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AMPLICON SEQUENCING ANALYSIS OF BACTERIAL AND ARCHAEAL COMMUNITY IN HEAVY METAL(LOID) POLLUTED SOILS

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Soil heavy metal(loid) contamination is a well-known and widespread problem, especially in the vicinity of industrial and mining sites. Such areas are located in the northern Hungary where the former heavy industrial cities were polluted mainly with Pb, Cd, Hg, As, Zn and Cr. So far, there are only some researches to indicate the survival and the shift in bacterial and archaeal species in polluted soils. Hence, the current research was addressed to identify the impact of known metal(loid) contaminants on the species as well as reveal the core communities in urban soils. In this regard, four heavy metal(loid) polluted urban and an unpolluted brown forest soil samples were subjected to amplicon sequencing analysis (16S rRNA amplification) as well as soil chemical components and physical parameters were considered to assess the impact on communities and their richness. The sequencing was performed on Illumina MiSeq platform and analyzed via MOTHUR pipeline and R v3.5 software. It was found that in the higher level taxonomic ranks the abundant phyla Acidobacteria, Actinobacteria, Proteobacteria, Planctomycetes, and Bacteroidetes are dominated at all sampling sites. Whereas at the rare taxa, it is observed that particular group of operational taxonomic units (OTU) (in the level of the genera) exist only either in non-contaminated (*Lactobacillus*, *Streptococcus*, *Filomicrobium*, *Nitrobacter*, *Methylococcus*, etc.) or in contaminated samples (*Rhodococcus*, *Dyadobacter*, *Singulisphaera*, *Planctopirus*, *Peredibacter*, *Sulfurifustis*, etc.). Most of the latter taxa are known for their heavy metal(loid) resistance or ability to degrade hardly degradable organic substrates. The examination of the less abundant Archaea community described that two genera (Nitrososphaeraceae and Woesearchaeia) are the most abundant in all soil samples regardless the contamination degree. Substantially, the influence of Zn, Hg, NH_4^- and soil sand texture was proofed on bacterial, and NO_3^- content on archaeal community by non-metric multidimensional scaling (NMDS) analysis. Moreover, applied regression analysis reflected the high correlation of Zn and Cr concentrations with bacterial diversity richness ($r^2 = 1$, $r^2 = 0.9$, respectively). However, no any impact of enriched Pb, Cd and As concentrations were recorded on bacterial community, though at the same sampling areas high Pb concentration affected the archaeal diversity richness ($\rho = 1$, $p<0.0001$). In conclusion, the long-term environmental factors adapted the abundant species to metal(loid) contamination due to the similarity in urban and brown forest soil samples as they shared many common operational taxonomic units. Nevertheless, the variability of soil properties especially metal(loid)s affected the community of the rare taxa.

STOWAWAY OR GOOD MATE? - ISOLATION OF FUNGAL STRAINS FROM STOCK CULTURES OF CYANOBACTERIA

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Associations between algae and fungi (or bacteria) have been known and studied for a long time. These organisms exhibit a wide range of relationships, from mutualism to parasitism. Symbiotic relationships reach their highest levels in the form of lichens. However, free-living cells could also be beneficial to each other, even if they do not have strong physical or metabolic associations. On the other hand, presence of bacterial or fungal cells in the algal and cyanobacterial cultures often considered as contaminants, such as in the case of two cyanobacterial strains *Synechococcus cedrorum* (SAG 88.79) and *Synechococcus elongatus* (SAG 89.79), which we purchased from the SAG culture collection. SAG itself stated that the samples have fungal contaminants. We believe it would be beneficial to the scientific community if we identified those fungal "free-riders" and established the nature of their relationships with the cyanobacterial strains. According to their taxonomic positions based on their ITS sequences, two of the fungal strains turned out to be a *Cyphellophora olivacea* and a *Simplicillium aogashimaense*. To obtain further evidence for their taxonomic positions, we carried out thorough phylogenetic analyses using different methods. Besides molecular analyses, we also examined the colony and microscopic morphology of the fungal strains in different media and conditions. In order to understand their interactions (whether they are advantageous or disadvantageous), we separated the cyanobacterial strains from the fungi with serial dilutions, and then these strains were cultured in separate and co-cultivated manners, in different media and circumstances. Our preliminary studies suggest that cyanobacterial strains may grow faster in the presence of fungal strains than in monocultures.

COMPARATIVE BIOINFORMATICS ANALYSES OF THE EXTREMELY CONSERVED FUNGAL SPLICEOSOME COMPONENT CWF14 (BUD31) PROTEIN AND ITS OTHER COUNTERPARTS

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The spliceosome is a dynamic multiprotein complex with several counterparts that catalyze excision of introns from the pre-mRNAs. Thereby, mutation in any of its subunits may cause intron retention, which could cause harmful effects on the most distinct cell processes. One group of the spliceosome proteins belongs to the Cwf group (Complexed With cdc Five), which has 29 members in *Schizosaccharomyces pombe*. These proteins are very diverse both in size and probably in their functions, too. However, their exact roles are still not known; the majority of the proteins can be involved directly or indirectly in the cell division, their deletions lead to abnormal morphology and mitosis or they produce inviable spores after meiosis. One member of the group, the *cwf14* gene encodes a short G10 protein whose deletion caused cell wall defect and decreased cell growth on glucose carbon source, besides the abnormal cell morphology in *S. pombe*. To reveal whether this specific protein is evolutionarily and functionally conserved or not, we previously investigated the complementation ability of the *C. albicans* and *H. sapiens* Cwf14 orthologues in the *cwf14Δ S. pombe* strain. Our preliminary results suggested that the proteins encoded by the foreign genes partially rescued the gene deletion phenotype of *S. pombe* despite the large evolutionary distances of the genes. Based on these experiences, we wanted to examine the overall conservativism of this specific protein throughout the eukaryotic kingdoms. We managed to find more than 1,000 similar proteins within the Eukaryotes, but none was found among Bacteria and Archaea. We decreased the number of the protein sequences with reciprocal BLASTp and with HMMER searches to ~130 sequences, which covered all the main lineages of Eukaryotes from Protists to Primates. Almost every sequence contained the five signature motifs of the G10 domain. The C-terminal cysteine residues proved to be the most conservative sites of the protein sequences (zinc ion cluster). Secondary and tertiary structure predictions revealed that the structure of the Cwf14 protein is almost the same in the different eukaryotes. The phylogenetic analyses showed that the evolution of the *cwf14* sequences broadly coincided with the evolution of the main eukaryotic kingdoms and divisions.

DETOXIFICATION POTENTIAL OF LACTIC ACID BACTERIA ORIGINATED FROM SILAGE

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During ensilage, a complex process is taking place in silos involving Gram-positive lactic acid bacteria. Lactic acid bacteria can convert fermentable carbohydrates present in fodder plants into lactic acid. It is known that mycotoxins that are contaminating the raw agricultural materials mainly eliminated from the silage until the end of the fermentation [1]. In our project, lactic acid bacteria were identified from ready silages and their mycotoxin resistance and binding capacity was investigated in microtiter plate. The studied isolates were identified in Bruker MALDI-TOF system. Based on the identification higher number of isolates were Lactobacillus plantarum, Lactobacillus pentosus, Lactobacillus brevis, and Lactobacillus coryniformis, which were all analyzed further. Aflatoxin B1, aflatoxin M1, deoxynivalenol, zearalenone, and ochratoxin resistance and elimination was tested and, interestingly, only ochratoxin caused significant changes in cell density as it reduced the culture growth in MRS medium under 20 hours of incubation. Mycotoxin binding capacity studies revealed a significant binding of the toxins into the cell wall peptidoglycan.

[1] Driehuis et al (2018) J Dairy Sci 101:4093, https://doi.org/10.3168/jds.2017-13836

RECYCLING SPENT MUSHROOM COMPOST AFTER MICROBIOLOGICAL PRE-TREATMENT IN CHAMPIGNON CULTIVATION

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Besides the production of good quality spawn and mushroom compost, the basis of the efficient and economic white button mushroom (*Agaricus bisporus*) production is the ensurance of excellent quality casing material. In mushroom growing houses, the casing material, the role of which is primarily to ensure the formation of fruiting bodies and provide high water retaining capacity, covers the mushroom compost colonized by the mycelia of white button mushroom. The

nutrient-rich mushroom compost has low water retaining capacity, thus the role of the casing material with high water retaining capacity is to compensate this deficiency of the compost. The depletion of peat mines in Hungary and Europe, as well as the environmental problems resulting from peat mining are resulting in an increasing need for the development of alternate opportunities for the production of good quality casing materials. Recycling of spent mushroom compost has been attempted in some cases by vermicomposting; furthermore, microbiological recomposting may be a potential alternative. Our working hypothesis is that due to the outstanding fiber content of the harvested mushroom compost, it can serve as an excellent raw material for the development of casing materials. To this end, during our research we select and characterize fungal and bacterial strains that can be used for the controlled exploration and transformation of spent mushroom compost, in this way recycling it to casing material. As part of this, microorganisms (bacterial and fungal strains) are isolated from samples deriving from the experimental recomposting process of spent mushroom compost, which are deposited in a strain collection after species-level molecular identification. Microorganisms (strains of bacteria and fungi) will be isolated from samples taken during the natural recomposting process, subjected to species-level molecular identification and deposited in culture collection. By the examination of high priority traits from the point of view of mushroom compost colonization in the case of the isolated microorganisms, we will create the opportunity to select microbial strains aiding the recomposting of spent compost and its conversion to casing material. The spent mushroom compost is also tested alone and in mixture with commercial potting soil in seed germination experiments to evaluate its potential applicability as a natural fertilizer in horticulture.

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ABERRANT CHITIN DEPOSITION OF HMBA HMGB PROTEIN DEFICIENT ASPERGILLUS NIDULANS

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The „High mobility group box domain proteins" (HMGBs) are non-histone architectural components of chromatin of eukaryotes by their ability to bind DNA with no sequence-specificity and interact with other protein components of the chromatin. Our research group in *Aspergillus nidulans* identified three architectural HMGB proteins, namely HmbA (AN2885), HmbB (AN1267) and HmbC (AN10103), and we showed that HmbB is essential for the maintenance of mitochondrial DNA (produces cellular energy), the viability of fungal spores, fungal toxin production, and other metabolic processes. Regarding HmbA and HmbC, we have limited knowledge about their functions in terms of their physiological roles and transcriptional regulation despite HMGB proteins are regulators of gene expression and players of DNA recombination, replication and repair, as well as development and differentiation processes and tumor genesis. Based on the functions of the yeast orthologue of HmbA, Nhp6Ap, it is reasonable to think that HmbA modulates the expression of a wide range of genes and affects various biological processes. To address this question, we executed a complementation test by the heterologous expression of *Saccharomyces cerevisiae nhp6A* gene in *A. nidulans hmbA* deletion mutant (and vice versa) followed by the thorough study of the complemented strains. The study included the investigation of carbon- and nitrogen source utilization, stress responses against cell wall, osmotic and oxidative stressors, secondary metabolite production and *matA*/*matB* expression of *A. nidulans*, and temperature sensitivity and nitrogen starvation tolerance of *S. cerevisiae*. In this recent presentation, we show the aberrant deposition of chitin at the hyphal tips of *hmbA* deletion strain that explains growth rate reduction of the mutant and the observed sensitivity against calcofluor white stressor.

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METRONIDAZOLE-RESISTANT *BACTEROIDES* STRAINS FROM KUWAIT: A MOLECULAR STUDY

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Metronidazole resistance constitutes a significant issue among anaerobic pathogens, particularly in countries where antibiotics are not prudently used. Its best-described resistance mechanism is mediated by *nim* gene-insertion sequence (IS) element pairs, which can be questioned, but most of the metronidazole-resistant clinically important *Bacteroides* isolates do harbor them. Metronidazole resistance among *Bacteroides* strains is also a major concern along with the prominent

rise of multidrug-resistant strains as well. 421 clinical *Bacteroides* strains were collected during 2006-2018 in Kuwait. Antibiotic susceptibilities were recorded by ETESTs and *nim* genes and IS elements were detected by PCR in metronidazole-resistant strains. *Nim* gene types were characterized by nucleotide sequencing and the localization of the *nim* genes was determined by plasmid DNA isolation, sequencing and Southern blotting. The genetic similarity of the metronidazole-resistant *Bacteroides* strains was investigated by Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR) typing. Carbapenem resistance mechanisms were also analyzed using comparable methods. Out of the 421 *Bacteroides* isolates, 12 were proven to be metronidazole resistant (10 *B. fragilis*, 1 *B. dorei* and 1 *B. thetaiotaomicron*). All but one was *nim* gene-positive harboring the *nimE* gene. Of these, nine were activated by ISBf6 and 5.7, 8.3, 10 and 11 kb plasmids harbored the *nimE* genes. Interestingly, six of the *nim*-positive strains were also *cfiA*-positive with five silent and one with phenotypic resistance. By means of ERIC-PCR typing, our *cfiA* and *nim*-positive *B. fragilis* strains were different from other *cfiA*-positive *B. fragilis* strains. The prevalence of metronidazole resistant *Bacteroides* strains is around 4 % in Kuwait and it is mainly due to *nimE* -carrying plasmids, which in our cases represented four molecular weight classes. Since most of the examined strains were also *cfiA*-positive, therefore possible treatment options are fairly limited.

PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF CARBAPENEM HETERORESISTANT *BACTEROIDES FRAGILIS* STRAINS

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The insertion sequence-regulated, high-level carbapenem resistance of *B. fragilis* strains mediated by the *cfiA* gene is already well known. However, besides this latter and the low-level ('silent') carbapenem resistance, strains are regularly found to show reduced susceptibility to imipenem (MIC 1-8 µg/ml) and gave heterogeneous resistance profiles with IP ETESTs. We aimed to characterize those resistance mechanisms because *B. fragilis* strains with this phenotype might not be treated effectively by carbapenems. It was also assumed that the *GNAT* and *XAT* acetyltransferase genes found in the vicinity of the *cfiA* genes are involved in this resistance mechanism as a toxin-antitoxin (TA) addiction pair. Agar dilution and ETEST MIC values were determined along with population analysis profile (PAP) experiments using 10 heterogeneously imipenem resistant and five control (three susceptible and two fully resistant) *B. fragilis* strains. These latter values, imipenemase production and the expression data of the *cfiA*, *GNAT* and *XAT* genes measured by qRT-PCR were then correlated with each other. Heterogeneously imipenem resistant strains tended to give broader growth ranges, which were ≥3 in 2-fold dilution steps. They also produced higher specific imipenemase activities than silent strains. Parameters of the PAP curves, imipenem MICs, imipenemase activities and gene expressions of the *cfiA*, *GNAT* and *XAT* genes correlated well. We conclude that higher imipenemase production is responsible for imipenem heterogeneous resistance, which is most likely regulated by the GNAT-XAT TA pair inferred from the high correlation between the expression of *cfiA* and the GNAT toxin.

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OCCURRENCE OF *ESCHERICHIA COLI* PRODUCING EXTENDED SPECTRUM B-LACTAMASES IN FOOD-PRODUCING ANIMALS

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Multiresistant Enterobacterales producing extended-spectrum beta lactamases (ESBLs) is a crucial problem worldwide, both in animal and human healthcare. ESBL producing bacteria can colonize the human and animal microbiomes and may be transmitted through the food chain or with direct contact in accordance to the One Health theorem. Along this concept, we examined the appearance of ESBL producer strains in food-producing animals and compared these to animal strains. One hundred porcine and 114 poultry samples were collected. The samples were cultured on eosin-methylene-blue agar supplemented with 2 mg/L cefotaxime, the isolates have been identified by MALDI-TOF-MS. Antibiotic susceptibility testing was performed by Kirby-Bauer disk-diffusion test based on EUCAST guidelines and ESBL production was confirmed by double-disk synergy test. *E. coli* isolates were characterized for better comparison. ESBL gene families and the phylogroup distribution were investigated with PCR. Seventeen contemporary human clinical isolates were chosen for comparison. Among the porcine and poultry isolates 43 (30.7%) and 39 (15.9%) *E. coli* produced ESBL. The co-re-

sistance pattern of porcine and poultry *E. coli* isolates was similar; colistin (0.0% vs. 2.6%), amikacin (39.5% vs. 35.9%) and tobramycin (39.5% vs. 35.9%). In case of gentamicin and co-trimoxazole (SXT) the resistance was higher among porcine isolates (81.4% vs. 35.9% and 79.1% vs. 12.8%, respectively). All poultry isolates, while 68.9% of the porcine *E. coli* were resistant against ciprofloxacin. The most common ESBL gene family was *CTX-M-1* in animal isolates (90.7% and 69.7%). All but one porcine isolate and all poultry *E. coli* carried the *blaCTX-M-1* gene, the remaining one porcine isolate harbored *blaCTX-M-3*. The *CTX-M-2* gene family was found only in porcine isolates (4.7%), while the *CTX-M-9* family was found in case of poultry *E. coli* (7.7%). Most isolates belong to phylogroups commensal in humans, but their distribution was variable. Porcine isolates belonged to A, B1 and C phylogroups, while the poultry isolates were more diverse, only phylogroup D was absent from animal samples. In case of human isolates, the most common ESBL gene was the *CTX-M-15* (11/17; 64.7%), four of these was ST131 clade C2, one isolate carried *CTX-M27* and belonged to the clade ST131/C1-M27. Most isolates belonged to the B2 and D phylogroups (58.9% and 23.5%, respectively). Our results suggest that the gene flow is continuous between humans and animal isolates but the proportion of shared genes or isolates is small and unimportant in terms of public health epidemiology in Hungary.

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

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The degradation of monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (BTEX) are of general interest because these are widespread contaminants in groundwater, having toxic or even carcinogenic effects. Due to their relatively high-water solubility, cleanup of the polluted ecosystems is always obligatory by law in Hungary to avoid any such contamination of groundwater reserve. In subsurface BTEX-contaminated environments where the oxygen availability is the main factor affecting degradation, it was observed that indigenous bacterial community adapted to hypoxic BTEX-degradation, which may have a key role in this process. Previous studies of oxygen-limited, BTEX-contaminated sites of Hungary have shown the large diversity of subfamily I.2.C-type extradiol dioxygenase (*C23O*) genes in these environments. Although a broad diversity of microorganisms can degrade mono-aromatic hydrocarbons in the absence of oxygen, benzene, along with p-, and o-xylene are among the least degradable BTEX-compounds under anaerobic conditions. Only one study has reported a pure culture, which is able to degrade p-xylene anaerobically, and only a handful of studies have reported on stable enrichment cultures with confirmed utilization of p-xylene under denitrifying or sulfate-reducing conditions. To reveal those *C23O* genes, which have a key role in the hypoxic degradation of xylene isomers, aerobic and microaerobic enrichment cultures, degrading solely xylene, were established and thoroughly analyzed. Accordingly, in the first step of investigation aerobic and microaerobic enrichment cultures were set up by using groundwater sample of the Siklós hydrocarbon contaminated site with mix (1:1:1) of xylene isomers as sole source of carbon and energy. Results of the study have showed that considerably different microbial communities were present under aerobic and microaerobic conditions. Though in case of both aerobic and microaerobic enrichments *Pseudomonas* was the main player in the community by occupying approximately half of the total community, but remarkable change was noticeable for the other community members. Furthermore, results also showed that genus *Sphingobium* which is present in the aerobic enrichment was absent in case of microaerobic and that segment of the community was taken over by *Rhodoferax*. Regarding subfamily I.2.C-type *C23O* genes it was observed that completely different genotypes were detectable under aerobic and microaerobic conditions. These primary data from the enrichments raise our interest in investigating the community to reveal further interesting insights in future.

COMPARATIVE ANALYSIS OF BACTERIAL ENRICHMENT CULTURES DEGRADING BENZENE UNDER MICROAEROBIC CONDITIONS

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Petroleum hydrocarbons seriously damage the ecosystem or even the human health. Due to the relatively high-water solubility of aromatic hydrocarbons, benzene, toluene, ethylbenzene, and xylene (BTEX) are the most common contaminants of the groundwater and thus the drinking water. The bioremediation has proven to be the most success in solving a

problem of a widespread contamination, because it is cost-effective and environmental friendly. Aerobic microorganisms can utilize aromatic hydrocarbons as carbon and energy sources. Due to their presence and metabolism, the concentration of dissolved oxygen in the soil decreases rapidly. Aeration of subsurface can be a solution but this method also has environmental burden especially if the contamination is extensive or deeply located. The microbial biodegradation of toluene and ethyl-benzene can also occur under anaerobic conditions but benzene, para and orto-xylene can be persistent underground. Under aerobic conditions the catechol 2,3-dioxygenase enzyme is responsible for the cleavage the aromatic ring. A group of this enzyme is active even at low concentrations of substrate (oxygen), resulting the *C23O* gene shows the highest diversity in microbial communities of contaminated subsurface environments. Bearing in mind, the environmental sustainability the exploration of bacteria and functional genes that can participate in biodegradation processes even in oxygen-limited subsurface has a current importance. Accordingly, microaerobic enrichment cultures were set up by using groundwater sample of the "Siklós" hydrocarbon contaminated area. In three parallel enrichment cultures, the benzene was the sole carbon and energy source and in other three enrichments, the benzene was mixed with a small amount of other BTEX-compounds to reveal the efficiency of benzene degradation in the presence of other aromatic hydrocarbons. Taking into account the degradation efficiency and the diversity of the enrichment bacterial communities, one sample was selected from both of the two different type of enrichments for 16S rDNA amplicon sequencing. The results of the study showed that there was no significant difference in the efficiency of benzene degradation between the enrichments containing only benzene and the mixture, besides a large similarity in the microbial diversity was experienced between them. The composition of the bacterial communities of the different enrichments were similar, however, differences in the dominant genera were detected. The benzene-degrading enrichment was dominated by the genera *Rhodoferax* and *Acidovorax*, while in the BTEX-mixture-degrading enrichment beside the *Acidovorax*, members of the genus *Pseudomonas* were also dominant. In addition, in the benzene-degrading enrichment, members of the genus *Azoarcus* and *Rhizobium* appeared in smaller quantities, and in the enrichment containing the BTEX-mixture, members of the genera *Sediminibacterium* and *Geothrix* accounted a smaller part of the community.

ENGINEERING A BACTERIAL CONSORTIUM FOR THE COMPLETE AND RAPID BIODEGRADATION OF ALL THE SIX BTEX-COMPOUNDS

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BTEX-compounds (benzene, toluene, ethyl-benzene, o-, m-, para-xylene), often associated with carcinogenic, mutagenic or teratogenic properties, are one of the most widespread and toxic groundwater pollutants among petroleum hydrocarbons. The biological degradation of these pollutants still is the most environmentally friendly and sustainable approach to remove these harmful chemicals from the polluted environments (bioremediation). Although microbial degradation of BTEX-compounds is widely known, in the scientific literature there are only a handful of bacterial strains or consortia, which are capable of degrading efficiently the whole spectrum of BTEX-compounds simultaneously. In our recent research a bacterial consortium was engineered, which can be applicable in the complete and rapid degradation of all the six BTEX-compounds. The BTEX-biodegradation capability of the selected bacterial strains was tested separately for each isolate either by using individual BTEX-compounds as sole source of energy and carbon or by using a mixture of BTEX-compounds (testing co-metabolic BTEX biodegradation). The BTEX mixture biodegradation capability of the bacterial consortium was also tested. By the end of the microcosm experiments, the relative abundance of the co-inoculated was assessed by using a cultivation dependent approach, as well as T-RFLP. Based on the obtained results strain A even alone was capable of degrading all the six BTEX-compounds in both settings. In contrast, regardless of the type of BTEX addition (individual or mixture) strain B could degrade only toluene, m- a p-xylene. Although strain A was capable of degrading all the six BTEX-compounds, simultaneously the application of the bacterial consortium proved to be more efficient and a faster BTEX degradation could occur in mixed-cultures. While strain A completely eliminated the BTEX from the test solutions only after ~168 hours of incubation, the engineered consortium could degrade BTEX in an initial concentration as high as 20 mg/L after 6 hours. By the end of the experiment, both strains could be re-isolated in high number (10^8 cells/mL) from the microcosms, in addition the T-RFLP electropherograms showed co-relatable data.

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SELECTIVE ENRICHMENT AND IDENTIFICATION OF DICLOFENAC, IBUPROFEN AND CARBAMAZEPINE DEGRADING BACTERIA FROM A GROUNDWATER BACTERIAL BIOFILM

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Nowadays pharmaceutical residues, as part of the group of emerging organic water contaminants (EOCs), are one of the most frequently reported environmental hazardous substances worldwide. Wastewater effluents are the main sources of contamination; surface and groundwater contain the greatest load of them. Pharmaceutical residues have already been reported also being present in drinking water in Germany, UK, Italy, Canada and U.S. The conventional wastewater treatment plants are generally insufficient and frequently unable to completely remove these compounds, often associated with ecotoxic effects, from the wastewater. Therefore, there is a high demand for the development of auxiliary and alternative wastewater treatment solutions to completely get rid of these pollutants from the environment. The sensu lato goal of our research is to develop a biotechnological approach, namely SBP encapsulated pharmaceutical residues degrading bacteria applied in form of a permeable reactive bio-barrier system, by using which the pharmaceutical residues from the wastewater effluents can be removed. To achieve the main goal of our research first the selective enrichment and identification of diclofenac (DIC), ibuprofen (IBU) and carbamazepine (CBZ) degrading bacteria was conducted from a subsurface bacterial biofilm by applying "omics" techniques. A strain collection of potentially pharmaceutical degrading bacteria was also established. DIC, IBU and CBZ were used as target compounds because they are the most frequently detected pharmaceuticals in aquatic environments. According to the so far obtained results members of the genera *Ferrovibrio*, *Hydrocarboniphaga*, *Sphingopyxis* and *Starkeya*, and bacteria affiliating with *Pimelobacter*, *Starkeya*, *Hydrocarboniphaga*, *Sphingosinicella* and *Methylibium*, as well as members of the genera *Pseudonocardia*, *Sphingopyxis*, *Achromobacter*, *Rhodococcus* are most probably capable of DIC, IBU and CBZ biodegradation, respectively. Tested members of the established strain collection like *Starkeya*, *Variovorax*, *Pseudomonas*, *Bacillus*, *Stenotrophomonas* and *Brevundimonas*, potentially capable of pharmaceutical biodegradation, may be used in the future for the development of the envisaged biotechnological approach.

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INVESTIGATION OF THE MANGANESE ION SENSITIVITY OF THE *ASPERGILLUS TERREUS* ITACONIC ACID FERMENTATION

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Aspergillus terreus is a filamentous Ascomycete fungus used for technical-scale itaconic acid production by submerged fermentation. Itaconic acid is an unsaturated dicarboxylic acid with a high potential as a platform for chemicals derived from sugars. Itaconic acid is known to accumulate to high levels only when a number of nutritional parameters are carefully adjusted, of which strict manganese(II) ions deficiency (<5 ppb) in the growth medium is particularly important. Metal parts of the 2-L scale bioreactors used (stirrer attachment, aeration system, sampling tube) are built of stainless steel that may contain up to 2 % of manganese. Corrosion of the steel surface may lead to metal ion leaks. In this study, we have tested the hypothesis if the detrimental effect of manganese(II) ions on itaconic acid formation are dependent on the cultivation time and the actual stage of growth.

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A NOVEL APPLICATION OF THE EXTRACELLULAR ORGANIC MATTER FROM *MICROCOCCUS LUTEUS* TO ENHANCE THE BIOREMEDIATION OF SOILS POLLUTED BY USED LUBRICANT OILS

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Lubricant oils (LOs) are conventionally derived from crude oil to reduce friction between mechanical moving parts of machines, and accumulate harmful compounds (e.g. combustion products, heavy metals, polychlorinated and polyaromatic hydrocarbons, etc.) even upon normal operation. Hence, used lubricants oils (ULOs) are crucial targets of pollution research and environmental rehabilitation procedures. Compared to the great number of physicochemical remediation approaches, bioremediation represents a less destructive, environmentally friendly and cost-effective choice, since it exploits the natural degradative capabilities of microorganisms and plants to neutralize environmental pollutants. Biostimulation is performed via nutrient addition in order to enhance the biodegradation of contaminants by the native microbiota, while bioaugmentation involves the inoculation of degrader strains into the polluted site. However, the application of living organisms in the field can be difficult due to the transition of inoculated bacteria into a viable but nonculturable (VBNC) state when facing environmental stresses. Extracellular organic matter (EOM) from *Micrococcus luteus* can reactivate these cells or enhance the biodegradation performance both of the native and augmented strains. In this study, the ex situ bioremediation of a long-term ULO-polluted site was modelled in bench-scale soil microcosm set-ups through conventional and EOM-supplemented approaches, including natural attenuation (NA), biostimulation (BS), biostimulation-combined bioaugmentation (BAS), biostimulation supplemented with EOM (BS+EOM) and biostimulation-combined bioaugmentation supplemented with EOM (BAS+EOM). A mixture of *Rhodococcus erythropolis* PR4 and *R. quingshengii* KAG C cells (1:1) was used as inoculum for bioaugmented soils. By monitoring the changes in soil respiration, ULO-bioconversion, culturable cell counts, taxonomic composition and ecotoxicological parameters (soil catalase, soil dehydrogenase and soil phytotoxicity), we conclude that compared to conventional bioremediation methods, EOM addition resulted in an increased microbial activity and biomass, a more diverse soil microbiota and an enhanced biodegradation performance. After 60 days, the most effective ULO-biodegradation was achieved in BS+EOM (37%) and BAS+EOM (45%), nevertheless, germination indexes of Indian mustard (*Brassica juncea*) seeds in EOM-treated soils reached only 13% and 24%, respectively, presumably due to inhibiting breakdown products of ULO-biodegradation. These results indicate that a decreased hydrocarbon concentration does not necessarily correlate with reduced soil phytotoxicity.

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THE EFFECT OF ACQUIRED AZOLE RESISTANCE ON ABIOTIC STRESS TOLERANCE AND VIRULENCE IN CASE OF *CANDIDA AURIS* STRAINS

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The prevalence of invasive fungal infections caused by *Candida* species increasing worldwide. Although *Candida albicans* is still the most significant human pathogen among the *Candida* genus, the frequency of isolation of non-albicans species among cases of candidiasis are also rising rapidly, shifting the attention towards these lesser-known pathogens. One of the emerging species, *Candida auris* was described in 2009, and quickly recognized as a serious threat, since high percentage of *C. auris* isolates demonstrated resistance towards the regularly used azole class antifungal, fluconazole. Since its early occurrence, several *C. auris* outbreaks have been reported worldwide, associated with isolates displaying decreased susceptibility towards all three classes of antifungal drugs clinically authorized for bloodstream *Candida* infections. The effect of the development of antifungal resistance on this emerging pathogen was investigated by generating resistant strains via in vitro microevolution method. During the process two azoles susceptible *C. auris* clinical isolate was treated with increasing concentration of azole class antifungals (Fluconazole, Posaconazole, Voriconazole). Changes in antifungal susceptibility of the evolved strains and cross resistance between drugs were determined by microdilution method, utilizing azoles as well as other class of antifungals, such as the echinocandin type Anidulafungin, Caspofungin, Micafungin and a polyene, Amphotericin-B. Abiotic stress tolerance of the generated strains compared to the initial isolate, were examined by spotting assay, using osmotic stressors, cell wall perturbants and membrane detergent. To evaluate the impact of the acquired resistance on sterol biosynthetic pathway, ergosterol composition was measured for all generated strains. Significant differences were found between the membrane compositions of the strains implying a different

resistance mechanism towards the used antifungals. The connection between the virulence and the acquired antifungal resistance were also studied both in in vitro and in vivo settings. Phagocytosis of the generated strains by J774.2 mouse macrophage-like cells were measured and analyzed by flow cytometry. To study the effect of the changed susceptibility in vivo, both non-conventional *Galleria mellonella* survival model and murine intravenous infection model was used. In the murine infection model fungal burden of the Fluconazole and Posaconazole evolved strains were determined in spleen, kidney, liver and brain, and compared to the fungal burden associated with the initial azole susceptible strain. Significant differences in virulence of the initial and the generated strains were identified suggesting a connection between the virulence and antifungal susceptibility of the emerging fungal pathogen, *Candida auris*.

TAXONOMIC AND FUNCTIONAL DIVERSITY OF PROKARYOTES IN HUNGARIAN HYDROTHERMAL KARST SYSTEMS

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Hydrothermal karst systems have traditionally been considered environments with temperature above 20°C and where most of the water is of hypogenic origin. Thermal waters containing different minerals and microorganisms are the source of various karstic dissolution (e.g. cave formation) and precipitation processes around the world. In Hungary, the hydrogeological conditions resulting from the nature of the basin and the thinning of the earth's crust are very favorable for the formation and discharge of thermal waters, in addition to the appropriate aquifers. Hydrothermal activities have already led to the development of various karst phenomena in the earth's historical past. The unique thermal water supply of Hungary (e.g. Budapest baths, Hévíz, Harkány) is also the basis of the now world-famous medical and health tourism. The nature of karstification processes is determined by the complex interaction of hydrogeological, physical-chemical and microbiological characteristics of thermal waters (e.g. natural thermal springs, thermal water of wells). Microorganisms with diverse lifestyles (e.g. planktonic and biofilm-forming) and those capable of adapting to extreme environmental conditions (e.g. high temperature, radioactivity, low organic matter content, darkness, anoxia) are also present in hydrothermal karst environments. The presentation provides an insight into the composition and supposedly diverse metabolic relationship of this special and hidden prokaryotic world.

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SCREENING AND MEDIA OPTIMIZATION OF BIOSURFACTANT PRODUCTION WITH *BACILLUS* SPECIES

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Biosurfactants are amphiphilic molecules that are applied in several fields (e.g. pharmaceutical industry, cosmetics industry, food industry, agriculture (plant protection), and microbial enhanced oil recovery). Biosurfactants are lowering the surface and interfacial tension like the chemically synthesized surfactants. Nowadays biosurfactants gained interest because they are environmentally friendly, have lower toxicity, and higher biodegradability compared to the chemically synthesized surfactants. Several microorganisms produce Biosurfactants. Bacilli are producing lipopeptide type biosurfactants. This biosurfactant group includes the surfactin, iturin, and fengycin families. These molecules are built from an amino acid oligopeptide ring attached to a fatty acid residue. In our experiments, we compared six *Bacillus* species (*Bacillus circulans* B.02324, *B. coagulans* DSM1, *B. firmus* B.01087T, *B. licheniformis* DSM13, *B. subtilis* DSM10, *Geobacillus stearothermophilus* DSM2313), in order to determine their biosurfactant production. The screening experiments were performed in shaking flasks with three parallel measurements. The cell growth was monitored by optical density and cell dry weight measurements. The glucose consumption was followed by HPLC measurements. Surface tension measurements were performed to indicate the presence of the biosurfactants in the fermentation broth. To isolate the biosurfactant from the broth the acid precipitation method was applied. The downstream processing of the fermentation broths was performed throughout five steps to get the final product. The best candidate, which reached the highest biosurfactant concentration, was the *Geobacillus stearothermophilus* DSM2313. Media optimization was performed with the selected best-producing bacterium. A central composite statistical design was planned and performed to determine the optimal carbon and nitrogen source concentration and the optimal initial pH value.

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INSIGHTS INTO THE MECHANISM OF SULFIDE OXIDATION IN A TYPE VI SULFIDE:QUINONE OXIDOREDUCTASE

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Despite its toxicity, sulfide plays various physiological role in eukaryotes and prokaryotes such as neurotransmitter or electron donor. Sulfide detoxification, homeostasis and sulfide dependent energy conversation processes are catalyzed by sulfide:quinone oxidoreductases (SQR) via electron transfer from sulfide to the membrane quinone pool. SQRs are ancient membrane bound flavoproteins and members of disulfide oxidoreductase enzyme family. For catalysis, SQRs require FAD cofactor and a redox active disulfide bridge formed between conserved essential cysteines in their active center. Based on phylogenetic and structure-based classifications there are six types of SQR proteins. Some types of SQRs may have diverse enzymatic mechanisms due to the variances in catalytic cysteines. The photosynthetic purple sulfur bacterium *Thiocapsa roseopersicina* possesses a type VI SQR enzyme (SqrF). Biochemical and enzyme kinetic analysis of homologously expressed and purified recombinant wild type TrSqrF revealed significant differences between SqrFs and other type SQRs indicating a diverse catalytic mechanism of type VI SQRs compared to the known SQR mechanistic models. TrSqrF protein contains four cysteines presumably involved in the catalysis. To identify the role of cysteine residues of TrSqrF in the catalytic process cysteine mutant enzymes were created. Based on biochemical and kinetic characterization of these TrSqrF variants, Cys121 essential for enzyme activity. The cofactor is covalently bound via a heterodisulfide bridge between Cys121 residue and the C8M methyl group of FAD. Mutation of another cysteine presented in all SQRs (Cys332) causes remarkably decreased enzyme activity proving that the role of Cys332 is important, but non-crucial in TrSqrF enzyme catalysis. Iodoacetamide as sulfhydryl-blocking agent can irreversibly inactivate TrSqrF if the enzyme is actively catalyzing sulfide-dependent quinone reduction. FAD cofactor can be released from iodoacetamide-inhibited enzyme indicating the split of the cofactor binding covalent bond. The inhibition studies support the catalytic mechanistic model that entails opening and reforming of the heterodisulfide bridge between Cys121 and FAD during the catalytic cycle of TrSqrF. The structural model for the sulfide oxidation site and proposed function of active site cyteines of TrSqrF enzyme reveal a distinct mechanism for group VI of SQRs.

STUDYING FUNGAL STRESS RESPONSES – A TRANSCRIPTOMICS BASED APPROACH

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In mycology, stress is generally defined as environmental conditions that threaten the survival of fungi or at least prevent their optimal performance [1]. Understanding fungal stress responses can help us to prevent unwanted fungal growth on food, feed, and art treasures, in buildings and even in the human body. Secondary metabolism is highly influenced by stress therefore, prevention of mycotoxin formation or overproduction of pharmaceutically important secondary metabolites can also benefit from stress microbiology studies. Since fungi, like other creatures, always respond to the stress as whole organisms, holistic approaches are needed to get a real picture from stress responses. Not surprisingly, omics (e.g. transcriptomics or proteomics) are widely applied in stress microbiology studies. In the last decade, we used DNA chips and RNA sequencing in combination with proteomics or physiological methods to elucidate stress responses in *Aspergillus* species. This presentation focuses mainly on how the chosen reference culture; the small changes in the culturing conditions or the genetic background of the studied strain can influence the stress response in *Aspergillus nidulans* and *Aspergillus fumigatus*. I would like to show that: i) Stress responses depend on both how fungi try to adapt to the stress and how they have adapted to the reference conditions. Therefore, the more special the reference conditions are, the less characteristic the stress response is for the stress. ii) Stress responses are sensitive to the culturing conditions. Due to it, predicting in vivo behavior of pathogenic fungi is challenging when we use only in vitro data. iii) Deletion of genes can substantially modify stress responses even if they are likely not involved in the stress response. Despite we know how a laboratory strain responses to a stress we cannot easily predict the behavior of (industrial) strains with several mutations in their genome.

[1] Hallsworth JE (2018) Fungal Biol 122:379.

PURIFICATION OF ERGOMETRINE FROM *CLAVICEPS PURPUREA* ISOLATES BY CENTRIFUGAL PARTITION CHROMATOGRAPHY

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Claviceps purpurea is a plant pathogenic fungus that grows on a spike of cereals and it forms 2-5 cm long, black sclerotia. Members of this taxon produces numerous alkaloids, specifically ergot alkaloids, from which the most important are the ergometrine and ergotamine, that are considered drug precursors, which confirmed to have hallucinogenic effects. Therefore, in the European Union their concentrations are limited to extremely low levels in various cereals (2012/154/EU). For the monitoring of these compounds, purified material is needed. In the purifications, centrifugal partition chromatography – which is a form of liquid-liquid chromatography – can provide a cheap and quick solution. This technique uses a two-phase solvent system, in which the desired components are partitioned by their different affinity of the two phases. As part of our research, *C. purpurea* isolates were collected from the spikes of contaminated triticale, from which the best ergometrine producer isolate was selected. This strain was used to determine the optimal conditions for ergometrine production, including the selection of the culture media and the duration of cultivation. The optimized conditions were used for a pilot scale cultivation of the isolated fungus. From this, the fermentation material was extracted to gain a crude extract, which was utilized to start the method development for liquid-liquid chromatography using the „best solvent method". Based on these tests, the compounds of the crude extract were partitioned well in an ether-alcohol-water system, which was applied also for the instrumental separation resulting a successful isolation of ergotamine from the fermentation material.

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INVESTIGATION OF AFTATOXIN PRODUCTION OF *ASPERGILLUS* SPECIES

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Pier Antonio Micheli firstly described the genus *Aspergillus* in 1729. Currently, the genus consists of more than 250 recognized species, which are widespread worldwide. One of their importance is that certain *Aspergillus* species could cause enormous losses in the food industry due to their mycotoxin production (fumonisins, ochratoxins and aflatoxins). Various food- and feedstocks, which are contaminated by these toxic secondary metabolites, are not safe for consumption, thus it is important to evaluate the toxin-producing capability of isolates belonging to different species. It is also known, that from the genus *Aspergillus* aflatoxins are mostly produced by fungi belonging to the sections Flavi, Nidulantes and Ochraceorosei. In our work, aflatoxin production of *Aspergillus* isolates representing the above mentioned three sections were investigated. Initially, the optimal cultivation parameters of toxin production were determined; for this, an *A. parasiticus* isolate (SZMC 2473) as a known high-level mycotoxin producer strain was selected. In the first experiments, the optimization of the cultivation media was carried out: effects of 12 solid and liquid media were investigated. Then the temperature dependence of the toxin production was examined in the previously selected medium, and then the production kinetics of the aflatoxins were recorded during a 16-days experiment (carried out at the optimum temperature) with a daily determination of mycotoxin level. Finally, using the optimized cultivation parameters, the aflatoxin production of 56 *Aspergillus* strains acquired from the Szeged Microbiology Collection were determined quantitatively with HPLC-MS/MS method.

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CHANGES IN THE MICROBIAL STRUCTURE DUE TO WATER PURIFICATION: REPORT ABOUT THREE DIFFERENT, SMALL WATER PURIFICATION SYSTEMS

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The aim of the present study was to investigate the water quality of three Hungarian water purification systems (WPS1, WPS2 and WPS3) by molecular techniques (taxon-specific PCR and amplicon sequencing) as well as microscopic cell counts (after DAPI staining with epifluorescence microscopy). Water samples were collected from four sites at each

purification system during the whole treatment process: from the well before treatment, after GAC adsorption, after breakpoint chlorination and from the consumer point tap of the system. Taxon-specific PCR or amplicon sequencing did not reveal fecal indicator bacteria. *Pseudomonas* spp. and *Stenotrophomonas maltophilia* were detected in almost all samples, which is not surprising as they are widely distributed in aquatic ecosystems. *Acidithiobacillus ferrooxidans* was significant in each systems, especially in WPS1 and WPS2, which may refer to corrosion processes in the system. Microscopic cell counts with the exception of WPS3 showed that chlorination decreased the number of microbes at least one magnitude, but their number has highly increased at the GAC adsorbent. Amplicon sequencing data of WPS1 and WPS2, in strong connection with cell count results, revealed the abundance of methanogenic Archaea and methylotrophic Bacteria after chlorination. Ammonia-oxidizing archaeal group was the most dominant among Archaea indicating nitrification based processes in the water treatment systems showing that it is essential to take into account the effect of chlorination. Supposedly, it is not completely adequate in the system. *Stenotrophomonas maltophilia* as an opportunistic pathogen was found in each sample of WPS2, but other pathogens, as *Neisseria* was also detected sporadically in the samples of this water purification system.

BACTERIAL COMMUNITY COMPOSITION OF DANUBE RIVERBANK IN THE NORTHERN WATER SOURCE OF BUDAPEST

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Riverbank filtration is a naturally occurring complex biological, mechanical and chemical process, when river water slowly filters through the gravel riverbed, and particulate and dissolved contaminants are removed, then drinking water appears in nearshore wells. This study aimed to determine the taxonomic composition of biofilm bacteria and those living in the pores surrounding the gravels. To achieve this 16S rRNA gene-based amplicon sequencing was applied on an Illumina MiSeq platform. Results showed that a significant fraction of bacteria belonged to yet-uncultivated lineages, and a notable proportion of organic material degraders was detected, furthermore remarkable differences were revealed between the biofilm and water-inhabiting communities. Members of the phyla Proteobacteria, Chloroflexi, Acidobacteria, Planctomycetes and Actinobacteria and e.g. the genus *Gaiella* were characteristic for the former, while in the water surrounding the gravels the phyla Proteobacteria and Bacteroidetes and the genera *Flavobacterium*, *Rheinheimera*, *Rhodoferax* and *Pseudomonas* had the highest relative abundance.

DECIPHERING THE ROLE OF NOVEL SMALL SECRETED PROTEINS (SSPS) AND PUTATIVE 7 TRANS-MEMBRANE RECEPTORS IN FUNGAL MULTICELLULARITY

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With the exception of fungi, the evolution of multicellularity is almost universally associated with the expansion of receptor-encoding genes, especially G-protein coupled receptors (GPCRs). GPCRs are cell-surface seven trans-membrane (7TM) receptors, which play a role in environmental sensing and cell-cell communication. Based on our genome annotations, the canonical GPCR diversity of complex multicellular fungi is surprisingly low (2-70, mean 14.7), while 7TM putative receptor genes are abundant (2-539, mean 85). We hypothesize that these novel putative receptors associate with diverse fungal functionalities, including multicellularity. The ligands of these certain orphan receptors are also unknown. 443 fungal proteomes were analyzed by a comparative genomic approach, and according to these results, the examined putative 7TM receptors' diversity shows a correlation (Pearson 0.7) with that of small secreted proteins (SSPs). This finding suggests that SSPs are potential ligand candidates of orphan receptors. SSPs are defined as less than 300 amino acids long, cysteine-rich polypeptides with an extracellular secretion signal. Some of them are particularly interesting because they show a high expression dynamic during the fungal ontogeny. An additional potential link between GPCRs and SSPs is provided by mating pheromones, which are also small peptide ligands of pheromone sensing canonical GPCRs. To examine these questions, we use the inky cap mushroom (*Coprinopsis cinerea*), the main model system for fungal multicellularity. We would like to prove that the predicted 196 putative 7TM receptors are GPCRs, by testing their interaction with G-proteins. On the other hand, we would like to decipher the possible impact of SSP candidates on development, by examining how externally administered SSPs influence the morphogenesis of the complex multicellular fungus, *C. cinerea*. We expect the results of these experiments help to decipher novel signal transduction pathways in fungi.

TRANSCRIPTIONAL RESPONSES IN CARBON-LIMITED CULTURES OF ASPERGILLUS NIDULANS

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Microorganisms are often facing carbon stress (either limitation or starvation) both in natural and industrial environments. The availability and quality of carbon sources are essential factors that influence the vegetative growth, differentiation, pathogenesis as well as extracellular hydrolase production and secondary metabolism of filamentous fungi. In this study, carbon stress responses of *Aspergillus nidulans* as a model organism were recorded by next generation RNA-sequencing. Stress conditions were induced by transferring exponentially phase mycelia, pre-grown on glucose, into carbon source free (carbon starvation), lactose or arabinogalactan containing (carbon limitation) media as well as into glucose containing (reference) media. The transcription of approximately 3,000-4,000 genes in each case was significantly depended on the applied stress. Among them 1,192 genes showed up-regulation and 921 genes showed down-regulation during all the three carbon stress treatments. Up-regulation of secondary metabolite cluster genes and genes encoding carbohydrate-active enzymes (CAZymes) as well as down-regulation of genes involved in glucose utilization, respiration, and oxidative phosphorylation were observed in each stress treatment. Up-regulation of autophagy-related genes, genes encoding hemicellulose and pectin hydrolyzing enzymes and of D-galactose oxidoreductive pathway genes were characteristic for carbon starved, arabinogalactan and lactose containing cultures, respectively. Interestingly, up-regulation of autolytic cell wall degradation genes were observed in both carbon starved and arabinogalactan containing cultures. Meanwhile, up-regulation of lactose permease and β-galactosidase genes (including *lacpA*, *lacpB*, and *lacD*) were observed in all the three carbon stressed cultures. Our results support the view that adaptation to carbon stress starts with the secretion of various hydrolyzing enzymes. Later, the compounds liberated by these enzymes can up-regulate further genes needed for the utilization of the "discovered" nutrients. This mechanism explains why the early stress responses to different carbon stresses are very similar and elucidates how fungi can easily adapt to grow on special carbon sources like lactose.

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POTENTIAL IMPLEMENTATION OF LIQUID-LIQUID CHROMATOGRAPHY FOR PREPARATIVE PURIFICATION OF OCHRATOXIN A

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Ochratoxins are toxic secondary metabolites of different *Aspergillus* and *Penicillium* species and there are strict regulations on their levels in food. Qualitative and quantitative measurements of this compound require high amounts of pure ochratoxin as a standard. This large amount of pure compound can be obtained by purifying the fermentation medium of the producing microorganisms. Liquid-liquid chromatography (LLC) seems to be a suitable method, which is becoming more common in the purification of natural compounds. One instrumental implementation of LLC is centrifugal partition chromatography (CPC). In our work an *A. albertensis* SZMC 2107 strain was selected for the ochratoxin production. The fermentation was performed in yeast extract-sugar medium and for sample preparation, three-step liquid-liquid extraction was applied. The resulting crude extract was used for solvent system testing to find a suitable biphase for liquid-liquid chromatographic purification. During the "shake flask" testing step the resulted samples were analyzed by HPLC-UV and HPLC-FLD. After the application of various three- and four-component systems, the distribution coefficients were in the proper range for both ochratoxin A and ochratoxin B in the hexane-isopropanol-water system. With this system, the instrumental separation was carried out at 10 ml/min flow rate and 2,000 rpm rotational speed. The purities of resulted ochratoxin A and B were more than 99% and 55 %, respectively. The purity was calculated based on the areas of HPLC-UV chromatogram on 333 nm.

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INVESTIGATION OF THE GROWTH AND HYDROGEN PRODUCTION OF MICROALGAE STRAINS IN STARCH-CONTAINING MEDIA

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The aims of our experiments were the investigation of growth and hydrogen production of *Chlamydomonas reinhardtii* cc124 and *Chlorella* sp. MACC 360 algae strains using starch-containing media and bacterial partners. The products of starch degradation can serve as extra nutrients for photoheterotrophic algae growth. The *Bacillus amyloliquefaciens* bacterial partner degraded completely the applied amount of starch (6 mM) within 48 hours, while *Enterobacter* sp. had limited capability for starch degradation. The applied bacterial strains did not produce hydrogen either in TAP (Tris-Acetate Phosphate) or starch-containing TAP. The presence of *B. amyloliquefaciens* in starch-containing TAP clearly stimulated the growth of *C. reinhardtii* strain, while it did not exert such an effect in TAP media. At the same time *B. amyloliquefaciens* inhibited the growth of *Chlorella* sp. both in TAP and in starch-containing TAP. The heterotrophic bacteria efficiently respire oxygen during growth, thereby creating ideal conditions for the green algal hydrogen production. *Chlorella* sp. was capable of producing hydrogen both under axenic conditions and when co-cultured with bacteria in either TAP or starch-containing TAP and it was able to degrade starch. However, *C. reinhardtii* algae was capable of producing produces any hydrogen both under axenic conditions and when co-cultured with bacteria either in TAP or starch-containing TAP, but did not degrade starch. The *Enterobacter* sp. partner stimulated hydrogen production to the greatest extent in both algae strains. The experiments served as a laboratory model for the investigation of the photoheterotrophic wastewater treatment efficiency, in our experimental system, we applied a combination of biodegradation of organic substances and concomitant biohydrogen production.

POSSIBLE DEVELOPMENT STRATEGIES OF YEAST PROBIOTICS USING CRISPR/CAS9 SYSTEM

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The use of probiotics is continuously on the rise due to conceived positive health effects and increased microbiome awareness. However, the popularity of probiotic products has not been accompanied by scientific risk assessment in many cases. An increasing number of infections originating from probiotic use are reported worldwide, with the majority of such cases caused by the yeast *Saccharomyces 'boulardii'*, a subtype of *Saccharomyces cerevisiae*. Because of their frequent use, they pose a significant health risk, especially to severely ill or infant patients, and patients with prolonged hospitalization. We subjected a collection of clinical *Saccharomyces* isolates from Hungary to whole-genome sequencing and compared those to previously published probiotic-derived human isolates and to probiotic products. This revealed the existence of numerous, very closely related probiotic-derived infectious yeasts in local hospitals. Globally, we found very low intra-clade divergence for the probiotic yeasts and a surprising rareness of genome structure variations in contrast to other pathogenic *Saccharomyces*. The probiotic yeast is thus apparently relatively widespread and homogeneous among clinical isolates and efforts should be made to make it less likely to cause infections. To facilitate probiotic strain improvement and risk assessment we investigated the adaptations that help *S. 'boulardii'* to survive in the mammalian host. Thus, we investigated yeast isolates of both commercial and clinical origin and tested subclones of these samples re-isolated from experimentally infected immunosuppressed BALB/c mice. We conducted stress-phenotyping in order to reveal the traits under selection during pathogenic lifestyle and survival in the bloodstream. Our data showed that the clinical isolates and their subclones from experimental infection were more tolerant against LiCl and NaCl compared to the commercial probiotics leading to increased survival in our mammalian model. Since these adaptations may be related to increased virulence, we used the CRISPR/Cas9 system for gene deletion in order to create a yeast probiotic strain that is unable to adapt to these stress factors and consequently to the host environment outside the gut, especially in the bloodstream. After gene deletions we infected BALB/c mice again with the mutant strains and followed the animals' and the mutant strains' survival. As a result, all of the mice remained alive until the end of the experiment, and the CFUs decreased dramatically compared to the wild type strains. Thus, we could create a strain that is unable to survive in the mammalian bloodstream. In the future, these strains might be a foundation for future probiotic products that are not only safer but have enhanced or additional probiotic traits that they have never had before. The new generation of yeast probiotics should combine the proven beneficial effects of the species on gut health with safety, to eliminate the risk of infection.

FUNGAL ROOT ENDOPHYTES OF DIFFERENT HOSTS AND FERTILIZATION SYSTEMS FROM THE LONG-TERM EXPERIMENTS AT MARTONVÁSÁR

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Endophytic fungi represent one of the various groups of microorganisms that live together with plants. They are found within internal tissues of living plants without any immediate, overtly negative effects. Dark septate endophytes (DSE) may have various effects on plant survival under different stress conditions such as drought, which was the main factor behind lower harvests of key crops in many regions of central and northern Europe in 2018. The effect of a DSE species strongly varies, and these functional differences may be derived from a certain degree of habitat adaptation of an isolate to the host or to the conditions of the sampling 'home' area. We have information on main grass associated DSEs of natural grasslands, however our knowledge on DSEs of agriculturally important gramineous crops are limited. In this study, we investigate the habitat and host adaptation of DSE fungi. In the present work, our goals were to (1) screen the DSE community of gramineous crops, and to (2) isolate endophytic fungi, which are also present in natural grasses and can be used for in vitro inoculation experiments. In addition, to (3) carry out molecular identification of the common DSE taxa. Root samples were collected from experimental monoculture plots of ATK Agricultural Institute in Martonvásár, where only wheat (*Triticum aestivum*) or maize (*Zea mays*) have been cultivated since 1960, enabling the proposed adaptation of the fungi to the habitat or host. For molecular identification of the isolates gained from the surface sterilized root sections, total DNA was extracted and the internal transcribed spacer (ITS) region of the nrDNA was amplified. Besides the ITS region, which is accepted as the barcoding region of fungi, the partial translation elongation factor 1-α (TEF) gene, the large subunit (LSU) and the small subunit (SSU) region of rDNA was also amplified and sequenced in case of isolates representing different lineages. Altogether 361 isolates were collected from the two plants, 197 isolates from maize and 164 from wheat. The isolates represented 21 fungal taxa dominated by ascomycetes and five lineages, which have been detected previously in grass roots worldwide. Representatives of these clades are chosen for inoculation experiments to test the hypothesized different effects to the 'home' conditions or to the original hosts. These results may provide important information on the inoculum production for better survival of agronomically important gramineous plants.

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ROLE OF SURVIVAL FACTOR GENES IN THE PATHOGENICITY PROCESSES OF *MUCOR CIRCINELLOIDES*

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Mucor circinelloides is a filamentous fungus belonging to the order Mucorales. Some members of this fungal group may cause fatal invasive infections called mucormycoses. The survival factor protein (SVF) plays a crucial role in the protection of cells from oxidative and other stresses (e.g., cold stress) in *Saccharomyces cerevisiae*. Furthermore, this protein participates in the sphingolipid biosynthesis of the cell membrane. Transcriptomic studies showed the upregulation of the encoding genes in several human pathogenic fungi during the host-pathogen interactions. However, the function and regulation of the SVF protein and the encoding gene(s) are still quite unknown in zygomycotic fungi. In the *M. circinelloides* genome, two hypothetical *svf* genes were identified and named as *Svf1* and *Svf2*. We have studied the expression of the genes after culturing the fungus under different conditions by real-time quantitative reverse transcription PCR. Using the CRISPR/Cas9 technique, single gene disruption mutants were constructed for each gene and we have started the characterization of the resulting strains. Macromorphology and sensitivity to different stressor chemicals (e.g., acetate, H_2O_2, Congo red and calcofluor white) were tested. Mutants showed altered characteristics compared to the original strain suggesting that the cellular integrity may be damaged in the mutants. Pathogenicity of the mutants was also examined in alternative *Drosophila melanogaster* model and a decreased virulence was detected. In *Galleria mellonella* model organism we observed increased virulence. We also carried out susceptibility test of our strains against various antifungal drugs (e.g. different azoles, amphotericin B). We also studied the differences of carotene content after treatments with various stressors.

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THE DELETION OF THE FUNGUS SPECIFIC CAPPZ1 PHOSPHATASE ENHANCES THE OXIDATIVE STRESS SENSITIVITY OF *CANDIDA ALBICANS* IN THE PRESENCE OF BETAMETHASONE

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Corticosteroids are widely used in medical practice owing to their anti-inflammatory, immunosuppressive and anti-allergic effects. The topical glucocorticoid therapies however increase the susceptibility of the patients to superficial fungal infections. This side effect is due to the immunosuppressive properties, as well as to a direct interaction of steroids with *Candida* species. As we demonstrated earlier, side effects of glucocorticoid betamethasone (BM) can be counteracted with the superoxide generating agent menadione (MSB) since the steroid made this pathogenic yeast more sensitive to oxidative stress. In addition, we found that the fungus specific protein phosphatase Z1 (CaPpz1) enzyme has a pivotal role in the pathogenicity and in the oxidative stress response of *C. albicans*, thus it can be considered as a potential drug target for the treatment of mucosal infections. The aim of this study was to investigate the combined effects of the CaPPZ1 gene deletion and the MSB induced oxidative stress on the physiological and transcriptional responses of the BM treated *C. albicans* strains. We found that the combined stress exposure significantly reduced the growth rate and elevated the activities of antioxidant enzymes as well as the ROS contents in comparison to MSB treated cells. The oxidative stress treatment had only in a moderate effect on the transcriptome (698/848 genes up/down) in the deletion mutant strain, while the presence of BM increased significantly the number of differentially expressed genes (1,185/1,203 genes up/down). Gene set enrichment analyses demonstrated that the genes involved in translation, ribosome biogenesis, TCA cycle, iron ion transport and RNA metabolic processes were downregulated, while genes related to the glycolysis, ethanol fermentation, fatty acid metabolism, oxidative stress response and peroxisome-related processes were upregulated in the MSB and BM treated mutant cells. Taken together our observations suggest that the synergism between the fungus specific CaPpz1 inactivation and between the more general MSB oxidative stress treatment can be utilized in a novel combinatorial antifungal strategy for the elimination of the side effect of steroid therapy in the fields of dermatology and ophthalmology.

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THE *ASPERGILLUS TERREUS* ITACONIC ACID FERMENTATION: PHYSIOLOGY BEHIND TECHNOLOGY

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Itaconic acid is used as a bio-based, renewable building block in the polymer industry. It is produced by submerged fermentations of the filamentous fungus *Aspergillus terreus* from molasses or starch, but research over the efficient utilization of non-food, lignocellulosic plant biomass is soaring. The key parameters for obtaining high itaconic acids yields in standard technical-scale itaconic acid fermentation are a sucrose/glucose concentration of over 100 g/L, a pH below 2.5, a high oxygen tension and a strict deficiency ($<10^{-7}$ M) of Mn^{2+} ions. Some of them have been interpreted to influence the regulation of D-glucose catabolism and its energy balance. Importantly however, D-xylose breakdown proceeds via the pentose catabolic pathway and only at later stages feeds its intermediates into glycolysis. It is not known whether this alters the influence of the above parameters on itaconic acid production. Therefore, the objective of this study was to test whether two landmark nutritional requirements of the *A. terreus* itaconic acid overflow on D-glucose – Mn^{2+} ion deficiency and high concentration of the carbon source – also occur in a similar fashion on D-xylose, the principal monomer of lignocellulose as a sole source of carbon, and whether their respective optimization would give as molar yields close to the theoretical maximum from D-xylose as from D-glucose.

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MOLECULAR CHARACTERIZATION OF NOVEL DSRNA VIRUSES ISOLATED FROM DIFFERENT ZYGOMYCETE FUNGI

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Most of the mycoviruses have double-stranded RNA (dsRNA) genome and they can be transmitted only intracellularly. In some cases, mycoviruses can be responsible for the hypovirulence of the host, however, most of these fungal viruses are carried asymptomatically, so their presence often remains unexplored. Based on recent studies mycovirus-harboring are very common among fungi, however, our knowledge about them is still very limited, especially in Mucoromycota fungi. Our aim was to detect and characterize the dsRNA fragments in different fungal isolates belonging to the Mucoromycota. We would also like to purify the virus particles from the virus-harboring strains, as well as the elimination of these viruses from host fungal isolates. We found 36 dsRNA-harboring strains from the total 235 investigated isolates. For the molecular identification of the detected dsRNA elements of 7 *Umbelopsis*, 14 *Mortierella*, 4 *Mucor*, 7 *Rhizopus* and 3 *Lichtheimia* strains the full-length amplification of cDNAs (FLAC) and the whole genome sequencing technique was used. These isolates harbor different dsRNA patterns with 1 to 6 discrete and differently sized (0.7-10.0 kb) dsRNA bands. Some isolates have been examined in detail: the determined dsRNA genomes of the novel viruses contain two open reading frames, which encodes the coat protein and the RNA dependent RNA polymerase. Phylogenetic analyses confirmed their relationship with the Totiviridae family. Transmission electron microscopy revealed the presence of two different sizes isometric virus particles in the *Umbelopsis ramanniana* NRRL 1296 strain. Attempts to eliminate viruses from virus-harboring strains are in progress as well as the comparison of the morphology of the virus-harboring and the virus-free isolates.

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THE APPLICATION OF MALDI-TOF MS IN THE CHARACTERIZATION OF OPPORTUNISTIC PATHOGEN PSEUDOMONAS AERUGINOSA

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Mass spectrometry is a powerful technique to identify bacteria by their protein content and to assess the functional traits through the analysis of specialized metabolites. A commonly used method is Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) that was developed for the rapid identification of large biomolecules. MALDI-TOF MS coupled with software analysis allows bacterial identification at the genus, species, and strains level based on their proteomic spectra profile. The aim of this study was the optimization of the MALDI-TOF MS method for the detection of strain-level biomarker peaks of an environmentally relevant opportunistic pathogen microorganism, *Pseudomonas aeruginosa* using five reference strains of different (clinical and non-clinical) origins with various antibiotic resistance profiles. MALDI-TOF MS profiling was carried out in a Bruker Biotyper system. Biological samples were extracted with different protocols (direct upload, ethanol precipitation, acetonitrile/formic acid extraction) and were overlaid with two commonly used matrix solution (HCCA and SA). Spectra were preprocessed and analyzed by the free statistical software Mass-Up following the instructions and settings described previously. Antibiotic resistance profiles were determined with the quantitative ETEST. Based on our results, the MALDI-TOF MS method was optimized for the strain level differentiation of species *P. aeruginosa*. The recommended preparation and extraction protocol ensure the reliable and repeatable measurement of *P. aeruginosa* strains of various origins. Based on the hierarchical clustering analysis of bacterial spectra, MALDI-TOF MS is a promising tool to identify biomarker peaks for further comparative (phenotypic, molecular, and phylogenetic) analysis including the biomarkers of antibiotic resistance.

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RESPONSE OF AEROBIC GRANULAR SLUDGE TO THE PRESENCE OF CARBON BASED NANOMATERIALS IN SEQUENCING BATCH REACTORS: NUTRIENT REMOVAL, EXTRACELLULAR POLYMERIC SUBSTANCES, AND MICROBIAL POPULATION

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This study investigated the bioreactor performances (removal of COD, NH_4^- N, NO_2^- N, NO_3^- N, and TP), secretion of extracellular polymeric substance (EPS) and microbial population dynamics of aerobic granular sludge (AGS) under chronic exposure (10 days) to graphene nanoparticles (G NPs), graphene oxide (GO) NPs, single-walled carbon nanotubes (SWCNT), and multi walled carbon nanotubes (MWCNT) at concentrations of 1, 5, and 10 mg/L in sequencing batch reactors (SBRs). The results showed that nanomaterials at 1 mg/L did not influence negatively the bioreactor performances; however, in the case of higher concentrations, the removal rate of COD, NH_4^- N, and TP decreased after 10 days. Both contents of EPS and the ratio of protein (PN) to polysaccharides (PS) changed significantly. Results of 16S rRNA gene deep sequencing showed that nanomaterials at all applied concentrations caused changes in microbial community of the sludge compared with the control bioreactor. Proteobacteria, Actinobacteria, and Bacteroidetes were the top three predominant phyla in all the sludge samples. Several genera that were found in the control reactors, such as Nakamurellaceae, Streptophyta, *Devosia*, and *Lysobacter* disappeared or reduced to below the detection limit in the contaminated reactors. These microorganisms play an essential role in nutrient removal and in the secretion of EPS. However, many genera appeared during the nanomaterial exposure, e.g. *Zoogloea*, *Aeromonas*, *Gemmobacter*, and *Raoultella*.

STUDIES ON THE DIVERSITY AND PHYSIOLOGY OF *CHAETOMIUM* SPECIES ISOLATED FROM CLOSED ENVIRONMENTS

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The genus *Chaetomium* is the most common representative of the family Chaetomiaceae in indoor environments. *Chaetomium* species represent ubiquitous cellulolytic fungi that are able to degrade moist cellulose-containing building materials, such as timber and plywood, as well as synthetic building materials like plastics and gypsum wallboard. Members of the genus produce more than 500 bioactive metabolites including various mycotoxins. Several *Chaetomium* species have been described as the causal agents of onychomycosis and some of them are capable of opportunistically causing systemic infections. Our research aims were to identify *Chaetomium* strains isolated from Hungary and Finland, to examine their morphological properties, and to compare *Chaetomium* species and their properties isolated from different sites. During the research, *Chaetomium* sp. strains were examined. 16 strains were isolated from flats and offices in Hungary, while another 19 strains from schools, flats, and offices in Finland. We studied the colony morphology of *Chaetomium* sp. strains by culturing them on different media. The phenotypic properties of the fungi were examined by optical- and scanning electron microscopy. The species-level identification of the isolated *Chaetomium* sp. strains was carried out by the sequence analysis of diagnostic DNA-regions. The dominant species among the identified strains was *Ch. globosum* (80% of the identified strains) both in Hungary and Finland. In addition, we identified three isolates as *Ch. cochliodes* (one from Hungary and two from Finland), and two isolates as *Ch. rectangulare* from Finland.

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INVESTIGATION OF VANCOMYCIN RESISTANT FECAL *ENTEROCOCCUS* SPP. IN THE DANUBE AND DRINKING WATER

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The prevalence of antibiotic-resistant bacteria has increased in both the clinical and the natural environment. Drinking water supply in Hungary is highly dependent on bank-filtered water resources, so it has become important to study the prevalence of antibiotic-resistant organisms in the surface water bodies, which provide most of the bank-filtered water

flow. The Clean Drinking Water Project aims to investigate water quality changes, including the presence of antibiotic resistant bacterial strains in a bank-filtered water supply system from the Danube to the consumer's tap. This work presents only the results of vancomycin resistant Enterococci (VRE) analysis. Sampling was carried every two weeks from the Danube upstream and downstream to Budapest as well as from bank-filtered wells and the drinking water supply system. Samples were processed by membrane filtration on vancomycin-containing and vancomycin-free agar. The bacterial colonies were identified by MALDI-TOF MS. Fecal Enterococci were detected in the majority of samples from the Danube. The highest colony counts were found in the winter period, and counts were systematically higher in the Southern sampling area than in the Northern one. During the entire study period, only two *Enterococcus* strains were isolated from the water supply system. VRE were present in detectable numbers in the Danube, but were not cultured from the drinking water supply system. From the 27 isolates, 26 belonged to the species *E. faecium* and one was identified as *E. casseliflavus*. The strains - which phenotypically proved to be VRE in culture – were further investigated with disk diffusion test and PCR-based antibiotic resistance analysis. The *E. faecium* strains responded similarly to most antibiotics. Differences were observed mainly for teicoplanin; the strains, which were sensitive to teicoplanin, were also sensitive to gentamicin. On the other hand, *E. casseliflavus* differed from the other strains in its antibiotic resistance profile. The presence of *vanA* and *vanB* genes is associated with vancomycin resistance. Twenty-one of the 27 VRE strains carried the *vanA* gene, and *vanB* gene was detected from the remaining six strains. The enterococci strains isolated from the water supply samples were sensitive to vancomycin and the other nine tested antibiotics. Neither the *vanA* nor the *vanB* gene were detected in these strains. Our investigations confirmed that Budapest is a source of microbiological load for the Danube. The colony count of the bacteria showed seasonality and correlation with the water level. Our work demonstrated the presence of VRE strains in surface waters, which is a growing problem for human health. However, bank-filtration proved to be efficient in removing antibiotic resistant strains.

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SHIFTS IN SOIL MICROBIAL COMMUNITY COMPOSITION AS A FUNCTION OF TRACE ELEMENTS IN THE IRRIGATION WATER

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The aim of the project is to investigate the effects of toxic arsenic and boron and essential selenium and iodine trace elements on the soil-water-plant system. This study tries to answer the question of how the trace element content of irrigation waters affects the diversity of microbial communities in different soils. Institute carried out outdoor rhizobox experiments for Soil Sciences and Agricultural Chemistry-Centre for Agricultural Research. The concentrations of trace elements in irrigation waters were chosen based on light-room experiments, as presented earlier. Three different concentrations were used for each trace element: values corresponding to the threshold and the 50% effective concentration, and a control. Based on this, the final concentrations were 0.1 mg/L and 0.5 mg/L for B, I and Se, and 0.05 mg/L and 0.2 mg/L for As. Three various soil types (sandy, loamy and sandy-loam soils) and six different vegetables (cabbage, carrot, bean, pea, potato and tomato) were used, and at the harvest, phase, rhizoplane and bulk soil samples were collected. From the 975 soil samples, 486 representative samples were chosen for molecular based community investigation. Total community DNA was extracted, and the partial sequence of 16S rDNA was multiplied by nested PCR. Following the DGGE, similarities between the unique fingerprint patterns were analyzed using the Pearson correlation coefficient. Similarities were displayed graphically as a dendrogram. The clustering algorithms used to calculate the dendrograms was an UPGMA method. Based on these, it is possible to determine what is the effect of the soil types, the added trace elements or the grown plants in the vessels on the microbial community of the soil. Although in silico analyses are ongoing, some conclusions can already be drawn. In the most cases, not all parallels cluster together and no correlation was observed between diversity of the microbial community and soil type. Rhizoplane samples were expected to separate from the bulk soil samples, but this hypothesis was only confirmed for a minority of the setups (bean:Se; tomato:Se; pea:As and cabbage:As). The effect of trace elements was also seen only for some of the combinations: e.g. bean:rhizoplane, pea:bulk soil and pea:loamy soil, the samples that were treated with boron and iodine separated from those irrigated with arsenic or selenium. Further analysis is necessary to resolve the reasons behind the observed diversity. DGGE profiles will be also correlated to data from the plants treated with different trace elements.

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STUDY OF THE BIODEGRADABILITY OF POLYLACTIC ACID-BASED BIOPLASTICS

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For the comfort of modern living, the use of plastics has become essential (clothing, medicine, packaging, food industry) and the production of plastic has preceded the production of any other material. Due to beneficiary properties of plastic (strength, stiffness, etc.) the availability is versatile and easy, although after usage the natural degradation of plastic is very slow, or partly undegradable, causing serious pollution to the environment. It is our own interest to maintain a healthy habitat and reduce the consumption of plastic. According to a regulation adopted by the European Parliament in 2019 (Directive 2019/904), the use of petroleum-based plastics in the European Union should be phased out from 2021 to the use of alternative biodegradable plastics in order to reduce pollution. Such an alternate and environmentally friendly solution can be to switch to the use of bioplastic made of biological resources (renewable energy sources) and biodegradable materials. In this field, it is resolved to produce, heat tolerant PLA (polylactic acid) from lactic acid with poliesterification in industrial conditions. Meanwhile there is less information available about the degradation of polylactic acid, it is the reason why this has been chosen as the research topic. First of all, it has to be determined if the different type of natural polymers could be used in the production of PLA-composites. After this can be determined the correct mixing ratio of the right biocomposites, and as well the optimization of the production steps. It is crucial to get to know the background of the biological degradation process next to the knowledge of preparations of production process. For this first, the acquisition of microorganism from the natural specimens needed. Because of the strain, selection pre-experiments are necessary for testing the degradation of polylactic acid, which examines the applied microorganism's PLA utilization on solid medium. Another important step is to map the enzyme set of the applied microorganisms especially because there are references in the scientific literature about the degradation of natural polymers are coherent with proteolytic and lipolytic enzyme activity properties of certain microorganisms. The examination of the microbiological degradation of PLA and bioplastic made of PLA composite can be followed by the released lactic acid and the mass change by the help of previously selected microorganism strains. Hopefully, by selecting new microorganism strains suitable for biodegradation, then increasing the efficiency of these strains and expanding the knowledge gained on the subject, it will provide a promising basis for developing and increasing the biodegradability of polylactic acid-based bioplastics.

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INHIBITION OF BIOFILM FORMATION OF FOOD-CONTAMINATING BACTERIA AND YEASTS BY NATURALLY OCCURRING PHENOLICS

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Plants contain phenolic compounds are secondary metabolites. They can contribute to pigmentation and adaptation processes and act as inhibitors against pathogens as well. The food-contaminating microorganisms can alter the quality and sensory characteristics of foods, as well as can cause infections by entering to the human body. Most of them can form biofilms resulting in increased resistance to antibiotics and other environmental adverse factors. Certain phenolics have gained attention in the food research as natural antimicrobials, and some compounds have been tested for the anti-biofilm activity as well. However, there is a need for comprehensive analysis and testing of the effects of new compounds against food-related yeasts and bacteria. Here, we evaluated the anti-biofilm activity of hydroxybenzoic (4-hydroxybenzoic, gallic, vanillic, syringic) and hydroxycinnamic (cinnamic, p-coumaric) acids and other phenolic derivatives, namely (−)-epicatechin, resveratrol, vanillin and quercetin, against food pathogens and spoilers. Four yeasts, i.e. *Debaryomyces hansenii*, *Pichia anomala*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, and four bacteria, i.e. *Escherichia coli*, *Pseudomonas putida*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were involved to the tests in which we applied the phenolics at 500 µg/mL concentration. Cinnamic acid, coumaric acid and vanillin demonstrated the highest activity against most of the biofilms tested. Inhibitory effect of the other phenolics tested varied depending on the biofilm investigated. The phenolics that were effective can be suitable candidates for the development of natural preservatives and/or sanitizers.

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CHARACTERIZATION OF A GLYCEROL-PHOSPHATE DEHYDROGENASE GENE *GFDB* IN *ASPERGILLUS NIDULANS* AND *ASPERGILLUS GLAUCUS*

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Glycerol-3-phosphate dehydrogenase catalyzes the conversion of dihydroxyacetone phosphate into glycerol 3-phosphate, which is subsequently converted into glycerol by a phosphatase. The genome of *Aspergillus nidulans* harbors two putative glycerol 3-phosphate dehydrogenases encoded by the genes *gfdA* and *gfdB*, while the genome of the osmophilic *Aspergillus glaucus* accommodates only the orthologue of the *A. nidulans gfdA* gene. Our aim was to decipher the role of *gfdB* therefore, we constructed a gene deletion mutant in *A. nidulans* by the double-joint PCR method and inserted the *gfdB* gene into the genome of *A. glaucus* with the adaptation of the *Agrobacterium tumefaciens*-mediated transformation protocol. The ΔgfdB mutant showed oxidative stress sensitivity in the presence of tert-butyl-hydroperoxide (tBOOH), diamide as well as hydrogen peroxide and showed moderate sensitivity towards the cell wall stress-inducing agent CongoRed. The increased oxidative stress sensitivity of the mutant also manifested in elevated intracellular oxidative species concentrations and reduced activities of the antioxidant enzymes catalase and glutathione peroxidase. The accumulation of intracellular oxidative species resulted in reduced viability and eventually, early onset of apoptosis in the ΔgfdB hyphae. In *A. glaucus* the *gfdB* complemented strain showed increased stress resistance in the presence of the oxidative stress generating agents including tBOOH, H_2O_2, menadione, as well as the cell wall integrity stress-eliciting Congo Red and the heavy metal stress inducing $CdCl_2$. Our results elucidate the fact that the *gfdB* gene from *A. nidulans* is also involved in the stress responses of the complemented *A. glaucus* strains strengthening our hypothesis on the role of *gfdB* in the oxidative stress defense in the *Aspergilli*. Nevertheless, the osmophilia of *A. glaucus* cannot be interpreted solely by the absence of the *gfdB* gene as we hypothesized earlier.

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ROLE OF PLEIOTROPIC DRUG RESISTANCE PROTEINS IN AZOLE RESISTANCE OF MUCOR CIRCINELLOIDES

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Mucormycosis is a life-threatening systemic infection caused by certain members of the filamentous fungal order Mucorales (*Rhizopus oryzae*, *Lichtheimia corymbifera* and *Mucor circinelloides*). Mucormycosis has a high mortality rate, which can be 30-95% depending on the underlying condition. Mucoromycota species are intrinsically resistant to most of the routinely used antifungal agents, such as azoles, which makes treatment difficult. In case of *Candida albicans* and *Cryptococcus neoformans*, several members of pleiotropic drug resistance proteins were described as contributing in the azole resistance of fungi. In case of Mucoromycota species, the role of PDR proteins has not been examined. After posaconazole treatment, RNAseq analysis was performed and one up-regulated PDR protein was identified (pdr1). In the *Mucor* genome database, further seven *pdr* genes were found. The relative transcript level of the *pdr* genes was measured after azole treatment using real-time quantitative PCR. We have started to disrupt the *pdr* genes using a CRISPR-Cas9 system. Sensitivity of the isolated mutant to different antifungal agents was examined. Our result suggested that expression of the *pdr* genes respond to the azoles, especially *pdr1*. Disruption of *pdr1* and *pdr2* resulted in increased sensitivity to posaconazole, ravuconazole and isavuconazole.

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ANTITUMOR EFFECT OF VISCUM ALBUM

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Viscum album L. the European mistletoe, species of the Santalaceae family, is a hemi parasite evergreen plant and used as a pharmacognostical drug. Traditionally the drug has been used in the treatment of hypertension, anxiety, insomnia and complementary cancer therapies. ABCB1 efflux pump is a membrane-associated protein, member of the superfamily of ATP-binding cassette (ABC) transporters. These transporters primary responsibility is to transport various molecules across the cell's membrane, however, it is also accountable for the resistance to anticancer drugs. Our aim was to investigate the potential ABCB1 efflux inhibitor effect of *Viscum album* extract. With cold percolation method a 50 mg/mL solution was produced, after that using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide the IC50 concentration of the drug on A-549, HeLa, Hep2, BB-19, CaCo-2, HL-60, K562, THP-1, U937 and KATO-III cells were determined. The cells were treated with IC50 concentration for 24h and after with TRIzol reagent the DNA was extracted. With 2% of agarose gel, the potential apoptosis effect of the extractum was checked. Finally, RH123 accumulation test was performed with Flow-cytometry. The MTT-test revealed that the extractum has on U937, THP-1, Hep2 and A-549 cells linear toxicity, however, on the remaining cells, this was logarithmic. The solution induced necrosis on THP-1, U937, HL-60, K562, BB-19 and KATO-III and on A-549, HeLa, Hep2 and CaCo-2 apoptosis. Interestingly, the *Viscum album* extractum inhibited the efflux pump on THP-1, K562, HL-60 and BB-19 cells, but on CaCo-2, U937, KATO-III and HeLa induced the efflux pump. Based on our results, *Viscum album* has potential antitumor effect on K.562 and HL-60 cells.

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LACTIC ACID BACTERIA ISOLATED FROM RUMINANT ANIMALS

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Lactic acid bacteria were chosen as the focus of our study, because they are widely used in the food industry, mainly for production of dairy products, in the agriculture, animal breeding, and human health-care as probiotics. Beside their beneficial effect on health, several lactic acid bacteria can bind mycotoxins on their surface according to the literature, which implies their benefits as feed supplements. During the study, we isolated presumed lactic acid bacteria from faces of four ruminants on lactic acid bacterium selective MRS (de Man Rogosa and Sharpe Agar) plates. Thirty seven strains from faces of grey cattle (Bos primigenius taurus hungaricus) and eighty six strains from European bison (*Bison bonasus*) from the Miskolc Zoo, Miskolc, Hungary, eight strains from buffalo (*Bubalus bubalis*) from the Buffalo Reservation, Mórahalom, Hungary, and sixty three strains from American bison (*Bison bison*) from Pécs, Hungary were collected. Molecular microbiological methods were used for the taxonomic identification of the presumed lactic acid bacterium strains. Pure cultures of the isolated strains were prepared on MRS agar plates and the DNA was extracted with DNeasy UltraClean Microbial Kit (BioMarker). The DNA samples were used as template in REP-PCR analysis done with primer 5′-GTGGTGGTGGTGGTG-3′, followed by agarose-gel electrophoresis and the strains were grouped based on their patterns. Representatives of each group were analyzed by 16S rDNA sequencing and identified by comparing the sequences to online databases. The aim of our research was to isolate lactic acid bacteria from faces samples of ruminants, to find species specially adapted to the intestinal microbiome of herbivorous animals. Our plan is to investigate the toxin-binding ability of the isolated LAB strains. Strains with the best toxin-binding capacities will be used in animal feeding studies.

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DEVELOPMENT OF MICROBIOLOGICAL BIOAUGMENTATION SEED CULTURE FOR IN SITU REMEDIATION OF PAH, BTEX AND TPH CONTAMINATED SOIL AND GROUNDWATER

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Detection and elimination of organic hydrocarbon pollutants present in geological media is a great challenge in environmental and remediation practices. PAH contaminants (e.g. naphthalene, phenanthrene, anthracene, pyrene, benzpyrene, etc.) are slowly degradable, persistent substances that can enter soils from both natural and anthropogenic sources. PAHs

are formed during fires, occur in smoke and in the vicinity of petroleum refineries and pipelines. Several members of the PAH compound family are carcinogenic (e.g. benzpyrene), so the required remediation targets are extremely low (in ppm order). The use of environmentally friendly on-site processes developed for PAH pollutants in remediation procedures is still little known and not widespread on an industrial scale. PAH contaminants are persistently present in soil and groundwater. Remediation and regeneration of contaminated soils and groundwater is an intensively researched area worldwide. Fermentia Microbiological Ltd., Elgoscar-2000 Ltd., Budapest University of Technology and Economics and Eötvös Loránd University have combined their interdisciplinary resources within the framework of the "Development of inocula based on metagenomic methods for in-situ bioremediation of polycyclic aromatic hydrocarbons with the support of a new on-line monitoring system." R&D project (2017-1.3.1-VKE-2017-00013) to develop a new, marketable soil remediation method for the removal of polycyclic aromatic hydrocarbons (PAHs) and most common concomitant pollutant compounds typically found in areas contaminated with petroleum and its derivatives. The main goals of the project are to produce effective biological - microbiological inocula, to determine optimal remediation conditions and to develop an on-line controlling and monitoring technology for the removal of PAH compounds. Our project aims to create biological-microbiological inocula and technologies for an environmentally friendly, in situ remediation of soils and groundwater contaminated with mainly PAH and other accompanying fractions e.g. BTEX and TPH, based on well-integrated biodegradation-bioaugmentation processes, in order to protect the environment and our natural resources. In pilot scale experiments in contaminated areas of Hungary, inoculum prototypes have already yielded encouraging results. The bioaugmentation inocula developed and registered during the project, as well as the related technologies supporting field applicability, represent a new direction in the market of remediation services, which can play a key role in the remediation of many domestic or cross-border areas.

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SELECTION OF ABIOTIC STRESSTOLERANT SOIL BACTERIA ADAPTED TO DETERIORATED SOILS AND ESTABLISHING A STRAIN COLLECTION

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The project aimed at the isolation of bacterial strains isolated from deteriorated soils and the establishment of a strain collection from the strains that showed plant growth promoting, soil ameliorating and biocontrol properties. 10 different soil types were selected from various regions of Hungary. Low-fertility, deteriorated soil types (dystric and eutric arenosol, alisol, sodic solonchak, solonetz, dystric gleysol, dystric fluvisol) were sampled for the isolation of soil bacteria. Over 1,300 strains were isolated that are adapted to extreme abiotic conditions and – as part of the native microflora -are able to enhance soil microbial activity when reinoculated in deteriorated soils. Stress-selective isolation and classical microbiological methods were used to select abiotic stress tolerant (pH, salt concentration, low temperature), plant growth promoting and biocontrol effecting (nitrogen fixing, phosphate and potassium solubilizing, siderophore producing, phytohormone producing) and soil aggregation promoting (EPS producing) bacterial strains. The isolated strains were classified according to stress tolerance and biological activity. Strains showing high stress tolerance and plant growth promoting ability were identified by 16S rDNA sequence analysis and BLAST database. A strain collection was composed of strains that are able to the regional climatic conditions at extreme soil pH levels and high salt concentrations based on the Soil Bacterial Screening System [1]. The stress tolerant strains were assigned to target soil types that were categorized into three main categories: acidic, alkaline and neutral. The classification may serve as a basis for the composition of soil inoculants.

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[1] Kutasi et al (2015) WO 2015/118516.

THE BIGGER THE BETTER: SEQUENCING AND ASSEMBLING POLYPLOID, HIGHLY HETEROZYGOUS BAKER'S YEAST GENOMES WITH THE HELP OF ONT LONG-READ SEQUENCING

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The assembly of polyploid, highly heterozygous genomes presents specific challenges for scientists and bioinformaticians. The emergence of a new generation sequencing techniques, such as the Oxford Nanopore Technologies MinION long-read sequencing, gives us a unique possibility to combat these challenges. With the help of the combination of short- and long-read sequencing data, it is now possible to tackle a highly continuous, detailed de novo genome assembly and gene annotation. Here we present the sequencing and genome assembly of two commercially available baker's yeast genomes that are tetraploid and highly heterozygous. One such baker's yeast isolate originated from an Active Dry Yeast (ADY) product, while the other was collected from a pressed yeast product. We performed high molecular weight DNA isolation, DNA quality measurements using Nanodrop and Qubit, and library preparation using ONT kits SQK-LSK109 and EXP-NBD104. Sequencing took place on a FLO-MIN106 flow cell with a MinION device with an estimated coverage of 100×. The same samples were sequenced with deep coverage using Illumina technology with 150 bp paired-end reads with an estimated coverage of 400× of the nuclear genome. Genome assembly and annotation were executed using the LRSDAY v1.6.0. pipeline implementing Canu for long-read based assembly. The assembly was polished with long- and short-reads as well, using the softwares racon-medaka and pilon. GenomeQC was used for assembly quality check. In long-read sequencing statistics for the ADY isolate, we got 104,000 reads, the read length N50 was 18,252. For the pressed yeast, we got 104,000 reads, the read length N50 was 18,840. After assembly, for the ADY, we got 19 scaffolds (N50 = 952,013, L50 = 6% N = 0.33. BUSCO: 95%). For the pressed yeast, we got 17 scaffolds (N50 = 926,576, L50 = 6, %N = 0.37. BUSCO: 94.5%). Based on the sequencing summary statistics, the assembly statistics and the BUSCO analyses, we can say that the attempt to a continuous de novo assembly was successful and resulted in a high quality genome. Since our strains are tetraploid, it is of great interest to perform haplotype phasing on them, utilizing their own long+short read assemblies as reference genomes, and circumventing the use of the S288c haploid reference genome that is phylogenetically distinct from our isolates' genomes. This task is currently being optimized using the software nPhase, with the aim of completely describing every chromosome's every haplotype in these commercial tetraploid yeasts.

DE-NOVO GENOME PROJECT OF *THERMOBIFIDA ALBA* STRAIN DSM 43795

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The *Thermobifida* genus includes four species: *T. fusca*, *T. alba*, *T. cellulosilytica*, and *T. halotolerans*. *T. fusca YX* is the best-characterized strain of this taxon and serves also as a model strain for cloning several cellulases and hemicellulases for industrial purposes targeting paper industry, biofuel, and feeding applications. The hemicellulolytic enzymes including glycosyl-hydrolases of *T. fusca*, *T. cellulosilytica* and *T. halotolerans* was already identified and described while there was not any genomic data of *Thermobifida alba* yet, despite *Thermobifida alba* DSM 43795 is the genus type strain. Thus, the main focus of our work is the whole-genome sequencing of *Thermobifida alba* by NGS techniques and later on determining its hemicellulolytic enzyme system. The Illumina and ION-torrent based NGS techniques results were not satisfying for further analysis or even for publications because they both yielding a genome with 300 plus contigs. The genome of *T. alba* is high in GC content and contains high number of repetitive elements therefore made it difficult for these sequencing methods to decrease the number of contigs and to assemble the DNA fragments in the right way. To overcome these problems there is a new sequencing technique known as PacBio-sequencing, which works with 12,000 bp ORFs, but for this method, we needed fully intact, not-shattered, high quality DNA sample. Optimization of the isolation methods of the genomic DNA included different lysis protocols and treatments of the bacterial culture were done: sonifier with zirconia grinding beads and lysis buffer; mechanical lysis of lyophilized bacteria cells; combination of all-above complemented with a DNA isolation kit. Finally, by the use of our optimized DNA isolation technique, genomic DNA with appropriate length and quality for PacBio sequencing was obtained. For annotating the *T. alba* genome results of the NGS sequencing and of the PacBio sequencing were combined, yielding a 4.9 Mb (4,903,256 bp) genome with 72.1% GC content on one contig. The genome contains 4,274 CDSs/ putative coding sequences with 51 tRNAs, 3 ncRNAs and 4 -4 (5S, 16S, 23S) rRNAs in total. The analysis of the annotated genome revealed it possess 29 putative glycoside hydrolases

belonging to 18 different GH-families, 35 glycosyl-transferases, 30 proteases. Interestingly the 4.9 Mb genome size of *T. alba* is much bigger than its relatives measured by Illumina and ION-torrent based sequencing: the 3.6 Mb *T. fusca* genome and 4.3 Mb *T. cellulosilytica* genome. It regrets the possibilities that the former genomes were not complete. The control experiments are in progress.

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THE ROLE OF ALTERNATIVE OXIDASES IN CITRIC ACID FORMATION BY *ASPERGILLUS NIGER*

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Reduced co-factors NADH and $FADH_2$ formed during sugar catabolism are regenerated in the terminal oxidation process. In the inner mitochondrial membrane, electrons flow through a transport chain and ultimately reduce molecular oxygen to water by the cytochrome oxidase. In parallel, protons are being pumped into the intermembrane space of mitochondria, and the resulting proton gradient provides energy for ATP synthesis. Mitochondria of a variety of organisms including most fungi contain a cyanide resistant and hydroxamic acid sensitive terminal oxidase called alternative oxidase (AOX) that branches off the main pathway prematurely to reduce oxygen. This process is therefore not coupled to ATP synthesis (free energy changes turn to heat), hence enzymes of the glycolytic pathway are relieved from ATP inhibition, allowing carbon catabolism to proceed unabated. This is crucial for the metabolic overflow that lies at the heart of high-yield citric acid production. We observed that several Ascomycota genomes contain two or even three alternative oxidase paralogues, as exemplified by *Neurospora crassa* and *Candida albicans*. Two of the genes are the result of recent duplication. The third gene, based on sequence divergence, was branched off much earlier. This third gene was either lost in some lineages or acquired horizontally by some lineages. The Ascomycete filamentous fungus *Aspergillus niger* – a long-time workhorse of industrial fermentations – has two alternative oxidase genes, *aox1* and *aox2*. Both genes were disrupted individually in the high citric acid producer NRRL2270 strain, and fermentation profiles were compared using optimized conditions (>30% dissolved oxygen levels, 14% initial carbon source concentration, suboptimal phosphate levels and strict [<5 ppb] manganese(II) ion limitation) in 2L scale bioreactors. The utilization of D-glucose utilization and the accumulation of mycelial biomass were faster in the parent strain than in either of the two deletion mutants. As well, the citric acid yields were higher in the parent strains than in the two deletion mutants. There were significant differences in the citric acid yields of the two deletants, with the *aox2* deletant producing less citric acid. This finding suggests that an intact AOX1 could not compensate for the loss of AOX2, implying that the two alternative oxidases play distinct physiological roles in *A. niger*.

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COMPARISON OF THE EFFECTS OF FERTILIZATION AND CULTIVATION METHODS ON THE SOIL PROKARYOTIC COMMUNITIES OF A LONG-TERM FIELD EXPERIMENT

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The lasting effects of cultivation techniques on crop yield, soil properties and microbiome can be thoroughly monitored in long-term field experiments. This study aimed to monitor the composition of soil bacterial and archaeal communities for a growing season in a research initiated in 1961 in Martonvásár (Hungary). The prokaryotic communities of cultivated soils under different systems of crop rotation (maize monoculture and maize-wheat diculture) and fertilization (unfertilized control, inorganic fertilizer, and inorganic fertilizer supplemented with farmyard manure), and that of a fallow land were compared based on 16S rRNA amplicon sequencing data. Majority of the bacterial sequences belonged to the phyla Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia, Gemmatimonadetes, Planctomycetes and Chloroflexi, while the archaeal communities were dominated by ammonia-oxidizing Thaumarchaeota phylum. Compared to the cultivated soils a shift in the ratio of Acidobacteria, Actinobacteria and Nitrosophaeraceae-related sequences can be observed in the field set aside for several decades. The fertilization regime was found to be a stronger driver of compositional differences than the type of crop rotation and plant phenophase. The long-term application of inorganic fertilizer resulted in a significantly distinct community composition, for instance higher ratios of Acidobacteria groups, SC-I-84,

Sphingomonas, WPS-2 and *Candidatus* Nitrosotalea. These effects, however, were mitigated by the additional farmyard manure in the case of the combined application of inorganic and farmyard manure fertilizers. The differences between monoculture and diculture samples were pronounced the most in the inorganic fertilized soils. Though the phenological developmental stages of plants had smaller effects on the prokaryotic taxonomic diversity, several taxa (e.g. *Acidibacter*, *Pseudomonas*, Woesearchaeia, unclassified Sphingomonadaceae and Blastocatellaceae genera) showed seasonal changes in their relative abundance.

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EXTREMOPHILIC PROKARYOTIC COMMUNITIES FROM THE ACTIVE MUD VULCANOES OF BECIU (ROMANIA) REVEALED BY NEXT-GENERATION SEQUENCING

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The Vrancea zone of the Romanian Foredeep is one of the most active seismic and the largest terrestrial mud volcanic area in Europe. Viscous mud and gasses (thermogenic methane, and other short chain alkanes) are discharged from large reservoirs of hydrocarbons up to 3,000 meters deep accumulated under salt diapirs. Mud samples were taken from five gryphons near Beciu in 2018 and subjected to a detailed community analysis based on bacterial and archaeal 16S rRNA amplicon sequencing. The highest prokaryotic richness as well as the highest archaeal diversity was observed in the recently formed mud volcano. *Marinobacter* (Proteobacteria) clearly dominated the bacterial communities of the older mud volcanoes ahead of unclass. Halomonadaceae (Proteobacteria), unclass. Gracilibacteria (Patescibacteria), *Sulfurovum*, *Sulfurominas* (Campylobacterota) and *Tangfeifania* (Bacteroidota). In contrast, the genus *Sulfurovum* dominated the core of the young volcano. As for the archaeal communities, the vast majority of the sequences was related to anaerobic methane-oxidizing ANME-1b, ANME-2a-2b and ANME-3 groups (Halobacterota) in all sample. *Cand.* Syntrophoarchaeum, *Halanaeroarchaeum* (Halobacterota) and several unclassified genera belonging to Woesearcheales (Nanoarchaeota) and Methanofastidiosales (Euryarchaeota) were also detected. The presence of strictly anaerobic methylotrophic methanogenic, as well as sulfur reducing and -oxidizing prokaryotes adapted to the extremities of the studied environment suggests that microbial transformations of carbon and sulfur compounds may be the most important processes in the local biogeochemical cycles.

EFFECT OF ECHINOCANDINS IN COMBINATION WITH *NEOSARTORYA FISCHERI* ANTIFUNGAL PROTEIN 2 AGAINST *CANDIDA* AURIS BIOFILMS

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Candida auris is the first fungal pathogen to be termed a global public health threat due to its ability to spread patient-to-patient, cause invasive infections with high mortality. In addition, *C. auris* isolates frequently exhibit multidrug-resistance; furthermore, it has an excellent biofilm-forming ability to various polymeric surfaces. *Neosartorya fischeri* antifungal protein 2 (NFAP2) secreted by filamentous ascomycetes (*Neosartorya fischeri*). NFAP2 is a cysteine-rich antifungal protein, which can induce apoptosis, and interfere with the organization of the cell wall, in filamentous *Aspergillus nidulans*. Previous in vitro studies proved that NFAP2 effectively inhibits the growth of clinically relevant *Candida* spp.; furthermore, synergistic interaction was observed in combination with fluconazole against *C. albicans*. The aim of our study was to examine the in vitro interactions between three licensed echinocandins and NFAP2 against *C. auris* biofilms. We tested five *C. auris* clinical isolates (National Mycology Reference Laboratory, UK). In case of biofilm formation, strains were suspended in RPMI-1640 broth in concentration of $1*10^6$ cells/mL, and aliquots of 100 µL were inoculated onto flat-bottom 96-well sterile microtiter plates. Interactions between echinocandins and NFAP2 were evaluated against one-day-old biofilms by broth microdilution checkerboard method. The tested concentrations ranged from 1 to 64 mg/L and from 2 to 512 mg/L for echinocandins and NFAP2, respectively. Minimum inhibitory concentrations (MICs) of biofilms were defined as the lowest drug concentration resulting in at least 50% metabolic activity reduction compared to growth control. The nature of drug- drug interactions was assessed by the fractional inhibitory concentration index (FICI) interpreted as follows: ≤0.5, synergistic; 0.5 to 4, indifferent; and >4, antagonistic. The susceptibility results were confirmed by LIVE/DEAD viability staining using Syto9 and propidium iodide stain for living and destroyed cells, respectively. NFAP2

caused 16- to 128-fold, 4-to 128-fold and 64-to 128-fold decrease in median MICs for anidulafungin, caspofungin and micafungin, respectively. Moreover, median NFAP2 MIC values showed 8- to 128-fold, 16-fold, 4-to 256-fold reduction when combined with anidulafungin, caspofungin and micafungin, respectively. In case of the five tested *C. auris* biofilms, synergy was observed in case of all echinocandins in combination with NFAP2 (FICI≤0.5). Based on LIVE/DEAD viability staining, echinocandin exposed *C. auris* biofilms showed increased cell death in the presence of NFAP2 (32 mg/L echinocandin + 128 mg/L NFAP2) compared to untreated sessile populations, or echinocandin-exposed biofilms. In conclusion, NFAP2 enhanced the activity of echinocandins against the multidrug-resistant *C. auris* in vitro. Based on these findings, NFAP2 may be a potential adjuvant in newly defined *C. auris* biofilm-related alternative treatment strategies.

INVESTIGATING THE PREVALENCE OF CARBAPENEM RESISTANT ENTEROBACTERALES IN BLACK-HEADED GULLS (*CHROICOCEPHALUS RIDIBUNDUS*) AND IN THEIR FRESHWATER HABITAT

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During winter, large numbers of black-headed gulls (*Chroicocephalus ridibundus*) flock at the docks of Budapest. These birds frequently use anthropogenic food sources such as landfills and are associated with surface waters. Our aim was to investigate the occurrence of carbapenem resistant Enterobacterales (CRE) carried by these birds and to compare them to contemporary water-derived isolates. Between January and March 2020 105 fecal samples were taken from gulls captured for ringing at the docks of Budapest. In parallel, twelve water samples were collected from the river Danube both upstream and downstream of the wastewater treatment plant. Water samples were processed according to standard methods for water quality indicators using Colilert test supplemented with 10 mg/L ceftriaxone. Gull CRE isolates were recovered using eosin-methylene blue agar containing 2 mg/L cefotaxime and identified by MALDI-TOF-MS. Susceptibility testing was performed by EUCAST disk diffusion method. CRE types were further investigated by MASTDISCS Combi Carba test and multiplex PCRs. CRE isolates were found in 7% (7/105) of sampled gulls. One *Citrobacter* sp., one *Enterobacter* sp., one *Klebsiella pneumoniae* and eight *Escherichia coli* isolates were recovered. In water samples downstream of the wastewater treatment plant, a total of ten CRE isolates were found; one *Enterobacter* sp., two *Citrobacter* spp. and seven *E. coli*. Upstream samples were negative. Metallo beta-lactamases (MBL) were the dominant carbapenemases in gull and water *E. coli* isolates; NDM was the dominant type followed by VIM. Both *Enterobacter* spp. were porin deficient AmpC producers while *Citrobacter* spp and *K. pneumoniae* were NDM-producers. No *KPC*, *OXA-48* or *IMP* genes were found. Co-resistance rates were high, 10/11 and 9/10 to ciprofloxacin, 10/11 and 8/10 to co-trimoxazole, 5/11 and 6/10 to gentamicin, 9/11 and 9/10 to amikacin and tobramycin among gull and water isolates, respectively. Full susceptibility was observed towards colistin except for one gull *E. coli* isolate, which was resistant to all other tested antibiotics. The presence of CRE in gulls and River Danube is extremely worrisome. Our results suggest that gulls and River Danube may serve as reservoirs of CRE, which may be a consequence of environmental pollution with antibiotics or with resistant isolates. Nonetheless, gulls may play a role in the dissemination of carbapenem resistance because of their vagrant behavior underlining the importance of the One Health concept.

THE DOUBLE ROLE OF HIV PROTEASE DURING PRIMARY INFECTION (IN MEMORIAM DR. OROSZLÁN ISTVÁN 1927-2020)

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Retroviral protease (PR) is a virally coded enzyme, known to active at the late phase of virus replication cycle, cleaving the precursor viral proteins to their final form, rendering the virus infectious. The possible involvement of retroviral PR in the early stages of viral replication cycle was suggested on the basis of in vitro experiments with purified capsids. These studies demonstrated a novel retroviral protein-processing pathway mediated by the viral PR that involves the regulated in situ cleavage of mature nucleocapsid protein assembled with the viral RNA. Such a proteolytic event is required for the progression of virus replication in the early phase of infection. The results suggested, that in the presence of PR inhibitors not the initiation and progression of reverse transcription is blocked, but the stability of full-size unintegrated cDNA, which is affected in the presence of PR inhibitor. The original suggestion was made by István Oroszlán at the NCI NIH, based on works done in his laboratory, and confirmed on HIV experiments I had been involved. Dr. Oroszlán was a pioneer in studies related to the biochemistry and enzymology of retroviral replication and the biosynthesis of retroviral

enzymes and structural proteins. He served as Scientist Emeritus with the HIV Dynamics and Replication Program in the Center for Cancer Research. He received his early training in engineering in his native Hungary. Following his immigration to the U.S., he obtained a Ph.D. in Pharmacology from Georgetown University. After his postdoctoral training at the NCI from 1961 to 1963, he conducted biochemistry research on retroviruses at the Albert Einstein Medical Center, George Washington University School of Medicine and Flow Laboratories. In 1976, he returned to the NCI to serve as director of the Molecular Virology and Carcinogenesis Laboratory from 1983 to 1995. Dr. Oroszlán was perhaps best known for his groundbreaking research defining the role of retroviral proteases in the generation of mature Gag-related protein products, which comprise the virus particle structure. This work played a central role in the development of HIV protease inhibitors, which became a mainstay in the treatment of HIV-infected individuals. His laboratory also discovered that many retroviral Gag proteins are myristilated, a finding that was of great importance in understanding the assembly of retroviral particles. He published more than 250 papers and was named one of the most highly cited researchers in microbiology by the Institute for Scientific Information. His numerous honors include the award of a Doctor Honoris Causa Degree from the University of Debrecen Medical School in 1993, election to the Hungarian Academy of Sciences as a foreign associate in 1994, a Retroviral Retrospective Symposium held in his honor at Hood College in 1994 and the Mór Kaposi Research Foundation Award in 2000. With his death, a giant of the biomedical science and the retrovirology left us.

GENOME-WIDE MAPPING FUNGAL GENE REGULATORY NETWORKS UNDER THE FUNCODE PROJECT

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The timing of expression and combination of genes expressed in regulatory networks determine the cellular outputs organisms can generate. The post-genomic era has brought a revolution in mycology, by providing abundant genomic data for virtually all major fungal clades. However, to connect genomic data to phenotypes, it is necessary to understand how genes are being put to use to generate the variety of cellular behaviors that ultimately lead to the functional and ecological diversity of fungi. The FUNCODE project aims to experimentally determine transcription factor binding sites for genome-wide collections of transcription factors in five important model fungi (*Anaeromyces robustus*, *Aspergillus niger*, *Coprinopsis cinerea*, *Neurospora crassa*, *Phanerochaete chrysosporium*) and reconstruct gene regulatory networks that govern the expression of structural and regulatory genes. The project combines experimentally determined transcription factor binding sites (via DAP-Seq) with RNA-Seq analyses of transcription factor knockout strains to infer the wiring and directionality of regulatory interactions between genes. In this report, we summarize progress that has been made in the first two years of the FUNCODE project. Ultimately, we expect resources generated during the project to provide key functional data for understanding fungal traits, such as pathogenicity, wood decay or morphogenesis and for generating the basic knowledge that can be harnessed in synthetic biology and fungal biotechnology.

INVESTIGATION OF THE INTERACTION OF KERATINOCYTES AND *CANDIDA* SPECIES

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Candida species are opportunistic human fungal pathogens that can cause life-threatening infections if the host's immune system is compromised. It is well known that, cutaneous candidiasis – or *Candida* infection of the skin - is most commonly caused by *C. albicans*. *C. parapsilosis* is the second/third most frequently isolated *Candida* species from blood samples. This species is also known as a common commensalist of the healthy human skin. Our skin provides immunological protection against several pathogens. Keratinocytes (epithelial cells of the skin) in the epidermis are able to recognize pathogen-associated molecular patterns (PAMPs) through their pathogen recognition receptors (PRRs), leading to defense responses through the activation of various transcription factors, such as NF-κB. Skin epithelial cells can respond in various ways to microbial stimuli, such as through the production of antimicrobial peptides, phagocytosis or cytokine secretion. Our knowledge is incomplete regarding how skin keratinocytes differentiate between the presence of pathogenic and commensal fungi. Therefore, this project places an important emphasis on investigating the interaction of human skin keratinocyte cell lines with *C. albicans* (SC5314 and WO1 strains) and *C. parapsilosis* (GA1 and CLIB214 strains). Human HaCaT and HPV-KER cell lines were used in the experiments. Our main goals are to determine the extent to which *C. albicans* and *C. parapsilosis* damage human keratinocytes, their attachment to host cells, the keratinocytes'

ability to internalize these fungi and their cytokine production in response to stimuli. Our results suggest that *C. albicans* causes significantly more damage to human keratinocytes than *C. parapsilosis* and the HPV-KER cell line seemed to be more sensitive to the infection. In both HaCaT and HPV-KER cell lines, the production of IL-6, IL-8, and CCL5 increased mainly after *C. albicans* infection. Based on the adhesion studies, there was a low degree of association in case of *C. parapsilosis* GA1 and CLIB214 compared to *C. albicans* SC5314 and WO1. Regarding uptake, our results showed that both HaCaT and HPV-KER cell lines were capable of internalizing *C. albicans* and *C. parapsilosis* strains. Continuing the experiments will provide us more information about the interaction between keratinocytes and pathogenic/commensal *Candida* species.

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ADAPTATION TO TRIAZOLES OF *CANDIDA PARAPSILOSIS* IMPACT CROSS-RESISTANCE AND VIRULENCE IN A DRUG DEPENDENT MANNER

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Cases of infections caused by *Candida* species are increasing worldwide. In the past few years number of non-albicans *Candida* (NAC) infections, such as *C. parapsilosis* increased dramatically and now these species are responsible for more invasive cases than *C. albicans*. Not only the case numbers of NAC increased, but also in the case of *C. parapsilosis* incidence of azole resistant isolates also often occurs. We aimed to investigate the development of azole resistance mechanisms in *C. parapsilosis* using a directed selection and inspect the impact of acquired resistance on virulence and stress tolerance. We have found that azole microevolution leads to cross-resistance depending on the specific azole used as selection pressure: FLUEVO and VOREVO strains gained resistance to both fluconazole and voriconazole; however, POSEVO strain developed cross-resistance to all azol type antifungals and increased MIC values were detectable to echinocandins. Whole genome sequencing revealed the distinct resistance mechanisms of azole-evolved strains: FLUEVO and VOREVO strains harbored non-synonymous mutations in MRR1 gene, resulted in A808T and N394Y amino acid substitutions, respectively and POSEVO strain gained D14Y amino acid change in the Erg3p. By determination of enhanced efflux-activity of FLUEVO and VOREVO strains together with the altered sterol composition of POSEVO strain, we have validated the resistance mechanisms in the three azoles evolved strains. We demonstrated that the adaptation to fluconazole and voriconazole do not or only slightly decrease the virulence of *C. parapsilosis*, meanwhile posaconazole adaptation results in a significant decrease in the pathogenicity. Our results on cross-resistance pattern and virulence properties have the potential to help the optimization of antifungal treatment strategies.

INVESTIGATION OF FATTY ACID PROFILES FROM *ASPERGILLUS* STRAINS

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Aspergilli have been used for many years in fermentation industry for the production of organic acids and more recently for the production of a variety of enzymes and other secondary metabolites. The classification of fungi is performed mainly morphologically and molecular techniques. However, it is often difficult, especially when closely related fungi or non-sporulating fungi are studied. Fatty acid analysis has been widely used as a chemotaxonomic marker for the characterization of bacteria and yeasts. The characterization of cellular fatty acid composition also seems to be a useful approach for the identification of filamentous fungi. Therefore, in our study, we optimized this identification tool for the taxonomical investigations of *Aspergillus* species to determine a characteristic fatty acid composition for various members of the genus and to reveal the relationship between the taxa and fatty acid profiles. For this purpose, thirty-nine *Aspergillus* species were selected from the Szeged Microbial Collection. Firstly, the cultivating conditions were optimized and standardized in order to obtain reproducible fatty acid profiles. Then the change of amounts and the compositions of fatty acids were determined during the cultivation. Altogether, twenty fatty acid components were identified from the ferment broths, which were classified into two groups containing the major and minor components, respectively. The most abundant fatty acids in the major group were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2), while the most significant minor components were the heptadecanoic acid (C17:0) and linoleic acid (C18:3).

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REPLACEMENT OF AGAR BY GELATIN ENHANCES QUALITY OF RNA ISOLATED FROM *SCHIZOSACCHAROMYCES JAPONICUS* HYPHAE

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Mycelial growth is an important ability of fungi, which secures their survival and resistance under special circumstances. Filamentous growth of the pathogenic yeasts is tightly connected to pathogenesis, a process -–which can also be a good target of the fight against them. Several studies have demonstrated that elimination or suppression of mycelial growth resulted in a decreased or absent virulence in, e.g., *Candida albicans*. Consequently, several studies now focus on understanding the molecular background of filamentous growth and use transcriptomic analysis, an excellent method for identification of genes involved in mycelial growth. This method requires two different states of the cells (yeast-phase and filamentous-phase) and high quality RNA. A basic problem involved in these experiments is how to culture yeast cells and hyphae under the same conditions. The majority of these studies cannot solve this problem and hyphal growth is often induced by FBS (Fetal Bovine Serum) or higher temperature, while yeast cells are grown on standard medium. Thus, this approach shows not just the differences between the yeast-phase and hyphal-phase, but also the effect of the inducer agents (e.g. serum) as well, which produces questionable results. Another technical difficulty is that RNA isolation from hyphae is often difficult. In our work, we used the unicellular dimorphic fission yeast, *Schizosaccharomyces japonicus* as model organism. In order to eliminate the problem caused by the very different culture conditions of the yeast-phase and mycelial-phase, we used gelatin as solidifying agent. To improve the yield and quality of RNA we removed the hyphae tips from the media and submitted them to RNA extraction. The quality control of the RNAs showed that the samples isolated from gelatin plates were not degraded and yielded a good amount of RNA, in contrast to the samples isolated from hyphae grown on agar plates, which were highly degraded and yielded a lower RNA concentration. In summary, we found that gelatin was a good substituent for agar and enabled us to use the same culturing conditions for breeding of yeasts and hyphae.

SZEGED MICROBIOLOGICAL COLLECTION: A HUNGARIAN MICROBIAL GENE BANK

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Szeged Microbiological Collection (http://www.wfcc.info/ccinfo/index.php/collection/by_id/987) is a member the World Federation of Culture Collections; its main mission is to collect, maintain, identify, and characterize bacterial, yeast and mold strains. Presently, the collection maintains 10,426 isolates of more than 120 microbial genera, which correspond to 1,387 bacterial and 9,039 yeast and filamentous fungal strains. Strains with special environmental and agricultural origin (such as producers of mycotoxin and other metabolites, plant pathogenic microbes, strains causing postharvest decay, as well as biocontrol, bioremediation and bioaugmentation agents) constitute a valuable part of the collection. SZMC has significant sub-collections of the members of the genera *Aspergillus*, *Fusarium*, *Mucor*, *Mortierella*, *Penicillium*, *Rhizopus*, *Rhizomucor*, *Trichoderma*, *Cryptococcus*, *Saccharomyces* and *Candida*. The main body of our bacterial collection consists of strains isolated for soil quality improvement, bioaugmentation and biocontrol studies (e.g. *Bacillus*, *Pseudomonas* and *Streptomyces* isolates). Strains are maintained primarily by cryopreservation in deep (-80°C) and ultra-deep freezers (-140°C) and in liquid nitrogen. Besides the maintenance, molecular identification, and characterization of the strains, such as screening for metabolite and enzyme producing abilities, are also carried out. In the past decades, the collection has developed for an outstanding and valuable genetic resource, which can effectively help the biotechnological, agricultural and food industrial research and development efforts.

ROLES FOR STWINTRONS (SPLICEOSOMAL TWIN INTRONS) IN ALTERNATIVE SPLICING EVENTS IN ASPERGILLUS

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In the primary transcript of nuclear genes, coding “exons” usually alternate with non-coding “introns”. The latter intervening sequences are precisely excised from pre-mRNAs by the U2 spliceosome, a complex RNA-protein organelle unique to Eukaryota, to generate the open reading frame by joining the exons. The resulting mature mRNA translates into the correct peptide product once exported from the nucleus. Stwintrons (“spliceosomal twin introns”) are complex inter-

vening 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 standard splicing reactions. Nevertheless, position-conserved stwintrons are often present across whole taxonomical fungal families or orders implying they are evolutionary stable rather than deleterious features. Originally, alternative splicing was presented as a means to increase protein diversity in Eukaryota but more often it yields "dysfunctional" RNAs (not encoding the correct peptide), which are usually degraded rapidly once transported to the cytosol to prevent their translation. We investigated functional relations between genuine fungal stwintrons, and extant exon skipping and intron retention events. A donor-disrupted stwintron in a ubiquitous gene coding for a reticulon-like protein occurs broadly in the Pezizomycotina subphylum [1, 2]. The stwintron is crucially involved in "skipping" the microexon behind it in certain species, like *A. niger* and *Neurospora crassa*, by using alternative 3'-splice sites for its internal intron. This instance of alternative splicing thus yields two similar but different isomeric proteins. A second instance of alternative splicing involves a branch-point motif-interrupted stwintron found in *A. nidulans*. Orthologue genes in allied species specify a standard intron at the very same position as the internal intron of the *A. nidulans* stwintron, whose removal is necessary to produce protein. However, subsequent excision of the new external intron eliminates the AUG start codon, implying that it must be retained to deliver a protein. Here, stwintron excision may be crucial in post-transcriptionally modulating the steady state levels of the functional mRNA, and its protein product.

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[1] Kavalecz et al (2019) Sci Rep 9:9940, https://doi.org/10.1038/s41598-019-46435-x
[2] Ág et al (2019) Sci Rep 10:6022, https://doi.org/10.1038/s41598-020-63239-6

DISSEMINATION OF INTERVENING SEQUENCES OF COMMON ORIGIN IN THE GENOME / TRANSCRIPTOME OF *HYPOXYLON* SP.

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Introns (intragenic sequences or intervening sequences) are sequences that must be removed from primary transcripts to allow translation into the proper gene product. Spliceosomal introns are unique to Eukaryota and their excision requires the action of a highly specialized organelle, the U2 spliceosome. The origin of spliceosomal introns – presently disseminated across eukaryotic genomes at densities of 4–8 introns per gene – remains a vexing mystery. We study fungal stwintrons (spliceosomal twin introns) as model systems. These are unconventional intervening sequences where one U2 intron interrupts one of the canonical splicing motifs of another U2 intron: In a [D1,2] stwintron, the internal intron is integrated in the 5'-donor of the external intron between nucleotides (nt) 1 and 2. Stwintron excision typically requires consecutive splicing reactions, with an identifiable splicing intermediate. We have previously described that stwintrons can emerge by the appearance of a new intron within a pre-extant intron or by the intronisation of exonic sequences [1, 2]. Here we report the identification of more than 20 [D1,2] stwintrons in the genome sequences of two strongly related endophytic fungi (NCBI WGS database) that are highly similar in sequence. We experimentally verified the splicing intermediate of the double excision of 23 of them in one of these two fungi. These recently proliferated, structurally highly related "sister stwintrons" reside in 23 genes of highly diverse size, predicted function and intron-exon structure. There is no bias for phase or localization of sister stwintrons which, with one exception, all appear at newly occupied intron positions. On the other hand, a stand out characteristic of sister stwintrons is the presence of two copies of a 10-nt palindromic sequence located roughly halfway the internal intron and halfway the external intron. Somewhat surprisingly, we also identified (and experimentally verified) 10 canonical U2 introns with striking sequence similarity with the 5' half of the internal intron of the sister stwintrons and the 3' half of their external intron. These "solo sister introns" constitute a more homologous group of new intervening sequences which appear to have formed from sister stwintron ancestors by the deletion of the central sequence between the two palindromes, retaining only one copy. We thus established the existence of a completely new mechanism by which spliceosomal introns can form at the genome (DNA) level.

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[1] Kavalecz et al (2019) Sci Rep 9:9940, https://doi.org/10.1038/s41598-019-46435-x
[2] Ág et al (2019) Sci Rep 10:6022, https://doi.org/10.1038/s41598-020-63239-6

ASPERGILLUS MYCOTOXINS AND THEIR NATURAL ROLES

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Aspergilli a well-known group of molds producing main mycotoxins contaminate agricultural products. Soil is the natural habitat for *A. flavus*, and aflatoxin B1 production is considered to give a fitness advantage in that environment. Aflatoxins are produced by as much as 16 species, most notably by *A. flavus* and *A. parasiticus*; however, the selective forces that maintain the polymorphism of non-aflatoxigenic and aflatoxigenic colonies of *A. flavus* are mainly unknown. A wide range of *Aspergillus* spp. produces the aflatoxin B1 precursor sterigmatocystin, which is also a carcinogenic compound. The neurotoxic cyclopiazonic acid is an indole-tetramic acid produced by 13 species in *Aspergillus* section Flavi. It inhibits endoplasmic reticulum calcium ATPases at nanomolar concentrations and, therefore, it is an inducer of cell death in plants. Usually, cyclopiazonic acid and its derivatives are aflatoxin B1 concomitant mycotoxins. The cyclopiazonic acid serves as a critical pathogenicity factor that enables the saprophytic lifestyle of *A. flavus*. Tremorgenic mycotoxins (e.g., aflatrem) got low respect but these are also having critical veterinary significance as they are causing tremor of the grazing animals. Here, we collected recent knowledge on the possible role and fate of these secondary metabolites.

SYMMETRIC SELENOESTERS AS POTENT EFFLUX PUMP INHIBITORS IN COLON ADENOCARCINOMA CELLS

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There are several mechanisms for the development of resistance to antineoplastic drugs in tumor cells, which can lead to the failure of chemotherapy. This phenomenon is often due to altered membrane transport, such as the overexpression of ABCB1 (P-glycoprotein) in tumor cells. Selenium and its derivatives have been reported in several studies as antiproliferative, cytotoxic, and pro-apoptotic compounds that can also reduce drug resistance in tumor cells. The aim of our study was to investigate the anticancer effect of symmetric selenoesters on sensitive and resistant human colon adenocarcinoma cells expressing ABCB1 protein. The antiproliferative and cytotoxic effects of the compounds were determined by MTT assay on sensitive and resistant colon adenocarcinoma and normal embryonic lung fibroblast cells. The efflux pump inhibitory activity of selenoesters was investigated by flow cytometry based on rhodamine 123 accumulation. The apoptosis-inducing effect of the most active derivatives was detected by annexin V-FITC staining using flow cytometry. Furthermore, the interaction of the compounds with doxorubicin was assessed by checkerboard combination assay and the type of interaction was calculated by Calcusyn software. Compounds containing the methyl ketone side chain (EDAG-1, -5, -8) had the strongest antiproliferative and cytotoxic effects and EDAG-5 showed a synergistic interaction with doxorubicin. Compounds containing methyl carbonyl and cyano groups had very low cytotoxic activity on the normal MRC-5 fibroblast cell line. Methyl ketone- and methylcyano-selenoesters (EDAG-7, -10, 11) were more effective ABCB1 inhibitors than the reference compound verapamil. Regarding the induction of apoptosis, one methyl ketone selenoester (EDAG 1) was shown to be effective in the resistant Colo 320 cell line. It is hypothesized that the biological activity of these symmetrical selenocompounds is due to the toxic, DNA-damaging effects of the two selenium atoms on the backbone, which may be enhanced by the presence of symmetrical groups. Based on the results, it can be concluded that these derivatives may be important scaffolds for future antitumor therapy, therefore further preclinical studies may be warranted.

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ALLOPOLYPLOID PROBLEMS: PHYLOGENOMIC „TREES" AND BEYOND WITH THE YEAST *SACCHAROMYCES*

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The history of the yeast *S. cerevisiae* is deeply interwoven with that of human migrations, the developments in agriculture and food technology, and trade since ancient times. Its utilization in the production of bread, beer and wine had made it one of the most important and most beneficial microorganisms. Its roles have widened even further, to include probiotics, biological research, and biotechnology. The species is a minor and probably transient member of the human microbiome, while it may also cause opportunistic infections. The advancement of *S. cerevisiae* comparative genomics and global surveys on its diversity have significantly widened our understanding of the origin and the domestication of the species, the importance of inter- and intraspecies hybridization, and the species' various phylogenetic clades. As in the case of domesticated plant and animal species, human-driven admixture left a significant mark on the species' population structure, resulting in a plethora of admixed, often polyploid lineages adapted to various fermentation processes. These yeasts with mosaic genomes are challenging to be integrated into existing phylogenomic pipelines that aim to represent evolutionary history in a dichotomous fashion. Coalescent models also fail to correctly capture the hybridization histories behind the contemporary *Saccharomyces* diversity. Thus, phylogenomic network analyses can be considered as promising tools to unravel the species' recent evolution, but these are still limited by methodological constraints especially in the case of polyploids. In the present work, we developed a pipeline based on alignment and assembly-free methods to evaluate the shared ancestries of yeasts regardless of ploidy. With this, we can evaluate heterozygous SNPs with an arbitrary number of simultaneous alleles, as well as indels and repetitive regions. We benchmarked our approach by generating in silico admixed tetraploid genomes from heterozygous diploid yeasts. With this pipeline, we generated a robust phylogenomic network representing all described clades of *S. cerevisiae* using 600 genomes. This analysis helped us to show that the majority of human (including clinical) isolates in fact originate from commercially available (food and probiotic) yeasts, while we also uncovered unique human-infecting yeasts belonging to exotic and poorly known clades. We also report on the second worldwide discovery of the so-called B* 2-micron plasmid, in one of our newly sequenced isolates. Our phylogenomic analysis is supplemented by the ONT long- and Illumina short-read based assembly of two tetraploid isolates representing the two major baking yeast groups commercially available in Hungary. These almost complete genome assemblies provide further insight into how admixed yeast genomes arose by human activity and went on to be successful not only in fermentation applications, but in colonizing the human body as well.

DETECTION OF SARS-COV-2 FROM RAW SEWAGE SAMPLES

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Wastewater-based epidemiology is a widely used tool to detect prevalence of viruses in the population. In the current COVID-19 pandemic, many countries began to analyze the novel coronavirus in sewage samples, and it was found a reliable method to monitor the tendencies of COVID-19 infections in different areas. The viral titer was observed to increase 4-10 days earlier in wastewater than the number of clinical cases. Therefore, the method could be used for early prediction. The method development started in April 2020 at National Public Health Centre (NPHC). Various concentration (flocculation, ultrafiltration) and RNA isolation methods (commercial kits and classic precipitation methods) were compared. The flocculation method showed low recovery rate, while the quality of the ultrafiltration method depended strongly on the type of filter unit. For the national survey, a specially manufactured membrane was chosen, due to its good recovery and reliable availability. The results of nucleic acid isolation were similar with the different methods, a commercial kit (Zymo Research) recommended to feces and soil was chosen due to its higher inhibitor-removal ability. RNA concentration is quantified by quantitative RT-PCR (designed for the nucleocapsid protein 1 gene), similar to the method used for clinical diagnostics. Systematic wastewater sampling started in end of May in Budapest; the survey was extended to all county capitals by the beginning of July. The operators of the wastewater treatment plants (WWTP) from the raw sewage carry the sampling out weekly after the grid filter, and the samples are shipped to the laboratory within 24 hours. Most WWTP does not have composite auto-sampler; therefore, sampling is carried out in the peak-load in most places. The results are available in 36-72 hours and published to NPHC website within a week. A decreasing trend was observable

in the data from the end of May to the beginning of June, in parallel with the decline of the first wave of the epidemic. After that, the concentration of SARS-CoV-2 stagnated at a low level until beginning of August. The increasing trend in the wastewater was followed by an increase of the confirmed COVID-19 cases approximately 2 weeks later. Data processing is still ongoing for better modeling of the correlation between clinical data and SARS-CoV-2 concentration in wastewater.

WHOLE GENOME SEQUENCING OF COAGULASE POSITIVE STAPHYLOCOCCI ISOLATED FROM OWNERS AND THEIR DOGS IN HUNGARY

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Staphylococcus aureus and *S. pseudintermedius* are the two most common coagulase positive staphylococci (CPS). Both can be asymptomatically carried and can cause various community and hospital acquired diseases in humans and animals as well. *S. aureus* is more prevalent among humans, whereas *S. pseudintermedius* is more commonly isolated from dogs. In the current study, we screened 102 dogs and 84 owners for CPS carriage in Hungary. After phenotypic and genotypic identification of the isolates, we also tested the antibiotic susceptibility of the strains. In order to get a better picture of the genetic relationship of the isolates, we used pulsed-field gel electrophoresis. In addition, selected co-carried isolates (n = 6) were whole genome sequenced and their MLST and spa types were established. Carriage rate of *S. aureus* was 23.8% in humans and 4.9% in dogs and two cases of co-carriage were found among dogs and owners. *S. pseudintermedius* carriage rate showed an inverse pattern: 2.4% and 34.3%, respectively, with only one co-carriage. The majority of the isolates were susceptible to the used antibiotics, however *S. pseudintermedius* strains showed elevated resistance against tetracycline. The co-carried strains of dogs and owners shared almost identical antibiotic resistance genes (including *tet(K)*, *bla(Z)*, *norA*, *mepR*, *lmrS*, *fosB*) and virulence pattern. We found not only common staphylococcal enzymes and cytotoxins, but also enterotoxins and exfoliative toxins in the genome-sequenced isolates. *S. aureus* sample pairs from dogs and owners belonged to ST45-t630, ST45-t671 and ST15-t084, ST15-t328, respectively. The co-carried *S. pseudintermedius* isolates shared the same housekeeping gene alleles determining a novel sequence type: ST1685. Based on the results of whole genome sequencing, dog-owner co-carried strains displayed only insignificant differences therefore they provided evidence for potential human-to-dog and/or dog-to-human transmission.

EFFECT OF *LACTOBACILLUS* INOCULATION ON THE DIVERSITY OF YEAST COMMUNITIES IN THE EXPERIMENTALLY *ASPERGILLUS FLAVUS* CONTAMINATED CORN SILAGES

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The presence of *Lactobacillus* species has an emerging role in silages. It is presumable that the function of *Lactobacillus* not only affect the consistence and quality of the silage, but it has an effect on the composition and the levels of microbiome. In our research, we investigated the effect of the high dose of *Lactobacillus* treatment on other characteristic members of the silage, which is the community of yeasts. In the beginning of the experiment and after an 8-week-long maturing we quantitatively cultivated the samples from the silage, which was inoculated with the mixture of three strains of *Lactobacillus* on agar plates containing Rose Bengal and chloramphenicol. In the starting mixture, in which the majority of the community was phylloplane, the number of the yeasts was $4.8 * 10^7$ CFU/g. Despite the high yeast level in the raw material the 8-week-old silage had one scale smaller number of yeast propagule ($2.9 * 10^6$ CFU/g), which value has decreased after the *Lactobacillus* treatment ($7.1 * 10^5$ CFU/g). Based on the phenotypical attribution and nucleotide sequences of ITS1-ITS4 region and D1/D2 domain of the 100 isolates from the treated silages, we were able to detect 10 different yeast species. In the control silage, we detected only four species in contrast with the isolated eight species of the 8-week-old silage and we were able to separate only three species in the *Lactobacillus*-treated silage. In the starting mixture, the most common species were *Cystobasidium slooffiae* and *Kregervanrija fluxuum.* The amount of both of these two species significantly decreased and even disappeared in the control silage. The taxonomical diversity of the yeast community of the starting mixture was relatively high (Index of Shannon = 2.27), which amount during the maturing phase significantly decreased (control silage = 1.93; silage treated with *Lactobacillus* = 1.54). Besides the diversity decrease, the *Lactobacillus* treatment significantly changed the community of yeasts. *Saccharomyces cerevisiae*

and *Pichia kudriavzevii* were the most common species in the control silage, while in the lactobacillus-treated silage *Pichia fermentans* also became abundant beside *Saccharomyces cerevisiae.* Based on our results we can conclude that the *Lactobacillus* treatment, which is widespread in silage practice, has a moderate effect on yeasts considered as autochthon microorganisms in silages.

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PRODUCTION OF PIGMENT BY *YARROWIA*

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Nowadays there is a great interest of the market for the natural pigments; especially microbial pigments because of widely used synthetic pigments have harmful issues associated with the workers of industry as well as consumer. *Yarrowia lipolytica* is one of the pigment-producing microorganisms capable of producing a brown color pigment called piomelanin. Melanins have broad area of application, mainly in agriculture, cosmetics and pharmaceutical industry. Melanins have important biological activity, including antimicrobial, hepatoprotective, antitumor activity, as well as immunopharmacological properties, and may be promising for the treatment of AIDS. In this research, different *Yarrowia* strains (*Y. bubula*, *Y. divulgata*, *Y. porcina*, and *Y. lipolytica*) were examined for pigment production. Moreover medium optimization experiments were performed to increase dye production. During the fermentation, the development of pigment production was monitored spectrometrically at 400 nm. Six *Yarrowia* strains were screened for pigment production using tyrosine-containing nutrient agar [1] to isolate *Y. lipolytica*. The brown discoloration formed around the colonies on the agar indicated the pigment-producing ability of the strains. It was observed that each of the tested strains produced pigment but different extent. Based on the results the *Y. lipolytica* strain found to be the best, so *Y. lipolytica*, the less studied *Y. divulgata*, and *Y. porcina* strains were chosen for further studies of their pigment production. In addition to tyrosine, the effect of various amino acids and lactic acid were also examined on pigment production during submerged fermentation. According to the literature, the use of lactic acid increases the pigment production, however this effect was observable only in the case of *Y. porcina*. The effect of pH on both growth and pigment production was investigated by buffering the medium found to be the best in the pH range of 5-8. Based on the results, it can be concluded that the growth of the microbe is not significantly affected by pH in the given range. The medium with pH 7 proved to be the best for pigment production. The effect of manganese concentration (0, 1, 2, and 5 mM) on pigment production was also examined in medium prepared by distilled water or buffer (pH 7). In the absence of manganese, both growth and pigment production started later. The increasing manganese concentration resulted a proportional increase in the amount of dye. The effect of it was lower in the case of the media prepared with buffer. 5 mM manganese concentration resulted approx. five times higher pigment production in the control medium.

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[1] Carreira et al (2001) Appl Environ Microbiol 67:3463.

RETICULATING CHIMERIC GENOMES IN ANTAGONISTIC *METSCHNIKOWIA* SPECIES

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The highly heterogeneous genus *Metschnikowia* (Saccharomycotina) comprises over 70 species. *M. pulcherrima* and a group of related species (the pulcherrima clade) produce (mostly extracellularly) pulcherrimin, an insoluble pigment that turns the colonies and the surrounding medium reddish. Pulcherrimin is a complex of pulcherriminic acid secreted by the yeast cells and ferric ions available in the environment. Its formation depletes the medium of free iron. Since iron is essential for many microbes, the yeasts of the pulcherrima clade can inhibit their growth (antimicrobial antagonism). The analysis of the barcode sequences ITS1, D1/D2, ACT1, EF2, TEF1 and RPB2 of the type strains of the species and the genome sequences of four strains revealed chimeric (admixed) alloaneuploid genomes composed of mosaics of different phylogenetic histories. Three of the genome sequences have two sets of PUL genes (four genes involved in pulcherrimin biosynthesis) organized in two clusters of different phylogenetic origins. Since the type strains can form viable interspecies hybrids, the complex genome structures can be attributed to interspecies hybridization and reticulate evolution. The network analysis of the repeat sequences of the rDNA arrays and the occurrence of certain ITS and D1/D2 segments (alleles) in the genomes of several type strains corroborate this hypothesis [1].

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[1] Sipiczki (2020) Microorganisms 8:E1029, doi: 10.3390/microorganisms8071029.

ALLOSPECIFIC MAT HETEROZYGOSITY IN THE REPRODUCTIVE ISOLATION OF *SACCHAROMYCES* SPECIES

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Our previous studies revealed that the *Saccharomyces* species are biologically isolated by a postzygotic double-sterility barrier. The first barrier is due to the abruption of the meiotic process by the failure of the chromosomes of the subgenomes to pair in meiosis and the second barrier is assumed to be the result of the suppression of mating by allospecific MAT heterozygosity. The former is analogous to the major mechanism of postzygotic reproductive isolation in plants and animals; the latter seems to be *Saccharomyces* specific. To bolster the assumed involvement of MAT in the second sterility barrier, we produced synthetic alloploid two-species „cevarum" (*S. cerevisiae* x *S. uvarum*) and „kudvarum" (*S. kudriavzevii* x *S. uvarum*) hybrids and three-species „cekudvarum" (*S. cerevisiae* × *S. kudvarum* × *S. uvarum*) hybrids and examined their MAT loci using species- and cassette-specific primer pairs. We found that the allospecific MAT heterozygosity repressed MAT switching and the mating activity (conjugation), making the hybrids sterile. The loss of heterozygosity by meiotic malsegregation of MAT-carrying chromosomes in the allotetraploid two-species hybrids broke down the sterility barrier. The resulting alloaneuploid spores (nullisomic for one type of the parental MAT-carrying chromosomes) produced vegetative cells capable of MAT switching and conjugation, opening the way for GARMe (Genome Autoreduction in Meiosis), the process that leads to chimeric genomes characteristic of brewery and certain wine yeasts [1, 2].

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[1] Sipiczki et al (2020) Curr Genet doi: 10.1007/s00294-020-01080-0.
[2] Pfliegler et al (2012) FEMS Yeast Res 12:703.

DESCRIPTION OF A NOVEL CARBAPENEMASE FROM *BACTEROIDES XYLANISOLVENS*

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B. fragilis is the most frequent anaerobic pathogen among *Bacteroides*. It can be carbapenem-resistant with low prevalence carrying the *cfiA* gene, which is activated by insertion sequence (IS) elements. Some non-fragilis *Bacteroides* strains with carbapenem resistance are already known but these strains are poorly characterized. A carbapenem-resistant clinical *B. xylanisolvens* strain (*B. xylanisolvens* 14880) was isolated in 2016 in Münster, Germany. Its antibiotic resistance profile was determined by ETESTs. Whole de novo genome sequencing was performed for further characterization, antibiotic resistance genes were identified after RAST annotation and the genome was analyzed for genomic islands by IslandViewer 4. The proposed carbapenemase gene has been transferred to a susceptible host after PCR cloning and triparental mating using a shuttle plasmid. Carbapenemase gene PCR experiments were also carried out. The *B. xylanisolvens* 14880 strain was resistant to ampicillin, piperacillin/tazaobactam, cefoxitin, imipenem and meropenem, but susceptible to clindamycin, moxifloxacin, metronidazole, tetracycline, tigecycline, chloramphenicol and rifampicine. The strain proved to be a metallo-β-lactamase producer as it yielded a >8-fold decrease in imipenem MIC (128 μg/mL) by EDTA (1 μg/mL). However, it was negative for the *cfiA* gene after PCR detection. Whole genome sequencing of *B. xylanisolvens* 14880 resulted in a 5.9 Mb genome and after annotation, a Class B1 β-lactamase gene was revealed with 37 % identity to the *cfiA* gene. This novel gene was termed to *crxA* (as carbapenem resistance gene in *B. xylanisolvens*), in its upstream region, there was an IS1380-like IS element and it was located on a ca. 5 kb genomic element identified by bioinformatic searches. The *crxA* gene conferred carbapenem resistance to the susceptible *B. fragilis* 638R after PCR cloning and conjugation. In an in house PCR screening, a carbapenem-reistant *B. ovatus* strain from an earlier fecal study was positive for *crxA*. Three additional *B. xylanisolvens* strains from a recent Hungarian susceptibility survey have proven also to be positive for *crxA* but they were not resistant to imipenem and did not harbor IS elements in the upstream regions of the genes. Our findings proved that there is at least another metallo-β-lactamase (other than CfiA) among *Bacteroides*. The *crxA* gene should be taken into consideration in the routine due to the possibly increasing incidence of *B. xylanisolvens* strains.

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CHARACTERISATION OF BOVINE BACTERIOPHAGES EFFECTIVE AGAINST *ESCHERICHIA COLI* O157 REPRESENTING THREE VIRAL GENERA

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The increasingly prevalent antibiotic resistance among foodborne pathogens, including that of Shiga toxin producing (STEC) and enterohemorrhagic *Escherichia coli* (EHEC), is a worldwide concern, prompting research aimed toward alternative antimicrobial agents. Bacteriophages are increasingly coming into focus as potential agents for therapy of bacterial infections, or for biocontrol, i.e. their preventive application in food safety. As cattle is the main reservoir of STEC strains, in the present study, we isolated and characterized bacteriophages from cattle faces as well as from cattle farm environment of various Hungarian cattle farms, using the EHEC prototype strain O157:H7 Sakai for propagation. The whole genome sequence of altogether 11 phages was determined. Eight of them originated from cattle faces, two of them from the farm environment. Based on nucleotide-level homologies of the genomes, two phages were classified into the *Tequatrovirus* (T4-like), four into the *Vequintavirus* (rV5-like) and five into the *Dhillonvirus* (HK576-like) phage genera. None of them carried any known virulence or antimicrobial resistance determinants. The linear double-stranded DNA genomes had the sizes typical for the genera. Three phages, vb_EcoM_bov9_1 (T4-like), vb_EcoM_bov11CS3 (rV5-like), and vb_EcoS_bov25_1D (HK576-like) were chosen for detailed phenotypic characterization. All of them showed effective lysis on EHEC O157:H7 strains as well as other strains representing the O157 serogroup, including enteropathogenic (EPEC) strains and those of atypical pathotype besides STEC. They had a burst size of 127, 293 and 18/cell and a latent period of 35, 5 and 30 minutes, respectively. While all the phages had short latent periods, the higher burst sizes of phages 9_1 and 11CS3 indicate that they could be promising candidates for future biocontrol experiments aiming the reduction or eradication of *E. coli* O157 strains in animals or foodstuff.

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PLANKTONIC BACTERIAL COMMUNITIES OF EURASIAN AND NORTH AMERICAN AQUATIC HABITATS WITH DIFFERENT SALINITY AND CHEMICAL COMPOSITION

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Despite their worldwide importance, still little is known about the microbial communities of shallow inland water bodies. As a result of the ongoing climate change, these habitats are expected to become more concentrated and more often desiccated due to increased evaporation. These changes could result in a shift in many lakes from freshwater to saline conditions or in increasing salinity in the case of already saline aquatic habitats. But how the bacterial communities would respond to these changes? Do the habitats with different salinity and water chemical type have a distinct bacterial composition at all? To answer these questions, we compared several bacterial 16S rRNA gene amplicon datasets (n = 230) obtained from various freshwater, brackish and saline aquatic habitats. The selected samples were processed with approximately the same wet lab protocol targeting the V3-V4 region of the 16S rRNA gene. During the bioinformatic analysis, sequences were filtered rigorously to avoid artifacts. Our results showed remarkable differences in bacterial community composition of freshwater, soda and saline habitats, however transitions between these habitats were also observed. Interestingly various differences were detected in the community structure of similar environments separated by large geographic distances. High abundance of an uncultured member of the Rhodobacteraceae family was characteristic for soda lakes. In the case of Eurasian soda lakes, planktonic actinobacteria (acIII-A1, Luna1-A2, uc_Nitriliruptoraceae) were also typical taxa. The decrease in relative abundance of certain freshwater groups (LD12, *Flavobacterium*, *Aquirufa*, *Limnohabitans*, *and Hydrogenophaga*) indicated transition to brackish waters.

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CHARACTERIZATION OF A NEW KINASE FAMILY IN AN OPPORTUNISTIC HUMAN PATHOGENIC FUNGUS MUCOR CIRCINELLOIDES

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The fact, that CotH3 of *Rhizopus* mediates fungal invasion of host cells during mucormycosis have pointed out the importance of the CotH protein family in connection with the virulence. Thus, our research is mainly focused on the extensive analysis of the gene family and the clarification of their role in the virulence of *Mucor*. We attempted to perform the functional analysis of the CotH proteins, which involved tracking the phenotypic alterations of genetically stable *cotH* mutants. Six putative spore-coat genes (i.e. *cotH1-6*) were disrupted in *M. circinelloides* by an in vitro plasmid-free CRISPR/Cas9 method. To identify on- and off-target mutation in an edited fungus, whole genome sequencing was also performed. Lack of the appropriate transcripts was proven by qRT-PCR. Growth ability of the mutants under different conditions (anaerobic environment, cell wall stressors, hydrogen peroxide) were examined. Spore surface morphology was imaged with scanning electron microscopy and the inner spore structure was investigated by transmission electron microscopy. The phagocytic assay was performed with the standard macrophage-like cell line J774.16. Possible changes in cell wall structure were monitored using cell wall stressors Congo red and Calcofluor white as well as fluorescence microscopy and flow cytometry analysis. Pathogenicity of the mutants was examined in *Drosophila melanogaster*, *Galleria mellonella* and a murine model of mucormycosis. Cell wall stressors affected differently the *cotH* mutants. The *cotH3* and *cotH4* mutant strains exhibited reduced virulence in murine model, while the *cotH4* and *cotH5* mutants showed reduced virulence in *D. melanogaster* model. Deletion of some of the *cotH* genes resulted in variances in the structure of the inner spore coat, differences in spore size distribution, fungal growth and sporulation.

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SELENOESTERS AS POTENTIAL QUORUM SENSING-INHIBITING AND ANTI-BIOFILM COMPOUNDS IN BACTERIA

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The emergence of drug-resistant pathogens leads to a gradual decline in the efficacy of many antibacterial agents, which poses a serious problem for proper therapy. Multidrug resistance (MDR) mechanisms allow resistant bacteria to have limited uptake of drugs, modification of their target molecules, drug inactivation, or release of the drug into the extracellular space by efflux pumps (EPs). In previous studies, selenoesters have proved to be promising derivatives with a noteworthy biological activity. Based on these results, new selenoesters have been synthesized to achieve a more potent antibacterial activity. Thus, fifteen selenoesters (eight ketone-selenoesters and seven cyano-selenoesters) were investigated regarding their quorum sensing inhibiting and anti-biofilm effects in vitro. The minimum inhibitory concentrations (MICs) of the selenoesters were determined on sensitive and resistant *Staphylococcus aureus* strains. The eruption of mature biofilm and the anti-biofilm activity were tested on biofilms produced by *Pseudomonas aeruginosa* (CCM, 3955) and *S. aureus* (ATCC, 25923). For the evaluation of the anti-quorum sensing activity, two reference strains of *Vibrio campbellii* were used (BAA1118 and BAA1119). In this assay, the viability of the cells and the quorum sensing inhibiting effects were determined in the presence of the compounds to differentiate between the toxic concentration and the quorum sensing inhibiting concentration. After determining the antibacterial effects, the ketone-selenoesters proved to be more effective than the cyano-selenoesters. The biofilm inhibitory capacity and the mature biofilm disrupting effects of the derivatives were remarkable in all systems. In terms of QS inhibition four ketone-selenoesters and three cyano-selenoesters exerted remarkable effect on both *V. campbellii* BAA-1118 and BAA-1119 strains. These results suggested that ketone- and cyano-selenoesters could be effective compounds reducing the virulence of bacteria. Selenium-containing compounds could provide alternative and effective scaffolds to overcome MDR, and these new derivatives are promising anti-biofilm and anti-QS agents. However, the mode of action of the compounds needs further investigation.

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ENGINEERING A BACTERIAL CONSORTIUM FOR BIO-CEMENTING POROUS MATRIX APPLICABLE IN BIOREMEDIATION

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Numerous studies report on the construction and remediation applications of ureolytic, microbiologically induced calcium carbonate precipitation (MICP). MICP is an environmentally friendly and sustainable method that uses a biological metabolic process to increase the stability of porous media. The process is based on the hydrolysis of urea, during which the pH level of the medium increases and the formation of carbonate occurs. In the presence of sufficient Ca^{2+}, the carbonate precipitates in the form of calcium carbonate crystals. The precipitated crystals cement the particles of the porous medium, thereby increasing its resistance to environmental influences. Several scientific results have reported the use of MICP e.g. on improving the resistance and durability of concrete elements, on the development of bio-bricks, dust suppression, concrete remediation, soil stabilization etc. Furthermore, MICP proves to be applicable also in environmental remediation e.g. for the development of subsurface barriers or in groundwater remediation. From our previous research, a bacterial strain collection of several tens of prolific biofilm forming and hydrocarbon degrading bacterial isolates was established. The goal of our recent research is to engineer a bacterial consortium made up of biofilm forming, hydrocarbon degrading and MICP-bacteria applicable in the development of porous materials for the absorption and degradation of petroleum hydrocarbons (e.g. BTEX- eliminating MICP-bio-barriers). It has to be noted that, the biofilm-forming ability of the used bacteria may further increase the efficiency of MICP, as EPS serves as an additional nucleation site for calcium carbonate precipitation. Members of the strain collection were tested for their urea hydrolysis abilities and for their capability to grow in MICP-media. Based on preliminary studies, two isolates, a biofilm producer and a BTEX-degrader, were selected for co-cultivability experiments with the MICP model organism *Sporosarcina pasteurii* DSM 33. By using the engineered consortium, "bio-bricks" have been produced in a laboratory scale model experiment. Moreover, the BTEX-degradation capability of the consortium has also been proved. Members of the engineered bacterial consortium did not inhibit each other's activity, either the process of MICP or hydrocarbon degradation. By using the developed consortium, porous materials (e.g. paving stones, biobarriers) may be produced that, in addition to performing their original function, can also adsorb and eliminate any potential contaminant leakage by biological means (e.g. accidental leakages from fuel tanks).

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CHARACTERISATION OF KILLER TOXIN PRODUCED BY *DEBARYOMYCES HANSENII* STRAINS

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Fungal infections cause worldwide problems not only in human health care but also in agriculture and in food industry. The limited number of efficient antifungal drugs and the increasing number of the resistant strains provoke the research to find new, natural antifungal compounds because they are less harmful to human health and environment. Killer toxins produced by yeast species are potential candidates as natural antifungals. These secreted proteins are able to inhibit the growth of sensitive cells or even kill them [1]. *Debaryomyces hansenii* is an ascomycetous yeast species what can be isolated from fermented foodstuff. This species has several killer isolates. In this present work the *D. hansenii* strains of Szeged Microbiology Collection (SZMC) were screened for killer activity. Five killer strains were identified. Their activity was tested against human pathogenic yeast species (*Candida*, *Cryptococcus* and *Lodderomyces*). The activity spectrum of the toxins of the five *D. hansenii* strains was narrow, because only *Candida glabrata* and *Candida tropicalis* proved susceptible to them in plate assay. The toxins turned out to be pH- and thermo-sensitive protein because they acted in a narrow pH range, became inactive above 40°C and they lost their activity after protease treatment. The molecular mass of the protein purified from strain SZMC 26738 was in the range of 50 and 100 kDa. The toxins function by forming pores in the cell membrane of the sensitive cells causing the death of the cells. The affinity of the toxins to different fungal cell wall components suggests that their receptor can be β-1,6-glucan. The *D. hansenii* toxins are probably encoded by chromosomal genes as no DNA plasmids or RNA viruses could be detected in the strains. Further study is needed to identify the proteins and to evaluate their therapeutic potential against human pathogenic yeast strains.

[1] Bijender and Satbir (2017) Biology of Killer Yeast and Technological Implications. Springer, Berlin.

ANTIBIOTIC RESISTANCE DIVERSITY OF CO-HABITANT STRAINS OF SALMONELLA INFANTIS AND ESCHERICHIA COLI FROM BROILERS

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The global spread of multiresistant (MDR) strains of *Salmonella* and *E. coli* increases the food-safety risk and contributes to decreasing the quality and marketability of chicken meat Mobile determinants of antibiotic resistance are key players in the spread of MDR, and the interplay of mobile resistance elements could increase genetic diversity of the above co-habitant enteric bacteria in broiler flocks. Here we aimed to provide the characterization of resistance diversity and to reveal the relation between antibiotic resistance and integron content in populations of co-existant *S.* Infantis (SI) and *E. coli* (Ec). For this, an initial collection with approx. 500-500 of co-habitant colonies of SI and Ec was established. These colonies were simultaneously isolated from caecal, fecal and meet samples from broilers, representing 121, 83 and 44 broiler flocks respectively. To estimate the prevalence and diversity of MDR SI and Ec, the initial collection of co-habitant strains was screened against a set of 11 antibiotic compounds most of them being related to mobile resistance elements. Based on this, a set of co-habitant SI (n = 65) and of Ec (n = 206) strains were selected to represent antibiotic resistance phenotypes and were subsequently subjected for detection and typing of genetic determinants of antibiotic resistance, including class 1 and class 2 integrons. SI was detected in 8-21% of the broiler samples, of which the caecum was the most contaminated source of isolation. Antibiotic resistance phenotyping showed that MDR strains of SI and Ec frequently co-exist in all three sample sources. Broiler strains of SI were characterized by a low diversity of MDR phenotypes, of which the co-resistance of Nal-Sul-Tet was predominantly found. In contrast to this, Ec strains shared a large diversity of MDR phenotypes with the highest prevalence of Amp-Nal-Sul-Tet resistance. Resistance diversity was at least four times higher for Ec than for SI, i.e. in general one SI strains was accompanied by at least four co-habitant Ec strains with different resistance phenotypes from each individual sample. Molecular characterization of the representative strains showed the presence of class 1 integrons in 76% of the SI and 28% of the Ec strains, while class 2 integrons were detected in three Ec strains only. On the basis of analysis of the restriction profiles, four types of class 1 integrons were identified. Out of these, the 1 kb integron with the gene cassette aadA1 was commonly identified for co-habitant strains of *S.* Infantis and *E. coli*. The high prevalence of Nal-Sul-Tet phenotype indicate the continuing predominance of the PFGE clone B2 in recent populations of SI, and the potential exchange of corresponding genes between SI and Ec strains. Class 1 integron genes aadA1 and sul1 could be considered as the most important players in the interaction between these two co-habitant bacterial species.

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PLEIOTROPIC EFFECTS OF LIGASE 4 GENE DELETION IN *SCHIZOSACCHAROMYCES JAPONICUS* DIMORPHIC FISSION YEAST

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Homologous recombination (HR) is responsible for the proper integration of recombinant DNA. In some species, such as filamentous fungi or some plants, the cellular repair system "refuses" to replace genes with the foreign DNA, resulting in non-specific integration in most cases. This non-homologous recombination (NHEJ) is more common in these organisms than homologous recombination (HR) in spite of the fact that it can cause negative effects, such as unwanted gene disruption(s). NHEJ is an important and fastest way to repair DNA damage however, it is rather an undesirable pathway in the extensively studied model organisms or in industrial strains. According to genetic and biochemical studies, several genes are involved in the NHEJ pathway. One of them is the *lig4* gene, whose defect causes the human LIG4 syndrome. This disease is characterized by growth retardation, developmental delay and immunodeficiency. In order to expand our knowledge about the function of *lig4* gene, our aim was to investigate what kind of consequences the absence of the ligase IV (*lig4*) can have. The dimorphic fission yeast, *Schizosaccharomyces japonicus* was used for the experiments, because it is well suited for studying of the basic cellular processes and gene functions. In our study, we managed to prove that the *lig4*Δ cells were much more sensitive to UV radiation than the control cells. Furthermore, various phenotypic abnormalities were also found that affected the cell proliferation, yeast-to-hyphae transition and aging of the mutant cells. The *lig4*Δ cells were also characterized by an increased sporulation tendency. A high degree of flocculation was also noticed, which seemed to be in connection with changes in the glucose concentration of the culture medium. These results suggest that mutation of the *lig4* gene causes pleiotropic effect. The data suggest that the altered phenotype can be related to the nutrient supply and its sensation.

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

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The *Candida* species are among the most common causes of systemic nosocomial fungal infections worldwide. *Candida albicans* is the dominant - and consequently, the best studied organism of the group. Over the past three decades, however the incidence of other *Candida* species has increased apparently. Reports show that *Candida parapsilosis* is often the second or third most commonly isolated *Candida* species from blood cultures and is associated with outbreaks of infection in neonatal intensive care units. Both species have several virulence factors by which they can adapt to the host's environment and, therefore, can cause an infection. These factors include adhesins, as well as the ability to form biofilms, furthermore hydrolytic enzymes, such as the acidic proteinases and lipases. These *Candida* species also have the ability to obtain growth-limiting heavy metal ions from their environment, such as zinc. This capability is key since host niche represents a zinc-limited environment that is one way to inhibit microbial growth. Hence, these pathogens need to possess a zinc transport system that allows them to access bound zinc ions from the host environment upon infection. In contrast with this, a high zinc ion concentration can also be a way of microbial elimination as it occurs in the phagosomes of *Mycobacterium tuberculosis* infected macrophages. In the case of *C. albicans*, the way of zinc acquisition is intensively studied, but we lack any information on the components playing role in the zinc homeostasis of *C. parapsilosis*. Thus, we aimed to predict in silico potential zinc transporters in *C. parapsilosis*, create homozygous knock out mutants, and expose them to various types of stressors and zinc limiting conditions. We have identified six genes, but only removal of two of them (*CPAR2_210740* and *CPAR2_212100*) resulted in difference in zinc uptake compared to the control strain. We also analyzed the kinetics of uptake of these mutants by macrophages, their killing efficiency and investigated the zinc ion level in the phagolysosome during in vitro infection.

STUDIES ON MBM CELLS AS A MODEL FOR ACUTE FELINE IMMUNODEFICIENCY VIRUS INFECTION

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To study pathomechanism of AIDS, the feline AIDS is the only natural small animal model. Feline (FIV) and human (HIV) immunodeficiency viruses show extremely high homology. The course of disease in human and cats are overlapping. However, there are several aspects in feline AIDS awaiting for elucidation; especially the immediate processes upon acute FIV infection need more attention. Our aims were to study aspects of acute FIV infection in a new lymphoid cell line (MBM) obtained from a cat. Course of infection was followed by classical and molecular methods. Specific attention was paid on the chromosomal integration of FIV and characteristics of cell deaths. Materials and methods: MBM cells were maintained in RPMI-1640 medium. MBM and peripheral blood lymphocytes were immunophenotyped by human monoclonal antibodies. Both one of the European FIV isolate from Pisa (FIV-M2) and the prototype American Petaluma isolate (FIV-Pet) were used to infect cells at different multiplicity of infection. Studies on cytopathic effect and cell viability was complemented by quantitation of FIV replication using reverse transcriptase assay and p24 specific ELISA. Integration was detected by PCR using both FIV-M2 and FIV-Pet primers in a time course post infection. Programmed cell death was detected by low molecular weight DNA extraction and flow cytometry. Contrary to mouse monoclonal antibodies, human counterparts showed that MBM cells are immature T cells abundantly expressing CD34 and CD57 markers. Upon infection by both FIV strains integration has been detected from two hours post infection onwards irrespectively of virus load. Following infection at low multiplicity, cells show 4-5 days latency then both cell growth and viability decrease in parallel increasing FIV reverse transcriptase and antigen production. Replication of immobilized cells also decreases following infection. Production of FIV-Pet titered at 10^5 infectious unit/mL, while FIV-M2 did at 10^4 IU/mL. Infected MBM cells formed smaller clumps and these disintegrated gradually, cells became smaller, blebbing on the surface, granules in the cytoplasm and nuclear pycnosis were found; finally cells completely fell apart. Signs of apoptosis were verified by DNA ladder. Gradual accumulation of pycnotic cells was observed by daily flow cytometry. Sensitivity of MBM cells to human monoclonal antibodies strengthens the relationship between FIV and HIV. MBM cells are more sensitive to FIV-Pet than FIV-M2. Very early viral integration into MBM cells and gradually increasing virus production demonstrate extremely high sensitivity to acute infection. Apoptosis detected first in our experiments corresponds to HIV induced programed cell death, consequently MBM-FIV system is an excellent model for acute retrovirus infection.

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ISOLATION AND CHARACTERISATION OF NEW LIGNOCELLULOSE DEGRADING BACTERIA

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Plant biomass is being generated in huge quantities, a considerable part of which is considered waste. Unutilized parts of plants produced in the agriculture are potential sources of renewable energy and raw materials. For exploiting the biotechnological potential inherent in the metabolic diversity of bacteria, isolation and characterization of bacterial strains belonging to new taxa is indispensable. The aim was, therefore, to isolate and characterize new lignocellulosic bacterial strains. A total of 200 bacteria were isolated from agricultural soil samples, 20% of which (40 isolates) were able to degrade some lignocellulose-forming polysaccharide. As a result of the identification of these 40 isolates, four new species candidate strains were found. In our work, polyphasic taxonomic characterization and *de novo* genome sequencing of the four new species candidates were performed. Based on the data obtained, the four strains are members of the genera *Sphingobacterium*, *Flavobacterium* and *Cellvibrio*. According to the 16S rRNA gene sequence analysis, the strains showed highest similarity to *Sphingobacterium composti* DSM 18850 (94.71%), *Sphingobacterium composti* T5-12 (94.76%), *Flavobacterium artemisiae* SYP-B1015 (97.83%), *Cellvibrio diazotrophicus* strain E50 (97.13%). During the mapping of annotated genomes, we focused our search on glycoside hydrolases involved in lignocellulose degradation. Among the new species candidates, *Cellvibrio* sp. Ka43 strain has cellulase, xylanase and mannanase genes. Degradation experiments proved that this strain is very efficient in hydrolyzing plant polysaccharides. The genus *Cellvibrio* currently has 10 members, several of which are known for their remarkable lignocellulose degrading ability. From our experiments, we can also conclude that new species can be effectively isolated from well-researched areas, such as field soil, using atypical media.

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BIOFUNGICIDAL POTENTIAL OF ANTIFUNGAL PROTEINS AND THEIR PEPTIDE DERIVATIVES FROM FILAMENTOUS ASCOMYCETES

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As a consequence of the worldwide increase of enormous crop losses caused by pre- and postharvest pesticide resistant plant pathogenic fungi, there is an essential demand to develop new antifungal strategies in the agriculture. The small molecular weight, cysteine-rich and cationic antifungal proteins (crAFPs) secreted by filamentous ascomycetes are already considered as potential biofungicides. However, several factors still limit their direct topical application. These limitations are the high costs of production, narrow antifungal spectrum, and potential toxic effect on plant development and human/animal health. In our work, we proved the effective and safe applicability of two crAFPs (*Penicillium chrysogenum* PAF and *Neosartorya* (*Aspergillus*) *fischeri* NFAP) and their de novo rationally designed peptide derivatives spanning the evolutionary conserved antifungal so-called γ-core motif in the plant and crop protection. Additionally, our results from crAFP expression and γ-core peptide synthesis provide a proof-of-principle for industrial biotechnological production of protein-based biofungicides and bioactive peptides.

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COMBINATORIAL APPLICATION OF *NEOSARTORYA FISCHERI* ANTIFUNGAL PROTEINS AND THEIR PEPTIDE DERIVATIVES IN PLANT PROTECTION

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In recent decades, the control of the enormous crop losses caused by pre- and postharvest plant pathogenic fungi represent a worldwide challenge for the agriculture. The combined effect of climate change, the emergence of pesticide-resistant fungi, global trade and transport is the most possible reason of this phenomenon. Therefore, there is a substantial need to develop novel antifungal strategies for the agriculture. The small molecular weight, cysteine-rich antifungal proteins from filamentous ascomycetes and their synthetic peptide derivatives represent a rational solution for this problem. In the present work, we investigated the plant protective ability of antifungal proteins with different mechanisms of action from *Neosartorya* (*Aspergillus*) *fischeri* NRRL 181 (NFAP and NFAP2) and their de novo designed peptide derivatives spanning the γ-core motif (γNFAP, γNFAP2, γNFAP-opt, γNFAP2-opt). We observed that monotherapeutic application of these proteins and peptide derivatives exerts fungicidal or fungistatic activity on plant pathogenic fungi in vitro, except for γNFAP and γNFAP2. Furthermore, we demonstrated that, with exception of the γNFAP2-opt, they do not have a toxic effect on intact tomato plants in vivo. Their applicability as combined biopesticides was investigated by testing the antifungal efficacy of the NFAP + γNFAP-opt combination against *Botrytis cinerea* SZMC 21472 in an in vitro microdilution test, and in vivo on tomato plant leaf. We demonstrated a synergistic interaction between the two compounds in the combination as their monotherapeutic minimal inhibitory concentrations decreased in vitro. However, this combined application did not inhibit the infection development on tomato plant leaves; but decreased the symptoms despite the fact that the combination is not toxic to the plant. Based on results, the combined application of cysteine-rich antifungal proteins and their rationally designed peptide derivatives provides a new antifungal strategy for the agriculture.

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CANDIDA PARAPSILOSIS AND *C. ALBICANS* INDUCE DIFFERENT IMMUNE RESPONSES BY HEALTHY ORAL EPITHELIAL CELLS

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Candida species are common colonizers of the human oral microenvironment. However, in case of a suppressed immune status, - due to a shift in the local microbiota (or dysbiosis) - these fungi are able to overload the mucous membranes of the mouth and cause disease. Although the most prevalent species is *C. albicans*, non-albicans *Candida* species, such as *C. parapsilosis*, are also present in the healthy oral microbiota. Although both of these species are opportunistic pathogens, there are fundamental differences between their pathobiology. In this study, our aim was to dissect and compare healthy oral epithelial cell responses along with their regulation towards these fungi. For these experiments, a telomerase-immortalized oral epithelial cell line (OKF6/TERT2) was used. Based on the results of host cell damage, infecting doses were selected for later examination of epithelial cell responses: gene expression of pro-inflammatory cytokines, chemokines and antimicrobial peptides were examined. Whole transcriptome and miRNA analysis of host cells was also performed to reveal potential differences in host response regulation. Our results indicate that besides causing only mild host cell damage, *C. parapsilosis* was not able to induce a strong inflammatory response compared to *C. albicans*, regardless of the applied infection dose. One form of post-transcriptional regulation is performed by miRNAs that regulate gene expression by binding to mRNAs, leading to their degradation or translation repression, thus subsequent gene silencing. As a result of host miRNA profiling following fungal exposure, we identified several miRNA species, specific to *C. albicans* (miR-12136, miR-1293, miR-138, miR-34b, miR-4521, miR-16-1, miR-20a, miR2110) and *C. parapsilosis* (miR-1305, miR-4755) infections, suggesting their species-specific regulation. According to our knowledge, many of the identified miRNAs have not been previously associated with fungal infections. Together these results suggest that one possible reason why epithelial cell responses differ towards *C. parapsilosis* and *C. albicans* is due to these species' distinctive regulatory role in host miRNA response modulation.

IN VITRO ACTIVITY OF ECHINOCANDINS AGAINST DIFFERENT *CANDIDA AURIS* LINEAGES

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Echinocandins (caspofungin, micafungin, anidulafungin) are considered as the drug of choice in case of life-threatening invasive *Candida* infections, yet data on their in vitro and in vivo activity against the recently emerged ascomycetous yeast *C. auris* is scarce. To date four distinct lineages of *C. auris* have been described (Clade I – East-Asian, Clade II – South-Asian, Clade III – South-African and Clade IV – South-American) with a potential fifth from Iran. In our experiments we have compared the activity of the clinically approved echinocandins and an echinocandin type antifungal agent under development (rezafungin) against a panel of *C. auris* (n = 17) isolates belonging to the four globally prevalent lineages (Clade I-IV) using time-kill methodology. The examined concentration range was 0.25-32 mg/L in presence and absence of 50% of human serum in RPMI-1640 medium. According to our results, all the tested echinocandins showed fungistatic activity over their respective MIC in both media examined, however positive k-values (killing-rate) were less consistently achieved in normal RPMI-1640 compared to serum containing medium. For instance, in RPMI-1640 no echinocandin was able to reduce the number of living cells compared to starting inoculum for isolates of Clade III (South-African). In contrast, in presence of serum, at least a slight decrease was always observed, yet elimination of fungal cells was slow. Paradoxical growth was observed for Clade IV isolates for all echinocandins in RPMI-1640, but not in presence of serum. Overall echinocandins showed mostly concentration dependent fungistatic activity in both medium used. The better activity observed in presence of human serum may at least partially explain the discrepancy between the weak in vitro and the much better in vivo activity of echinocandins against *C. auris* isolates. Lineage dependent differences in echinocandin susceptibility were noticed, however whether these have any clinical implications is yet unknown.

SURVIVAL OF MICROBIAL COMMUNITIES IN NUTRIENT LIMITED CIRCUMSTANCES

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Microorganisms sustaining life in low nutrient content environments are characterized with novel strategies to survive such extreme conditions. During the present study, cultivation and cultivation independent methods were performed on different oligocarbophil aquatic environments in order to reveal the inhabitant microbial communities: the prokaryotic cell counts of the different samples were determined based on DAPI staining (Nikon 80i epifluorescence microscopy), bacteria were isolated and determined based on their 16S rRNA gene sequences, thereafter the growth of the isolated bacteria was tested by using micro titer plates. NGS metagenome analysis was done to check the uncultivable hidden diversity of the samples. The following sampling sites were examined: well waters of Tatabánya, Dandár bath, Szentendre, Szent Flórián and Ciprián. The results showed that the cell counts of Ciprián groundwater had the highest values at the opposite of Tatabánya and Szentendre samples, however, these two samples had the highest values of diversity indices based on amplicon sequencing. Szentendre and Ciprián samples showed remarkable similarity in their Archaeal community composition. These samples contained many Archaeal and Bacterial OTUs known to be involved in the nitrogen cycle. In case of Dandár, Altiarchaeia predominated its Archaeal community; their presence is common in anaerobic groundwater, where they have an important role in carbon dioxide sink. Previous studies showed that they are often in close association with sulfide-oxidizing bacteria, this was confirmed with the high presence of uncultured Thermodesulfovibrionia and high SO_4^{2-} content. Methane oxidizers are among the most abundant OTUs within the archaeal community of Tatabánya groundwater where Woesearchaeia dominated the water of Szent Flórián. Widespread presence of Omnitrophicaeota and Unclassified Parcubacteria could be also demonstrated in all samples except Ciprian where the majority of the bacterial OTUs were assigned to Flavobacteriaceae. Among the tested 100 bacterial strains, many behaved as oligocarbophil microorganisms but only 10 could be classified as obligate oligotrophic, most of them were originating from Szentendre and Ciprián samples. Based on literature we can conclude that many strategies exist to overcome the low nutrient content "barrier", e.g. having high-affinity transport systems, ability to degrade xenobiotic and recalcitrant poly aromatic compounds and possessing syntrophic metabolic model, despite that their taxonomy is different.

EPIDEMIOLOGICAL CHARACTERIZATION OF HEV INFECTION, IN HUNGARY

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Hepatitis E virus (HEV) is one of the most important causes of acute hepatitis infections worldwide. Several epidemiological studies have confirmed that the number of chronic cases is increasing continuously. The virus can spread through contaminated water and also by the consumption of undercooked pork or game meat in the developing and the developed countries. The international results verify the fact that HEV infection has become a global problem. In Hungary, only one study showed data about HEV epidemiology in human about 10 years ago, thus our aim was to characterize the recent epidemiological situation. Since June 2018, 802 sera samples from 767 patients admitted to various hospitals in the University of Szeged were analyzed. We used WANTAI HEV-IgM/IgG ELISA test (Wantai Bio-Pharm) to detect anti-HEV IgM and IgG antibodies. In case of IgM positive results, stool samples were also requested for the detection of HEV RNA. Nucleic acid isolation was effected by QIAsymphony DSP Virus/Pathogen Mini Kit (QIAGEN). The cDNA detection was performed by broad range nested RT-PCR as described previously, the presence of HEV specific PCR product was confirmed by agarose gel electrophoresis. Using serological methods, 216 sera samples proved to be positive for IgG, in these cases IgM was not detected, this indicated previous HEV infections. IgM+/IgG+ results were found in case of 47 patients. Acute HEV infections were confirmed mostly over the age of 40 (45 patients). In acute HEV infection group, the number of male patients (29 patients) was higher than female patients. HEV RNA was isolated from stool samples of 37 patients with IgM positive results. Using PCR, we detected HEV specific PCR product in eight patients. The majority of HEV cases occurred in middle-aged and elderly men, probably due to previous liver damage, caused by excessive alcohol consumption. HEV IgM positive results were obtained mainly in January and July, which can be explained by the increased consumption of pork, other smoked products in the winter period and contaminated fruits and vegetables in summer. Our investigations can help to make the diagnosis of HEV infection more efficiently and to improve health control measures.

PHAGE-INDUCED ANTIBIOTIC SENSITIVITY OF MULTIDRUG RESISTANT *PSEUDOMONAS AERUGINOSA*: A PERSPECTIVE ON COMBINED PHAGE-ANTIBIOTIC THERAPY

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In 2017, the World Health Organization issued a global priority list of 12 antibiotic-resistant bacteria that poses the greatest threat to human health. The objective was to warn and entreat to prioritize the research against multi-drug resistant (MDR) bacteria. The most common opportunistic MDR pathogen *Pseudomonas aeruginosa* is on this top list. Recently, some strains even show evolved resistance to 'drugs of last resort', resulting in emergent pan-drug-resistant (PDR) strains. The bacterium has high endogenous resistance to many antibiotics, because of the species' outer membrane barrier, endogenous antibiotic inactivation, and multidrug efflux pumps. The increasing prevalence and severity of MDR bacterial infections have necessitated novel antibacterial strategies. One alternative for treating MDR bacterial infections is phage therapy, the use of lytic bacteriophages as self-amplifying 'drugs' that specifically target and kill bacteria. The single infection bursts out to an exponential pattern, and so it has more effective killing effect than other therapeutic agents do. Phages and their bacterial hosts coexist in nature, leading to an endless co-evolutionary dogfight. This can result in phage resistant bacterial mutants: the most discussed demerits of phage therapy. We propose to exploit this co-evolutionary mechanism to treat bacterial infections caused by MDR *P. aeruginosa*. A *P. aeruginosa* phage. PIAS (family Myoviridae), was isolated and characterized. Interestingly, during infection it induced large genomic deletions in drug-resistant clinical strains of *Pseudomonas aeruginosa*, leading to a compromised drug efflux system in the bacterial host. It was shown that PIAS used multiple receptors including OprM of the MexXY and LPS to initiate the infection. The evolved mutants gained phage resistance through loss or modifications in these receptors. These genomic changes also led to the inactivation of genes playing an important role in antibiotic resistance. In vitro study indicated that combinational therapy of phage PIAS and antibiotics could effectively inhibit *P. aeruginosa* growth. We explored the potential use of such combined therapy as an alternative approach against multidrug resistance *P. aeruginosa* infections.

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PATTERNS OF GENOME EVOLUTION REVEALS INSIGHT INTO THE REGULATION OF FUNGAL LIGNIN DEGRADATION

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Saprotrophic Agaricomycetes play a major role in terrestrial carbon cycle. This group of fungi includes white- and brown-rot species that use different strategies to decompose lignocellulose, the main component of the plant cell wall. White-rot emerged early during the evolution of mushroom forming fungi and involves a complex enzymatic apparatus that facilitates the efficient degradation of lignin, while brown-rot fungi use a reactive oxygen species based strategy to degrade cellulose and hemicellulose modifying, but not removing lignin. Evolution of brown-rot fungi was accompanied by convergent losses of several carbohydrate active enzymes (CAZymes) known to be involved in lignin degradation, e.g. class II peroxidases. However, most of our knowledge on the delignification of the plant cell wall is limited to such CAZymes and we know less about the regulation of the process. Therefore, using comparative genomics we aimed to identify novel players of the process with a special emphasis on regulatory gene families. In order to do that, we have reconstructed the evolutionary history of all the gene families of 171 Basidiomycota species with diverse lifestyles. First, we have assembled a dataset of the whole proteomes of the 171 species. Protein sequences were clustered into gene families and then aligned. Next, we inferred maximum likelihood gene trees and used a concatenated alignment of single-copy orthogroups with at least 50% taxon occupancy to infer a species tree. Then we carried out gene tree-species tree reconciliation and reconstructed gene duplication/loss histories across all families using the COMPARE pipeline. Since we hypothesized that gene families associated with lignin degradation have higher loss rates in weak lignin degraders, we have compared the loss rates of each gene families of efficient lignin degraders to those of weak lignin degraders in five orders of Agaricomycetes (Agaricales, Boletales, Gloeophyllales, Polyporales, and Russulales) separately. Gene families that had significantly higher loss rates in at least three out of five orders and are conserved in at least 75% of the efficient lignin degraders were functionally annotated by assigning Interpro (IPR) domains to each proteins. Regulators were filtered out manually based on IPR domains. Our analysis revealed several gene families, including phosphatases and WD40 proteins with a gene loss pattern associated with the brown rot lifestyle suggesting a role in lignin degradation in white-rot fungi.

UNDERSTANDING THE PATHOGENICITY OF *SCEDOSPORIUM* SPECIES, THE EMERGING CYSTIC FIBROSIS PATHOGENS

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Scedosporium species are ubiquitous saprophytic hyaline filamentous fungi, which are commonly associated with anthropogenic habitats. As opportunistic human pathogens, these species are capable of causing a wide spectrum of infections (e.g., they are the second most frequently isolated filamentous fungi from the sputum of patients with cystic fibrosis), while known to be resistant against numerous clinically used antifungal drugs. The incidence of clinically relevant species does not reflect their environmental distribution, moreover, some species has not been associated with clinical samples at all. These observations suggest, that the different *Scedosporium* species might possess unique properties (e.g., a specific set of virulence factors) allowing them to colonize distinct environmental niches. These virulence factors might serve as novel targets for antifungal strategies. In this study, we investigate host-pathogen interactions using in vitro and in vivo model systems in order to compare the pathogenic potential of *Scedosporium apiospermum*, *S. boydii*, *S. aurantiacum*, *S. angustum*, *S. dehoogii*, *S. ellipsoideum*, *S. minutisporum*, *S. fusoideum* and *S. desertorum*. To reveal differences in the killing potential of macrophages against various *Scedosporium* species and to describe the immune cell damaging capacity of these fungi, in vitro cytotoxicity and imaging flow cytometry-based phagocytosis assays are performed. Alternative invertebrate infection models are also applied in order to compare the in vivo pathogenic potential of scedosporia. Our preliminary observations suggest differences between the pathogenic potential of the tested species. We observed that *S. angustum* conidia are differentially phagocytosed compared to the conidia of *S. apiospermum* and *S. dehoogii* by murine J774.2 macrophages. These results were also confirmed by using the *Galleria mellonella* infection model. The results of this ongoing project may reveal novel aspects of the background of pathogenicity mechanisms (e.g., virulence factors) and the main risk factors for the emergence and spread of scedosporia in our immediate environment, and may also facilitate the understanding of the pathogenicity of filamentous fungi through the investigation of the emerging pathogenic *Scedosporium* genus.

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CHARACTERIZATION OF CYCLOPIAZONIC ACID PRODUCING *ASPERGILLUS TAMARII* ISOLATES FROM HUMAN KERATOMYCOSES

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In South India, where fungal keratitis is among the leading causes of ocular morbidity and corneal blindness, *Aspergillus tamarii* appears to be an emerging etiological agent of this sight-threatening disease. Although it is a non-aflatoxigenic species, its representatives can produce cyclopiazonic acid (CPA), which is known to possess neurotoxic and immunomodulatory effects. As the number of keratitis cases where molecular identification, antifungal susceptibilities and clinical data are determined at the same time are very low, the clinical relevance of identifying *A. tamarii* at the species level is still unknown. Thus, studies like the present work are of great importance. Firstly, we compared the reliability of morphological and molecular identification methods in case of *A. tamarii*. Secondly, we determined the antifungal susceptibility profiles of the isolates and tested the in vitro efficacy of clinically relevant dual drug combinations against them. And finally, the CPA producing ability of the strains was also examined. Our results suggest that morphological identification is uneasy in case of *A. tamarii*: none of the strains has been identified morphologically as *A. tamarii*, while ITS and the calmodulin sequences of the six isolates showed ≥99% similarity to sequences of the CBS 104.13 type strain of *A. tamarii*. Antifungal susceptibility testing revealed that amphotericin B, clotrimazole, econazole, itraconazole, ketoconazole and terbinafine exerted good antifungal activity (minimum inhibitory concentration < 2 µg/mL) against *A. tamarii* isolates. Interestingly, natamycin, the commonly applied antifungal drug in the treatment of fungal keratitis, had no inhibitory effect on the in vitro growth of the isolates. Drug combination tests revealed synergism between natamycin and itraconazole and between natamycin and econazole in three cases. The other combinations presented indifferent activities against *A. tamarii* isolates. Finally, LC-MS/MS analysis revealed, that five of the six isolated was able to produce CPA.

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PRODUCTION OF PREBIOTIC OLIGOSACCHARIDES BY BETA-GALACTOSIDASE ACTIVE COCKTAILS

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Beta-galactosidases are important enzymes in the food and pharmaceutical industries. They catalyze the lactose degradation, which is utilized in the production of lactose-free foods for lactose intolerant diet. Beta-galactosidases can synthesize galacto-oligosaccharide (GOS) prebiotics through transgalactosylation reactions. Some microorganisms are excellent sources of beta-galactosidases with industrial potential, however, production and features of the catalytic activity are less studied in zygomycetes fungi. In our experiments, many beta-galactosidase active fungal strains were identified within the genera *Mucor*, *Rhizomucor*, *Rhizopus*, *Mortierella*, *Umbelopsis* and *Lichtheimia*. Two submerged fermentation conditions (with lactose or wheat bran as substrates) and a solid-state approach containing wheat bran as fermenting material were tested for the production. The *Rhizomucor pusillus* and *Lichtheimia ramosa* presented good production ability and high enzyme activity under these conditions. Our aim was to evaluate the oligosaccharide synthesizing capacity of beta-galactosidase active cocktails produced by *R. pusillus* and *L. ramosa*. Partial purification of the activity was performed, then solutions containing different types of glycoside donor and acceptor substrates were set up for the enzymatic synthesis reactions. HPLC-MS/MS analysis showed C18 and C24 GOS compounds as well as raffinose and nystose molecules in certain reaction mixtures. Many saccharide solutions produced enhanced the growth of *Lactobacillus acidophilus*, *Bifidobacterium animalis* subsp. *lactis*, and *Saccharomyces boulardii* probiotics in vitro.

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