and *S.* Infantis strains from broilers. For this, a total of 90 multiresistant *E. coli* strains (bone marrow, faeces, caecum) as well as 56 broiler and human isolates of *S.* Infantis were compared on the basis of whole genome sequences. Web-based tools ResFinder and PlasmidFinder were used for *in silico* detection of acquired antibiotic resistance genes and typing of plasmids. The clonal relation of *E. coli* and *S.* Infantis strains was determined by multilocus sequence typing (MLST), while core genome

(cg) MLST was performed to reveal the intraclonal genomic diversity of *E. coli* and *S.* Infantis. Antibiotic resistome analysis showed that *E. coli* strains shared a large diversity of resistance genes, with the highest prevalence of genes related to class 1 integrons (*aadA*, *dfr*, *sul*) and plasmids such as aph(3")-Ib, blaTEM-1 and tet(A). The co-existence of multiple plasmids was detected in the majority of strains. Plasmid prevalence and diversity was higher in commensal isolates than in pathogenic ones, and the emerging plasmid-encoded resistance genes *blaCMY-2* and *qnrS* were most prevalent in faecal strains. In contrast to this, broiler and human isolates of *S.* Infantis carried a reduced set of resistance genes and plasmid types. Frequently detected genes such as *aadA1*, *sul1* and *tet(A)* are known to be associated with the predominant multiresistance plasmid pSI54/04 characteristic to the epidemic PFGE clone B2. According to the clonal analysis, *E. coli* strains were allocated to 49 sequence types (STs). Commensal and pathogenic strains were both found in larger clones such as ST10, ST117 and ST162. In comparison to certain individual clones that were associated with an increased prevalence of resistance genes, larger clones seemed to be less important as reservoirs for multiresistance. All *S.* Infantis strains were assigned to ST32.
The cgMLST results showed that the dominant *E. coli* phylogroups A and B1 and the epidemic clone B2 of *S.* Infantis exhibit a high level of genomic flexibility. Our findings indicate that so far only a few plasmid types and genes of mobile resistomes of *E. coli* and *S.* Infantis could be considered as potentially exchangeable between both species. The interplay between IncI and IncX plasmid types could have the most important contribution to the microevolution of multiresistance in the interaction between *E. coli* and *S.* Infantis.

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MICROBIAL COMMUNITY ANALYSIS OF CRUDE OIL/GASOLINE MIXTURE AMENDED AEROBIC AND MICROAEROBIC ENRICHMENT CULTURES BY A MULTI-OMICS APPROACH

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Saturated hydrocarbons (alkanes) are among the most frequent groundwater contaminants in Hungary, since quantitatively these are the most abundant components of all petroleum hydrocarbons. The most rapid biodegradation of these compounds can be observed under aerobic conditions. Nevertheless, in subsurface ecosystems the availability of oxygen is often restricted. However, only a handful of studies have investigated alkane-degradation under microaerobic conditions or alkane-degrading microbial communities in microaerobic environments. The hypothesis of the present study was that different alkane-degrading microbial communities can be observed in aliphatic hydrocarbon-contaminated environments under aerobic and microaerobic conditions. To investigate this hypothesis, an enrichment experiment was performed by establishing aerobic and microaerobic bacterial enrichments amended with a crude oil – gasoline mixture. Based on the results of 16S rDNA amplicon sequencing it was found, that the representative aerobic enrichment community was dominated by Gammaproteobacteria (64.5%) with high abundance of

Betaproteobacteriales (36.5%), followed by Alphaproteobacteria (8.7%), Actinobacteria (5.6%) and Candidatus Saccharibacteria (4.5%). The most abundant alkane monooxygenase (*alkB*) genotypes in this enrichment could be linked to an uncultured bacterium and to members of the genus *Rhodococcus*. Genome-resolved metagenomics revealed that the most dominant *alkB* genotype belonged to a yet uncultivated Gammaproteobacterium, having a ~3 Mbp genome, and probably it is a member of a new family of Pseudomonadales.
In the microaerobic enrichment Gammaproteobacteria (99%) overwhelmingly dominated the microbial community with high abundance of the genera *Acinetobacter* (66.3%), *Pseudomonas* (11%) and *Acidovorax* (11%). Under microaerobic conditions, the vast majority of *alkB* gene sequences could be linked to *Pseudomonas veronii*. Consequently, the present study provides new evidence that the known diversity of alkane-degrading bacteria is still incomplete and that oxygen-limited conditions can facilitate the high abundance of *Acinetobacter* species in aliphatic hydrocarbon contaminated subsurface environments (e.g. oil reservoirs).

ALLERGY AND THE GUT MICROBIAL FLORA

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The metabolism and products of gut microbial flora profoundly influences the development and activity of the human immune system. Abnormal epigenetic regulation of the microbiota might sensitize to immunological disorders. During pregnancy, short chain fatty acids produced by intestinal bacteria contribute to the maturation of gut wall. Colonization of the intestine of newborn children following vaginal delivery result in the normal biodiversity. Cesarean section, abandoned breast feeding and using formulas, antibiotic treatment during pregnancy and infancy result in an altered microbial flora of the child. Consequently, bacterial diversity remains narrow, production of short chain fatty acids through fermentation and that of antimicrobial peptides are less, Th1, Treg, IgA stimulation is weaker. Altogether, the mucosal immune system and the mucosal homeostasis (Peyer patches, MALT, GALT) underdeveloped. Dietary trends usually are harmful. Life style of the pregnant female in the vicinity of farm animals can beneficially affect the gut microbiota. Establishment of immunotolerance in the gut and respiratory tract depends on the commensal and symbiotic bacteria. Asthma and several allergic conditions, especially in the developed countries, have been detected more frequently, and at young age.
Not only the altered species diversity, but modified composition of microbe variants in the same genus sensitize to allergy, mainly involving *Bacteroides* and Bifidobacteria. In school age children, dominance of Firmicutes species, *Bacteroides fragilis*, *E. coli*, *Clostridium difficile*, *Bifidobacterium catenulatum*, *B. longum*, *B. bifidum* have been described. On the contrary, *Bifidobacterium adolescentis* and lactobacilli were found in reduced number. During acute symptoms of allergic reactions, *Bacteroides adolescentis* was abundant, but the quantity of *Akkermansia municiphila*, *Faecalibacterium prausnitzi*, *Bacteroides catenulatum* and *Staphylococcus aureus* was diminished. *Haemophilus* spp., *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, yeast and mold infections also alter the normal bacterial flora contributing to allergy. Long term antibiotic treatment (e.g. cephalosporins, cefoperazon, macrolides, amoxiciliin) contribute to the exacerbation of asthma, atopic dermatitis, rheumatoid arthritis, and other allergic symptoms. Reconstruction of the gut microbiota has become the first line treatment modality: carefully selected probiotics, back-to nature lifestyle, faeces transplantation. Education is a major mean of risk reduction.

THE FELINE ADENOVIRUS ISOLATE

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Adenoviruses transactivate HIV and induce lethal opportunistic infection in AIDS patients. The feline AIDS model is suitable to study adenovirus - retrovirus intecaction, but no adenovirus infection has been revealed in cats. We conducted studies on adenovirus epidemiology, virus carriage, isolation of a replication competent virus and, basic characterization of the isolate. No diagnostic tools, guidelines are available on feline adenovirus studies. Adenovirus serology was conducted by a home-made ELISA containing human adenovirus type 1 (HAdV-1) antigen. Sera of free-roaming and experimental SPF cats were obtained from several European countries and the USA. Depending on geographical regions, 9.8 - 20.3% of European cats, 26% of American animals possessed detectable anti-adenovirus antibodies. Four of five FIV infected, but none of mock-infected experimental cats harboured adenovirus antibodies. Serial passage of FIV among experimental cats could transmit adenovirus infection. Adenovirus shedding in pharyngeal and rectal specimens of seronegative and seropositive cats were screened by hexon-specific consensus PCR. In one of the seropositive free-roaming cats with transient hepatic failure both types of specimens taken at one year interval were DNA positve. Beside showing virus shedding, it excludes cross-contamination from human sources. The first fecal specimen was used to infect HeLa cells, and following two blind passages, one culture showed cytopathic effect typical for adenovirus. The isolate was verfied by electron microscopy, direct immunofluorescence by using anti-adenovirus monoclonal antibody and hexon-specific PCR. The fiber and hexon genes of the isolate were sequenced and compared to a recent European HAdV-1 isolate. Sequence analysis shows that the feline adenovirus isolate is related to HAdV-1. Further phylogenetic analysis revealed that this isolate clusters with members of species Human mastadenovirus C, especially with the prototype, and several Asian isolates of HAdV-1. In the fiber region, seven alterations were found, one of them is an RGS motif in the alanine-rich region of the knob. Hexon sequence alterations are silent. Surprisingly, the feline isolate replicates in several cell cultures of mammalian origin, except in cells lacking expression of the Coxsackie-adenovirus receptor or alpha-v-beta-3 or -5 integrin molecules. A recent serosurvey in American cats by using cells infected with the isolate, resulted in 80% seropositivity. Difference in the two serological assays suggests that the isolate has an antigenic stucture of the fiber different from HAdV-1.
The presence of virus DNA identical to our isolate has been detected in Japan and Brazil. As a new biological entity, the isolate is named feline adenovirus (FeAdV). Further studies are warranted to reveal its human and feline pathogenesis, especially in immuncompromised hosts.

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INACTIVATED HHV-6B INDUCES CYTOKINE PRODUCTION DIFFERENT FROM THE EFFECT OF INFECTIOUS VIRUS

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Human herpes virus (HHV)-6A, HHV-6B and HHV-7 infect CD4 cells but bind different cell surface receptors consequently induce different messenger RNAs and cytokine/chemokine release. Of the three Roseoloviruses, HHV-6B seems to elicit the most severe immunocompromised conditions in both primary and reactivated infections. The cytokine production by Roseoloviruses have already partially described both *in vivo* and *in vitro*. Beside infectious particles, herpes viruses produce high quantity of non-infectious particles and extracellular vesicles. The latter can deliver complete and incomplete virus particles, regulatory polypeptides and nucleic acids to other target cells. These affect the biology of immune cells. This aspect has not been studied yet. Our aim was to investigate the effect of non-infectious particles on the production of key cytokines *in vitro*. We used heat- and UV-inactivated virus preparations to treat MOLT-3 cells. At different time intervals, aliquots of mock-infected, infected and inactivated-virus-treated cells as well as supernatants were collected during the whole infection cycle. Production of anti-inflammatory and pro-inflammatory cytokines was quantitated by protein release applying commercial ELISA and the synthesis of mRNA by commercial RT-PCR kits. Through the whole infection cycle, cytokine production by inactivated viruses significantly differs from the mock-infected and infected cultures. HHV-6B infection drastically altered the release of several cytokines, especially during the first four days post-infection. UV inactivated virus can enter cells, thus gene expression is limited, but proteins of the infecting virus can cause immunomodulatory effects. In the early phase of virus absorption this can be seen on the protein and mRNA levels of the pro-inflammatory IFN-γ.
On the other hand, in the late phase of infection anti-inflammatory TNF-β1 cytokine level increases parallel to the expression of the pro-inflammatory GM-CSF. Heat-inactivation damages the structure and enzymes of the viruses. In the early phase of heat-inactivated virus absorption the pro-inflammatory IL-12 level is decreased, meanwhile pro-inflammatory IL-1β gene expression is increased to compensate the loss by the heat-inactivation. In the late phase of treatment with heat-inactivated virus, anti-inflammatory IL-2 cytokine level decreases along with the expression of the anti-inflammatory IL-10 and IL-4. Our results suggest that cytokines elicited by non-replicating particles might regulate the immune system. Further studies are warranted to investigate the regulatory mechanism by single gene expression on the interleukin production.

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FUNGAL CONTAMINATION OF BOTTLED WATER DISPENSERS IN HEALTH INSTITUTIONS

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Bottled water dispensers (BWDs) are present in a wide range of public institutions as an alternative source of drinking water. It is of great importance to provide adequate quality drinking water, especially in health institutions. Previous researches targeting the microbial properties of drinking water derived from BWSs had contradictory results. The aim of this study was to examine the quality of water provided BWDs operating in healthcare facilities (hospitals, pharmacies, dental clinics) in Budapest, Hungary. In total 36 BWDs were sampled and for each a questionnaire was filled to assess

the physical and chemical parameters, the fungal and bacterial contamination of the water and the usage habits of the appliances. To assess the fungal contamination 100 - 100 mL of water samples were concentrated onto membrane filters. Then these filters were placed onto malt extract agar containing 2% chloramphenicol. The samples were incubated at 25°C for 5 days. After the incubation the number of colonies were counted to determine their concentration (Colony forming units (CFU)/100 mL). Colonies were classified based on their morphology and groups reaching 4 CFU/100 mL concentration have been isolated and identified by their micromorphology. According to our results all of the physical and chemical characteristics of the water from the examined BWDs have met the relevant national and European limit values. However, 86.8% of all BWDs were contaminated by different kinds of filamentous fungi and yeasts. The common fungal genera were: *Cladosporium* spp. (41.2%), *Acremonium* spp. (19.1%), *Penicillium* spp. (16.2%), *Rhodotorula* spp. (14.7%), *Aspergillus* spp. (8.8%), *Rhizopus* spp. (8.8%), *Geotrichum* spp. (2.9%), *Beauveria* spp. (2.9%). Total concentration of fungi had significant positive correlation with the number of days spent since the last disinfection process ($p = 0.0064$, $R = 0.62$). Filamentous fungi positively correlated with the number of days remaining until the expiration date of the bottled water ($p = 0.0037$, $R = 0.36$), possibly because of competition with heterotrophic bacteria, as the negative correlation suggested (22°C: $p = 0.0078$, $R = -0.61$; 37°C: $p = 0.0268$ $R = -0.46$). According to the questionnaire the brand of the bottles affected significantly the concentration of fungi ($p = 0.0347$). Several of the detected fungal species are potentially pathogenic for patients with immunodeficiency deseases (e.g. cystic fibrosis) and people belonging to sensitive age groups. Therefore the presence of such fungi in BWDs may pose a health risk to visitors of healthcare institutions, especially to patients with oral lesions in dental surgeries. Steps should be taken towards the minimization of fungal contamination. Frequent disinfection and change of the bottles are recommended.

EFFECTS OF ARTIFICIALLY MYCOTOXIN-CONTAMINATED COMPOST ON BROWN BUTTON MUSHROOM GROWTH AND ON COMPOST MICROBIAL COMMUNITY COMPOSITION

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Button mushroom (*Agaricus bisporus*) is produced on a composted substrate, in which process compost microbiota has a fundamental role. There is an actual risk of potential mycotoxin contamination of the substrate raw materials (wheat and barley straw, sunflower husk, poultry and horse manure). However, there is limited knowledge about the effects of mycotoxins on *Agaricus bisporus* and about the ability of the compost microorganisms to produce, degrade or neutralize these mycotoxins. In the present work artificially mycotoxin-contaminated compost was used to monitor its effect on mushroom yield and on changes of the total and active microbial community during a small-scale brown button mushroom production. Phase II compost from a local large-scale mushroom producer was supplemented with aflatoxin B1 (0.02 mg/kg), fumonisin B1 (0.81 mg/kg) and toxin T2 (1 mg/kg), and untreated compost served as a control. Composts were spawned and incubated for colonization and fruiting body production. The mushroom yield was registered. Samples were taken at the beginning just after mycotoxin treatment, during first and second flushes. To investigate total and active bacteria community DNA and RNA were isolated from the samples. To track the transformation of bacterial communities 16S rRNA gene based terminal restriction fragment length polymorphism (T-RFLP) was used. Mushroom yield of fumonisin B1 contaminated compost was

significantly lower compared to the control, whereas other treatments did not reduce significantly mushroom yield. Furthermore, it should be noted that many fumonisin B1 containing compost became infected with moulds, which could have affected the mentioned yield decrease. Microbial community structure showed definite temporal changes, whereas treatment effects were smaller. In the future microbial community members (Bacteria, Archaea and Fungi) will be identified via next generation sequencing and also effects of further mycotoxins (e.g. deoxynivalenol) will be tested.

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POPULATION SNAPSHOT OF THE CTX-M-PRODUCING *ESCHERICHIA COLI* ISOLATED FROM HAEMOCULTURE IN A HUNGARIAN HOSPITAL

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Recently the burden of disease caused by third-generation cephalosporin-resistant *Escherichia coli* increased the most, in terms of number of infections and number of deaths in Europe. Its global spread is associated with the C/H30 clade of the sequence type 131 (ST131) high risk clone. Of the C/H30 clade, the C1-M27 subclade with *blaCTX-M-27* was recently also discovered as an international clade, beside the worldwide disseminated C2/H30Rx clade with *blaCTX-M-15*. In 2015 25% (13/52) of invasive *E. coli* isolates submitted to the National Public Health Center belonged to the C1-M27 subclade, while in 2018 this proportion increased to 47.8% (33/69). The aim of our study was to i) determine the prevalence of extended-spectrum β-lactamase (ESBL)-producing *E. coli* collected, especially of ST131, from a Hungarian hospital in a short time period; ii) perform genomic typing of identified ESBL-producing isolates in order to characterize their genetic background. Between October 2018 - November 2018, all ESBL-producing *E. coli* isolates, isolated from blood samples, were collected from Central Hospital of Southern Pest, where the antimicrobial susceptibility testing was performed. The possible clonal relationships were investigated by core genome (cg)MLST (SeqSphere+ (Ridom)) using whole-genome sequencing (WGS) data of all isolates obtained from Illumina 251-bp paired-end sequencing. From WGS data acquired antimicrobial resistance and virulence genes were retrieved using ResFinder3.1 and VirulenceFinder2.0 online tools. 24% (6/25) of the total isolates proved to be ESBL-producing *E. coli* in the study period. According to the WGS: five *E. coli* isolates belonged to the ST131 clone: two to C1-M27 and three to C2/H30Rx with at least 75 allele distance between two groups. The remained one belonged to the minor ST1193 clone with *blaCTX-M-27*. Acording to cgMLST all three ST131 *E. coli* isolates producing BlaCTX-M-15 showed relatively close clustering (≤6 allele differences), suggested an undetected nosocomial outbreak. The C1-M27 isolates producing BlaCTX-M-27 differed at 35 alleles from each other, thus suggested lack of epidemiological link. The ST1193 isolates showed distant clustering to any others (≥2,224 allele differences). Both C2/H30Rx and C1-M27 isolates harboured almost identical virulence and resistance genes. However, all six isolates had tool of virulence genes which enable the bacteria to cause extraintestinal infections, like the adhesion associated genes (*sfa*, *mat*, *crl*), siderophores (*irp*, *esp*, *chu*) and high invasion associated gene *ibe*.

Considering the limitations of this study, this population snapshot analysis highlighted the dominance of the two major CTX-M-producing *E. coli* clones regarding invasive infections in Hungary: a currently rising CTX-M-27-producing *E. coli* ST131 C1-M27 subclade and the dominant CTX-M-15 producing ST131. Only the C2/H30Rx isolates showed close genetic relationship.

IN VITRO CYTOTOXIC EFFECT OF *PENICILLIUM CHYSOGENUM* ANTIFUNGAL PROTEIN, ITS DE NOVO RATIONAL DESIGNED PROTEIN VARIANT AND PEPTIDE DERIVATIVE ON MAMMALIAN CELLS AND PLANTS

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Nowadays, the world agriculture suffers significant crop losses due to infection caused by pesticide-resistant pre- and post-harvest plant pathogenic fungi. This problem if further exacerbated by the fact that some of these fungi contaminate feed and food crops with mycotoxins posing a severe risk to animal and human health. Therefore, there is a substantial demand in the agriculture for new and safely applicable antifungal compounds. The natural antifungal peptides and proteins provide feasible bases for this challenge. Several studies reported that the evolutionary conserved, so-called γ-core motif (GXC-X[3-9]-C) in their primary structure is responsible for the antifungal effect. We already observed that this peptide motif can be found in the extracellular, cationic and cysteine-rich antifungal protein PAF from *Penicillium chrysogenum*, and rational design of the γ-core (PAFγopt) modulates the antifungal efficacy and spectrum of PAF against plant pathogenic filamentous fungi. In PAFγopt protein variant specific amino acids in the γ-core motif were substituted to elevate the positive net charge and the hydrophilicity of PAF. For the agricultural use of these proteins it is essential to investigate their cytotoxic effect on mammalian cell lines and plants. In the present study we demonstrated that PAF and PAFγopt do not show cytotoxic effect on human keratinocytes, intestinal epithelial cells and leukocytes above their minimal inhibitory concentrations against plant pathogenic filamentous fungi. However, a rational designed synthetic γ-core peptide derivative of PAFγopt (Pγopt) influenced the viability of these cells.

In contrast to this, neither the PAF and PAFγopt proteins, nor the Pγopt peptide were toxic to *Medicago truncatula* seedlings. These promising results suggest the safe applicability of PAF and PAFγopt as biopesticides or food preservatives in the agriculture and food-industry.

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POTENTIAL ROLE OF THE EVOLUTIONARY CONSERVED Γ-CORE MOTIF IN THE EFFICACY AND STRUCTURAL STABILITY OF *NEOSARTORYA* (*ASPERGILLUS*) *FISCHERI* ANTIFUNGAL PROTEINS

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The evolutionary conserved γ-core motif (GXC-X[3-9]-C) is present in almost all small and cysteine-rich natural antimicrobial peptides and proteins, where it plays an important role for antimicrobial activity or structural stability. Previously, we demonstrated the improved efficacy of the *Penicillium chrysogenum* antifungal protein PAF variant PAFγopt, in which specific amino acids in the γ-core motif were substituted to elevate the positive net charge and the hydrophilicity of the protein. The γ-core modification did not influence dramatically the secondary and tertiary structure of PAFγopt. Based on these promising results, the present work aimed at elevating the antifungal efficacy of the *Neosartorya* (*Aspergillus*) *fischeri* antifungal proteins NFAP and NFAP2 by changing the negatively charged (NFAP) or neutral (NFAP2) γ-core motif into a de novo designed motif with increased positive net charge and hydrophilicity. A *P. chrysogenum*-based heterologous expression system and chemical synthesis were applied to generate recombinant or synthetic variants of NFAP (NFAPγopt) and NFAP2 (NFAP2γopt) with modified γ-core motif. Both protein variants were degraded during the recombinant production, which let us assume that the modification renders the protein structure accessible for extracellular proteases. However, electronic circular dichroism spectroscopy demonstrated that synthetic NFAPγopt and NFAP2γopt had unordered structures compared to the respective wild-type proteins, which showed a β-pleated conformation. The γ-core modification improved the antifungal efficacy of synthetic NFAPγopt against the plant pathogenic fungus *Cladosporium herbarum* compared the wild-type protein. In contrast, a strong decrease in antifungal activity was observed with NFAP2γopt against the human pathogen *Candida albicans* in comparison to the wild-type NFAP2. These results suggest a supporting role of the negatively charged or neutral γ-core motif of NFAP and NFAP2, respectively, in correct protein folding.

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MICROBIAL COMMUNITY CHARACTERIZATION OF LOW NUTRIENT CONTENT AQUATIC HABITATS - A CULTIVATION BASED APPROACH

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Oligotrophic microorganisms are adapted to grow and multiply in low nutrient content environments due to their ability to sustain life in such extreme conditions. During the present study, we determined the number of bacteria after DAPI staining by epifluorescent microscopy and to reveal the cultivable diversity of bacteria newly developed oligotrophic media were used by direct spreading and spreading after enrichment from the following sampling sites: Tatabánya, Dandár, Szentendre, Szent Flórián and Ciprián. Bacteria were isolated randomly then the isolates were grouped by MALDI-TOF MS allowing us to obtain different representative groups of the different samples making the identification of these isolates by sequencing their 16S rRNA genes.The microscopic cell counts showed of 1.33 x 10^5 cells/mL in Tatabánya (ground water), 5.58 x 10^5 cells/mL in Dandár (well water), 1.79 x 10^4 cells/mL in Szentendre (well water), 1.92 x 10^4 cells/mL in Szent Flórián and 1.35 x 10^6 cells/mL in Ciprián (well water). However only 0.70 x 10^2 CFU/mL (using agar) and 2.55 x 10^2 CFU/mL (using gelrite) were obtained in Tatabánya, 3.60 x 10^2 CFU/mL (using agar) and 10^2 CFU/mL (using gerlite) in Dandár, 1.6 x 10^4 CFU/mL (using agar) and 1.51 x 10^4 CFU/mL (using gelrite) in Ciprián, 6 x 10^2 CFU/mL (using agar) and 7.25 x 10^2 CFU/mL (using gelrite) in Szentendre and no reliable number of colonies in case of Szent Flórián sample: the microscopic cell counts were at least 2 magnitudes higher than CFU values in the counted cases due to the great plate anomaly. Regarding the identification results of the cultivated bacteria the members of the family Microbacteriaceae (*M. tumbae*, *M. keratanolyticum*, *M. paraoxydans*, *M. oxydans*, and *M. album*) were the most abundant among the samples (present in Szentendre, Tatabánya and Szent Flórián), they are widely distributed in various terrestrial and aquatic ecosystems and reported to sustain life in low nutrients containing environments, they also embraces psychrophilic bacteria, common characteristic features in the three samples. The most abundant taxa in the groundwater sample belonged to the genera *Microbacterium*, *Pseudoxanthomonas* and *Sphingomonas* among the isolated microorganisms. We could also detect that the genus *Sphingopyxis* was represented by most species (*S. fribergensis*, *S. chilensis* and *S. solisilvae*), commonly isolated from soils, freshwater and marine habitats - many of them are facultatively chemolitotrophs, often produce H_2 during their metabolic processes. These taxa can be responsible for degradation of xenobiotic compounds and the catabolism of recalcitrant molecules an important capability for the use of carbon and energy in the oligotrophic environments. Genus *Sphingomonas* is characteristic in oligotrophic environments.
Among the different isolates, some showed low similarity values of their 16S rRNA genes which present them as a new bacterial taxon (*Paenibacillus* sp., *Dyadobacter* sp., *Brevibacillus* sp.).

FORMATION OF NOVEL BIO-ANODE BY IMMOBILIZATION OF *SHEWANELLA XIAMENENSIS* IN POLYMERS – BACTERIA CELLULOSE COMPOSITES

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The microbial fuel cell (MFC) is a renewable energy source that can convert organic wastes into electricity. *Shewanella* species facultative anaerobic bacteria are reported to be able to produce extracellular electrons and transfer them onto electrode, thus these species can act as biocatalysts in

MFC systems. Conducting polymers – cellulose composites with nanostructure have received growing interest in recent years. This material has largely potential applications such as batteries, sensors and electrical devices. Cellulose is a natural material due to its unique characteristics such as renewability, biodegradability and biocompatibility. Bacteria cellulose (BC), a special kind of cellulose, was produced by fermentation of bacteria such as *Acetobacter xylinum*, *Gluconacetobacter xylinum*. In addition, BC can be attractive insulating polymer matrix for the preparation of conducting nanocomposite. In this study, free-standing films of BC and polyaniline (PANI) composites with immobilization of *Shewanella xiamenensis* DSMZ 22215 cell was investigated as a bio-anode in MFCs. Bacteria cellulose/PANI (BC/PANI) nanocomposites were prepares through the in situ aniline oxidative polymerization by using ammonium peroxide sulfate as oxidant. Increase of polyaniline concentration resulted an increase in the conductivity in BC/PANI. The conductivity of BC/PANI with 0.1 mol/L and 0.2 mol/L of aniline were 1.5-fold and 2.5-fold higher, respectively
In MFCs, the maximum power density peaked 12.7 W/m^3 with BC/PANI 0.2 mol/L aniline, compared with 3.6 W/m^3 with BC without PANI. These results are promising for development of an improved MFC with natural material for anode that have higher power density.

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SECONDARY METABOLITE BIOSYNTHESIS IN *ASPERGILLUS NIGER*: CONSEQUENCES OF OVEREXPRESSION OF TRANSCRIPTION REGULATOR GENES

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Filamentous fungi are a rich source of bioactive secondary metabolites (SMs). In fungi, genes involved in the biosynthesis of SMs are typically co-localized in the genome and they are referred to as SM gene clusters. These SM gene clusters generally consist of a “backbone” gene and multiple “tailoring” genes. The backbone genes encode enzymes including polyketide synthases, non-ribosomal peptide synthetases, polyketide/nonribosomal peptide hybrid enzymes, dimethylallyl tryptophan synthases, terpene cyclases, and fatty acid synthases. The backbone enzymes generate the core of a particular set of SM compounds, serving as a scaffold for further modifications by tailoring enzymes. Often co-localized in a SM gene cluster are a transporter gene thought to be involved in the export of the SM and a gene encoding transcriptional regulator presumed to facilitate the transcription of the entire cluster and start the process of SM biosynthesis. Manual curation of the *Aspergillus niger* strain NRRL 3 genome revealed 85 gene clusters predicted to be involved in the biosynthesis of SMs. Genes encoding fungal-specific transcription factors are found to locate within 40 of these SM gene clusters. We cloned and overexpressed these 49 transcription factor genes by integrating them into the glucoamylase gene locus to replace the glucoamylase coding region such that the transcription factor genes are under the control of the strong glucoamylase promoter. Extracellular medium of the overexpression strains were analyzed for the overproduction of SMs by mass spectrometry and nuclear magnetic resonance. Global gene expression of the overexpression strains were analyzed by RNA-Seq. Preliminary results from overexpression of transcription regulator genes showed the following: 1) in a few simple cases, the co-localized genes in the clusters with over-production of SMs; 2) some but not all co-localized genes in the clusters are upregulated with low levels of SMs produced; 3) no upregulation of clustered genes and no production of SMs; 4)

ectopic clusters are up-regulated; and 5) overproduction of SMs involves both in-cluster genes and off-clustered genes.

THE IMPACT OF *CANDIDA ALBICANS* AND *CANDIDA PARAPSILOSIS* ON ORAL SQUAMOUS CELL CARCINOMA

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A large number of commensal microbial species reside in the human body that have co-evolved with the human genome and adopted to the host immune system. However, defects in regulatory processes or alterations in the composition of the microbiota can lead to various diseases, including cancer. A previous study has shown that the colonisation of *Candida* cells is significantly higher in patients with oral squamous cell carcinoma (OSCC) compared to healthy individuals. Our hypothesis is that the dysbiotic microbiota has tumor promoting effects, and the microbes are able to promote the metastatic activity of the cancerous cells. In order to investigate the effects of yeast cells on the metastatic activity of the cancerous cells we used a metastatic (HO-1-N-1) and a non metastatic (HSC-2) OSCC cell line. Cell migration, matrix metalloproteinase (MMP), proliferation activity and 3D tumor spheroid formation of HSC-2 and HO-1-N-1 OSCC cells were investigated after fungal stimuli. The migration capacity of HO-1-N-1 cells was significantly higher if we treated the cells with *Candida* cells compared to the untreated samples. Prominent MMP activity and larger spheroid formation were detected after 24 h pre-incubation with *C. albicans*.
Both cell lines showed increased proliferation activity upon treatments, which clearly indicates that the presence of fungi can accelerate cancer cell proliferation. Next, HSC-2 cells were injected to the tongue of SCID mice. We could detect faster tumor growth if we pre-treated the tumor cells with *Candida parapsilosis* before the injection. To investigate the possible molecular mechanisms underlying the increased metastatic activity of OSCC cells after fungal exposure we analysed the gene expression of more than 600 genes related to tumors after fnugal stimuli. Our data suggest that heat-killed *Candida* cells are able to change around 40 genes involved in tumor processes.

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STUDY ON RESPONSE SURFACE METHODOLOGY (RSM) OF ALCOHOL FERMENTATION FROM APPLE JUICE BY *SACCHAROMYCES CEREVISIAE*

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Pálinka is a traditional Hungarian spirit produced exclusively by the alcoholic fermentation and distillation from native fruits. In Pálinka production, one of the most important processes affecting the quality and yield of spirits is the fermentation. Therefore, in this study, the fermentation process

from apple juice concentrate and *Saccharomyces cerevisiae* by using Response surface methodology (RSM) coupled with the central composite rotatable design was investigated to optimize fermentation conditions. Based on preliminary experiments, three variables in a defined range of pH (3.0 - 4.5), sugar concentration (21 - 30°Brix) and temperature (25 - 35°C) were selected. After 8 fermentation days, fermented apple fruit reached an alcohol content of 12.7% v.v with plentiful and characteristic aroma compounds such as 1-propanol, 2-butanol, ethyl acetate, n-butyl alcohol, 3-methyl 1-butanol and 3-methyl 1-butanol at an optimized temperature of 25.1◦C, pH of 4, and sugar concentration of 30°Brix. In addition, the model showed all significant p-values for the interaction of variance (<0.05), Q-square = 0.993 and R-square = 0.999. These results provided important information in serving the basic to develop standard Pálinka production from apple.

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THE EXAMINATION OF THE INTERACTION BETWEEN *CANDIDA ALBICANS* AND ORAL SQUAMOUS CELL CARCINOMA CELL LINES ON THE LEVEL OF EXTRACELLULAR VESICLES

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A previous study of our laboratory has shown that the colonisation and diversity of yeast cells was significantly higher in the patient's mouth diagnosed with oral squamous cell carcinoma (OSCC) compared to the healthy individuals. Furthermore, in the tumorous mouth the colonisation of yeast cells was higher on the surface of the tumor compared to the healthy surface. We are investigating the interaction between *Candida* and tumor cells which can not only occur directly – through different receptors – but also indirectly on the level of extracellular vesicles (EV), which is recently poorly understood. Our aim is to examine the interaction between *Candida albicans* and oral squamous cell carcinoma cell lines on the level of extracellular vesicles. In order to investigate the interaction between OSCC and *Candida* we used HSC-2 and HO-1-N-1 cell lines and *Candida albicans* SC5314 strain. We successfully optimized the fungal EV isolation protocol from liquid and solid media. The caracterisation of the EVs by Transmission Electron Microscopy and Dynamic Light Scattering showed round shaped particles with diameters between 50 and 150nm. We examined the effects of EVs released by *C. albicans* and heat killed *C. albicans* on the metabolic activity and cytotoxicity of the tumor cells by MTT, LDH assay and Fluorescence Activated Cell Sorting technique. The EVs significantly reduced, the heat killed *C. albicans* significantly increased the metabolic activity of the tumor cells and did not cause apoptosis or necrosis. Next, we examined the effect of exosomes released by HSC-2 and HO-1-N-1 cells on the growth capacity of *C. albicans* in YPD and LCM media by coincubation experiments. The tumor exosomes significantly reduced the CFU of *Candida* cells in YPD media, in contrast to this in LCM media the exosomes significantly increased the number of colonies. We investigated the possible interaction occurring between the EVs/exosomes and *Candida*/tumor cells by fluorescence microscopy.
Our data suggest colocalization and interaction between the interaction partners.

FUNGAL POPULATION OF ROTTED WALNUTS AND THEIR ANTIFUNGAL SENSITIVITY

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Botryospaheriaceae family and *Diaporthe* genus and are responsible for several plant diseases, of which walnut blight became widespread in Hungary in the last years. The purpose of our study was to monitor the infection of three orchards in North-East Hungary and test some possible protection methods on two *Diaporthe*, two Botryosphaeriaceae strains. Four commercial pesticide formulation were tested, which active ingredients were cyprodinil, fludioxonil, fluopyram, tebuconazole and trifloxystrobin. *In vitro* antifungal sensitivity was assessed determining mycelial growth inhibition on medium containing different fungicides. Biocontrol agents are frequently applied recently, which can complete or substitute chemicals. *Epicoccum nigrum* is well-known for its antagonistic effect against pathogen fungi, this species was studied by dual culture against the causal agents. *Diaporthe* spp. were present in most of the collected symptomatic walnuts, but samples colonized by Botryospaheriaceae were also detected. The isolates were identified on the base of ITS region as *Botryosphaeria dothidea* and *Diaporthe eres*. There were not differences in fungicide sensitivity among the isolates. Cyprodinil 37.5% + fludioxonil 25%, fluopyram 17.7% + tebuconazole 17.7%, and tebuconazole 25% were the most effective on reducing mycelial growth. The results of dual culture assay were similar for all three genera. Mycelial inhibition was higher in the case of the two *Diaporthe* strains, however antagonism was also observed between *E. nigrum* and the two studied Botryosphaeriaceae strains. Our study indicates that there are possible solutions against walnut blight causing fungi, whose effectiveness should be evaluated *in vivo* assessment. Not only chemical treatment, but also promising biocontrol agents can be applied on protection technology, further fundamental studies are required to develop the most successful strategy.