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TRANSCRIPTOME ANALYSIS OF *RHODOCOCCUS PYRIDINIVORANS* SOIL BACTERIA IN THE PRESENCE OF ZEARALENONE

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Our food and feed crops are under intense stress due to extreme weather changes that reduces the resilience of our crops and increases the probability of microbial contamination already during field production. The main food safety risk are mycotoxins produced by molds, such as zearalenone (ZEA) produced by members of the *Fusarium* genus. ZEA has estrogenic effect by binding to the estrogen receptor, when released into the human body and livestock. Due to the molecular structure, ZEA could cause reproductive disorders. In order to eliminate the negative effects of the mycotoxin ZEA major focus should be on the development and proper application of decontamination methods. In the interest of reducing the human and animal health risks, it is essential to develop a method that can provide an effective solution without inducing additional health risks. Several studies have shown that certain microbes and their enzymes can degrade and neutralize mycotoxins. Bacterial biodegradation and detoxification can be an effective and safe way to remove mycotoxins in food and feed. Previous studies of our research group revealed that the soil-dwelling *Rhodococcus pyridinivorans* K404 has outstanding the biodegradation capacity of zearalenone. The intracellular extract of strain K404 degraded 98% of ZEA after 7 days. The aim of this study is to determine the background of biodegradation of the bacterial strain ZEA by identifying the genes and enzymes involved in the detoxification process. Strain K404 was cultured in vitro in the presence of ZEA to establish the growth kinetics and the log phase of the strain. The total RNA was isolated from the culture based on the log phase of the strain to obtain the largest amount of total RNA. Following the transcript analysis of total RNA of strain K404, we identified differential expressed genes (DEGs). From a total of 4,798 expressed genes, 1,329 DEGs were identified. Many of these are upregulated, such as transcriptional regulators, stress proteins and the genetic code of many proteins involved in metabolic processes. ZEA induced the highest expression of cyclohexanone monooxygenase among the enzymes with metabolic function. Studying these genes and enzymes and testing their biodegradation capacity could lead to the production of a feed additive that could help to eliminate the harmful effects of ZEA in the future.

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MICROBIAL COLONISATION OF SPENT MUSHROOM COMPOST

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The microbial community of the materials used for white button mushroom (*Agaricus bisporus*) production is a highly complex and dynamically changing assemblage of bacteria and fungi. The quality of the casing layer used during the production is influenced by its microbiological composition. Bacteria present in spent *Agaricus* compost include *Bacillus*, *Alcaligenes*, *Pseudomonas* and *Microbacterium* species, while fungal components comprise of *Fusarium*, *Mucor*, *Trichoderma* and *Lecanicillium* species among others. Certain representatives of the genera *Bacillus* and *Pseudomonas* are considered as beneficial. Our aim is to use spent mushroom compost for developing alternative casing layers. Samples were collected from spent mushroom compost during its natural recomposting process. We performed metagenome sequencing of the samples, and monitored the colonization ability of microorganisms in the deriving casing layer alternatives in pot experiments. Our results showed in all cases a microbiota with strong presence of Bacillota, Gammaproteobacteria and Ascomycota. From the colonization, experiment running for 4 weeks we concluded that bacteria peaked during the second week in the casing layer, while fungi increased in number over time, but bacteria were present in higher numbers in the casing layer throughout the observation period. The white button mushroom cultivation experiment in pots was also used to monitor the effect of the recomposted spent mushroom compost as a casing layer alternative on yield. Neither pinhead nor fruiting body formation could be observed, when recomposted spent mushroom compost was used alone for casing, while both pinheads and fruiting bodies were formed when the recomposted spent mushroom compost was mixed with peat in a 1 : 1 ratio. Based on our results, recomposted mushroom compost could be developed to a promising alternative casing material in the future.

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ROOT COLONIZATION ABILITY OF DIFFERENT PLANT GROWTH-PROMOTING BACTERIA ON TOMATO AND MAIZE PLANTS

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Bacteria play an essential role in supporting plant growth and improving their ability to tolerate stress. The colonization potential of different plant growth promoting (PGP) bacteria can be tracked using fluorescent labeling techniques. By examining the effectiveness of root colonization, we will gain a better understanding of their impact and faith of the used PGP strains during the development of the seedling and plant. This study aims to comprehensively investigate the root colonization potential and efficacy of plant growth promoting strains isolated form different habitats. Based on literature data, 48 bacterial strains were selected and screened from our department strain collection for PGP properties such as osmotic stress tolerance, indole-3-acetic acid, exopolysaccharide, siderophore, and 1-aminocyclopropane-1-carboxylate deaminase production and phosphate solubilization. We tried to introduce a kanamycin resistance and green fluorescent protein encoding plasmid into the strains with multiple PGP properties. The kanamycin sensitive strains were cultured in liquid medium until the early log phase followed by centrifugation and washing with cold HEPES buffer before being resuspended in cold glycerol (10%). The plasmid was introduced into the competent cells through electroporation. The transformed cells were regenerated and subsequently cultured on selective agar medium. For the root colonization assay surface-sterilized maize and tomato seedlings were grown in sterile perlite for 7 days and inoculated with fluorescence-labeled bacterial suspension. Following a 16 hour the incubation period, fine roots were repeatedly washed with sterilized water and the level of colonization was evaluated through fluorescent microscopy after seven days. 28 strains from genus *Pseudarthrobacter*, *Kocuria*, *Brevibacterium*, *Brevibacillus*, *Stenotrophomonas*, *Agrobacterium*, *Priestia*, *Pedobacter*, *Pseudomonas*, *Variovorax* and *Erwinia* possess multiple plant growth properties at the same time. So far, the plasmid was introduced into two *Pseudomonas* and one *Erwinia* strain, of which *Pseudomonas* strain SSZ104-1R isolated from grassland rhizosphere microbial community was the most able to colonize the root surface of the seedlings.

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ANTIFUNGAL IN VITRO EVALUATION OF *TRICHODERMA* SPP. ISOLATED FROM YELLOW PITAHAYA (*SELENICEREUS MEGALANTHUS*) CROPS AGAINST *ALTERNARIA* SPP.

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Yellow pitahaya has a high nutritional value and economic importance but can be susceptible to plant pathogens. The genus *Alternaria* spp. is one of the primary pathogens for this crop, and its management without agrochemicals represents a challenge to agriculture. The use of native *Trichoderma* strains has been proposed as a sustainable alternative to control this pathogen in yellow pitahaya. This study aims to evaluate in vitro the antifungal capacity of six strains of native *Trichoderma* spp. against two *Alternaria* spp. native strains to yellow pitahaya cultivars located in Morona Santiago province, Ecuador, and to identify them molecularly. For molecular identification at the species level, 5 different regions were amplified. Antibiosis assays were performed at 5%, 10%, and 15% concentrations. Antagonism assays were performed by dual culture. According to molecular identification, it was determined that two strains corresponded to *Trichoderma asperellum*, four to *T. koningiopsis*, and the remaining two to *Alternaria burnsii* and *A. alternata*. Significant differences ($P<0.0001$) were observed in the antifungal evaluation among treatments. The highest percentage of inhibition in antibiosis was obtained by strain MS-P-03 with 48% and in antagonism by strain MS-P-07-1 with 79.4%. *T. asperellum* and *T. koningiopsis* species can control or reduce the growth of *A. burnsii* and *A. alternata*.

EMERGENCE OF CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE* ENCODING blaOXA-48-LIKE AND blaNDM CARBAPENEMASES

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Carbapenem-resistant Enterobacterales (CRE) is a major public health concern due to their ability to cause severe infections with limited treatment options. The most prevalent CRE-related carbapenemases include the *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo-β-lactamase (VIM), imipenemase or IMP-type metallo-β-lactamase and OXA-48-like enzymes. In 2022, a new carbapenem resistant *K. pneumoniae* strain encoding *blaOXA-48*-like and *blaNDM* carbapenemases appeared in our tertiary setting. Isolates were collected between April and December 2022. The susceptibility testing was performed using Kirby-Bauer disk diffusion method according to the annual recommendations of EUCAST (European Committee on Antimicrobial Susceptibility Testing). Moreover, standard broth microdilution (EUCAST) was used to determine the susceptibility of isolates to colistin. Subsequently, CRE isolates derived from urine, lower-respiratory tract or blood samples were examined regarding the presence of genes encoding carbapenemases using the Cepheid GeneXpert® Carba-R system. In 2022, 428 CRE isolates were detected from 161 patients. Among the isolates, 404 were *K. pneumoniae* and 24 belonged to the *Enterobacter cloacae* complex. Regarding specimen distribution, the highest positivity rate was observed among screening samples (189/428; 46.3%). However, in case of the

invasive samples, the majority of CRE isolates derived from blood samples (69/428; 16.8%), followed by urine (60/428; 14.9%), lower respiratory tract (38/428; 9.4%), surgical (25/428; 6.2%), and catheter-related (15/428; 3.7%) samples. All isolates were resistant to at least one carbapenem (ertapenem, meropenem, imipenem). Differences were found between *K. pneumoniae* and the *E. cloacae* complex in case of the prevalence of resistance to ceftazidime/avibactam (54.7% vs. 8.3%), amikacin (84.2% vs. 12.5%), gentamicin (80.7% vs. 12.5%), tobramycin (92.1% vs. 12.5%), and trimethoprim/sulfamethoxazole (96.5% vs. 8.3%). All isolates from the *E. cloacae* complex were susceptible to colistin, but 33 (8.2%) *K. pneumoniae* were resistant. The vast majority of isolates (83.3%) harbored both *blaOXA-48*-like and *blaNDM* genes, while 8.8% and 7.8% exclusively encoded *blaOXA-48*-like and *blaNDM* genes, respectively. In conclusion, the emergence of carbapenem-resistant *K. pneumoniae* encoding OXA-48-like and NDM-type carbapenemases in the tertiary setting highlights the urgency of addressing this issue. The high prevalence of resistance among *K. pneumoniae* isolates, and limited treatment options emphasizes the need for a multifaceted approach, involving prevention, rigorous surveillance and prudent antibiotic stewardship.

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COMPREHENSIVE INVESTIGATION OF PEPTAIBOLS PRODUCED BY *TRICHODERMA* FUNGAL SPECIES TO ESTABLISH THEIR PRACTICAL APPLICATION

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Members of the genus *Trichoderma* are widely used in various fields of agriculture and biotechnology. One of the important secondary metabolic products produced by *Trichoderma* filamentous fungal species are the peptaibols. Peptaibols are produced by non-ribosomal peptide synthetases (NRPSs), which can incorporate a high amount of non-proteinogenic amino acids into the sequences and facilitate a great variety of motifs. Peptaibol extracts are capable of inhibiting plant pathogenic Gram-positive bacteria and several fungal species, thus, they can have a role as biocontrol and plant growth supporting agents. Nowadays, in addition to laboratory and plant tests, modern computer modeling techniques such as accelerated molecular dynamics (aMD) simulation also provide important information about the possible modes of action of peptaibols. The main aim of our work is the comprehensive investigation of peptaibols: to study their bioactivity effects against microorganisms, to test their impact on plant growth promotion and to understand the connection between the exerted effects and the characteristic properties of peptaibol sequences through structure-activity relationships (SARs). Firstly, the peptaibol production of 6 *Trichoderma* species from clade Longibrachiatum, of two *T. rossicum* strains from clade Stromaticum and of 4 *Trichoderma* strains from clade Harzianum were determined. Purified peptaibol extracts were tested against eleven commonly known Gram-negative and Gram-positive bacterial strains, as well as four plant pathogenic fungal species. Minimum inhibitory concentration (MIC, mg ml^{-1}) and effective concentration (EC, mg ml^{-1}) values of the purified peptaibol extracts were determined using in vitro tests. Among the peptaibol extracts, strains *T. longibrachiatum* f. *bissettii* SZMC 12546 and *T. rossicum* TUCIM 889 exerted a stronger effect against most Gram-positive bacteria, and they could inhibit Gram-negative *Rhizobium radiobacter*. In parallel with the laboratory tests, aMD simulations were carried out with the peptaibol sequences produced in the largest amounts to gain a deeper insight into their mechanisms. We compared the in vitro MIC and EC values with our results obtained during the simulations and looked for correlations between the folding mechanisms, structural properties of the peptaibols and their exerted bioactivities.

Based on our results, different structural properties of the peptaibols, such as characteristic amino acid motifs, the location of hydrophobic and hydrophilic regions, and the peptaibol length can influence the expressed bioactivity. To investigate further correlations between SARs and plant growth-supporting effects, peptaibol extracts were selected for experiments on plants under plant growing chamber, greenhouse and field conditions. The comprehensive studying of peptaibols may lead to an effective selection of peptaibols for plant disease management and application in agriculture.

ERGOSTEROL BIOSYNTHESIS AND ALTERNATIVE BIOSYNTHESIS PATHWAYS IN *MUCOR LUSITANICUS*

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The members of the order Mucorales are mostly saprotrophic filamentous fungi found all over the world. Several species of this group are capable of causing systemic infections, often fatal, in immunosuppressed patients, known as mucormycosis. Infections are characterized by a high mortality rate and rapid clinical progression, which may be due to a number of risk factors and the setting of late diagnosis. Lipid formulations of amphotericin B are used for the therapeutic treatment of mucormycoses, while azoles, most commonly posaconazole and isavuconazole, are used for prevention. Ergosterol is an important component of the fungal cell membrane. The ergosterol biosynthesis pathway is a successful antifungal drug target in the treatment of fungal infections in humans. Azoles inhibit the function of ERG11. In addition to *erg11*, *erg6* may also play a role in resistance to azoles. These compounds are the most common

antifungal drugs used to treat fungal infections. The main goal of this study is to investigate the role of the *erg6* gene in ergosterol biosynthesis, azole resistance and virulence in *Mucor lusitanicus*. Genome of *M. lusitanicus* encodes three sterol C-24 methyltransferase genes (*erg6a*, *erg6b* and *erg6c*), which catalyzes the conversion of zymosterol to fecosterol. *erg6* plays role in growth at high temperature and virulence in *Cryptococcus neoformans* and it plays a role in the alternative ergosterol biosynthesis pathway in yeast. The alternative pathway is activated when ERG11 is inhibited, for example by azole treatment. We created *erg6* single and double knockout mutants using a CRISPR-Cas9 system. Growth ability, sporulation capacity, sterol content, virulence and sensitivity to azoles of the mutants were examined. After knock out of MS12-Δ*erg6b*, we found that ergosterol and eburicol levels were significantly decreased in the mutant strain, while lanosterol, zymosterol and 7-dehydrodesmosterol were significantly increased. Sporulation capacity, we found a significant decrease in spore number per plate after MS12-Δ*erg6b* knockdown. The lack of MS12-Δ*erg6b* resulted in reduced growth ability and increased sensitivity to azoles. Using a *Galleria mellonella* invertebrate animal model, we tested the deletion mutant strains and found that the virulence of the strain was significantly reduced following MS12-Δ*erg6b* knockdown. No changes were observed in MS12-Δ*erg6a* and MS12-Δ*erg6c* compared to the control strain under the conditions previously tested. The sterol composition of *erg6b* knockout mutants was significantly altered and revealed the presence of at least four alternative sterol biosynthesis pathways.

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THE EFFECT OF SALT CONCENTRATION AND ANION COMPOSITION ON THE METABOLITES OF MICROALGAE

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Soda lakes and pans are unique, multiple extreme environments. These shallow, moderately saline, permanently alkaline aquatic habitats have a great biodiversity, they are important feeding, resting and nesting place for birds, have a colorful insect world, and many rare, protected plants. Soda pans also can serve as a model system to understand the effect of environmental changes. Small planktonic algae are key components of these ecosystems, but their chemical interaction with other microorganisms is only poorly known. Therefore, we performed an experiment to analyze the pattern of released compounds by cultivating them with different salt types applied in various concentration values. Based on 18S rDNA sequencing, the studied green algal strain belongs to genus *Mychonastes*. We studied the effect of up to 15g L^{-1} (~100mmol) NaCl and $NaHCO_3$ on the cultures.
Compounds released to the liquid growth medium were concentrated with solid phase extraction, and analyzed with a liquid chromatograph coupled with a quadrupole time-of-flight mass spectrometer in a range of 100 - 1,000m z^{-1}. The metabolite profile differed comparing samples with different salt types and concentrations. Among others, the quantity of kainic acid, a well-known bioactive compound changed with the amount of the salt, and increased most remarkably with the increasing amount of $NaHCO_3$. Presence of dissolved salts has an important effect on the metabolism of planktonic microalgae.

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DESIGN AND CHARACTERIZATION OF A MULTISTAGE PEPTIDE-BASED VACCINATION PLATFORM TO TARGET *MYCOBACTERIUM TUBERCULOSIS* INFECTION

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Bacille Calmette-Guérin (BCG), the only available vaccine against tuberculosis (TB), provides limited and inconsistent protection against pulmonary TB in adults, the primary source of disease transmission. The development of a new vaccine is hampered by the complex immunopathology of *Mycobacterium tuberculosis* (Mtb), especially in terms of eliciting protection against both active and latent stages of infection. Multistage protein-based vaccines, which incorporate antigens expressed in both phases, have emerged as a promising strategy to address this issue [1]. In line with this approach, we designed a multistage peptide-based vaccine platform containing $CD4^+$ and $CD8^+$ T cell epitopes for inducing an effective T cell response against Mtb. Choosing T cell epitopes that elicit a pathogen-specific immune response is crucial to developing effective and safe peptide vaccines. This study reports an accurate selection process from previously identified epitopes, finalized by the established ex vivo antigen recall assay on Mtb-sensitized peripheral blood mononuclear cells (PBMCs) that proved that the selected peptide pool PM1 successfully stimulated the production of IFNγ and TNFα,

pivotal cytokines in the control of TB. However, a proper formulation is required to overcome the inherent weakness of synthetic peptide vaccines, such as low in vivo stability and poor immunogenicity. This study compared two approaches: PM1 formulation with an adjuvanted oil-in-water emulsion (Sigma Adjuvant System, SAS) and the development of a more complex structure (Pal-CGHP) obtained by combining peptide synthesis, chemoselective ligation, and palmitoylation. The latter previously proved to improve peptide-based vaccines' stability, cellular uptake, and immunogenicity [2, 3]. Pal-CGHP showed significantly higher cellular uptake in three different antigen-presenting cells models: the human MonoMac-6 cell line, the murine macrophage-like RAW264.7, and the murine bone-marrow-derived dendritic cells (BMDC). Moreover, confocal microscopy results showed a fast internalization and endo-lysosomal localization. In vivo immunogenicity study was performed to compare the immunogenicity of the formulated PM1 and Pal-CGHP. SAS formulation did not improve the overall immunogenicity of the peptide mixture, although its two main components, monophosphoryl lipid A and the trehalose dicorynomycolate, are widely known inducers of the Th1 immune response. On the contrary, Pal-CGHP immunization produced significant T cell proliferation, and IFNγ and TNFα production. Pal-CGHP merits further investigation as a multi-peptide self-adjuvating vaccine platform.

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EFFECT OF QS SIGNALING MOLECULES AND ESSENTIAL OILS ON BIOLOGICAL ACTIVITIES OF FOOD-CONTAMINATING YEASTS

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Despite the increasing technological breakthroughs in food science and technology over the years, spoilage of food remains a global problem. The presence of naturally occurring bacterial, yeast and fungal populations make food contamination inevitable leading to serious problems in food production and preservation. Quorum sensing (QS) controls many physiological mechanisms in microorganisms, and it is based on specific signal molecules like N-acyl homoserine lactones (AHL) for bacteria and farnesol or tyrosol for yeast species. Due to the involvement of QS in expression of virulence genes inhibiting this process with specific molecules called quorum quenchers (QQ) appears to be a promising strategy for preventing microbial contamination. The present study is part of a research project aiming to elaborate novel anti-QS therapies applicable in food preservative developments. Therefore, we aimed to explore the effect of AHL bacterial signal molecules (C4, C12) and plant essential oils (thyme, cinnamon, lemon) as potential inhibitory agents against yeast growth and biofilm formation. Ten yeast strains were selected from our culture collection and ten were selected form meat sample isolates. C4 and C12 were used in 2μM while essential oils in MIC/2 concentrations to investigate biofilm formation. Bacterial signal molecule C12 reduced biofilm formation for most of the culture collection strains to 60% while for *Kluyveromyces marxianus* and *Saccharomyces ludvigii* reduction was to 20%. C4 signal molecule reduced biofilms to 60% and no effect was recorded for *Saccharomyces rouxii* and *Kluyveromyces lactis*. In case of isolated strains biofilms were more resistant, signal molecules inhibited biofilm formation only for *Debaryomyces hansenii*. Cinnamon EO was the best inhibitor with MIC values between 3.1 - 6.2mg ml^{-1} for isolates and 0.4mg ml^{-1} for collection strains. Thyme resulted in MIC values between 1.5 - 3.1mg ml^{-1} for all strains while for lemon MIC was between 3.1 - 6.2mg ml^{-1}. Biofilm formation of all strains was significantly inhibited mainly by cinnamon and thyme EOs. Our results indicate that bacterial signal molecules and EOs can affect growth activities of food-related yeasts and the compounds that were effective may influence the shelf life of certain foods.

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DETERMINING MICROBIAL NUTRIENT NICHES IN THE GUT

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The success of a microbe in colonizing the gut environment is determined in part by its ability to competitively acquire available nutrients. This "nutrient niche" is key not only to commensal establishment and abundance, but also to the microbiome's colonization resistance against enteric pathogens. Although the physiological potential - or fundamental niche - of a microbe can be studied in pure culture, its realized niche within a microbiome is more challenging to experimentally determine. In this lecture, I will discuss our efforts in developing and applying approaches based on stable isotope probing to shed light on the realized nutrient niches of a range of dietary

and host compounds. I will discuss how this can be used to screen for prebiotics with desired properties as well as to identify candidate probiotic organisms for therapeutic applications.

OSMOTIC STRESS ELICITED GENE EXPRESSION CHANGES IN *ASPERGILLUS WENTII* WILD-TYPE AND 'c *gfdB* AND *ASPERGILLUS NIDULANS* WILD-TYPE AND Δ*gfdB* MUTANT STRAINS

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Industrial applications of the fungal genus *Aspergillus* are steadily increasing. The osmophilic *Aspergillus wentii* is one of the most promising *Aspergillus* species, which has already found its applications in enzyme and biodiesel productions. In *A. wentii*, osmophily has been connected to the lack of the *gfdB* gene, putatively encoding a NAD^+-dependent glycerol-3-phosphate dehydrogenase in many *Aspergillus* spp., including the filamentous fungus model organism *Aspergillus nidulans*. To shed light on the molecular background of the osmophilic phenotype of *A. wentii, A. wentii* wild-type and 'c *gfdB* as well as *A. nidulans* wild-type and Δ*gfdB* strains [1, 2] were exposed to 1M NaCl (ionic osmolyte), 2M sorbitol (non-ionic osmolyte) and 1M NaCl + 2M sorbitol in submerged liquid cultures, and the global transcriptome changes were recorded using RNAseq technique and compared. Sorbitol exposure upregulated more genes and downregulated less genes in both the *A. wentii* wild-type and 'c *gfdB* strains in comparison to untreated controls but the number of downregulated genes was twice higher in the *gfdB* supplemented strain. Sorbitol-responsive genes highly overlapped in *A. wentii* wild-type and 'c *gfdB* cultures. NaCl treatment increased further the number of upregulated genes in the *A. wentii* 'c *gfdB* cultures but smaller phenotypes were observed in the presence of 1M NaCl than with 2M sorbitol in our previous stress assay studies [1]. Unexpectedly, combined NaCl + sorbitol exposures caused the least transciptomic changes in both the *A. wentii* and *A. nidulans* strains. In wild-type *A. nidulans* cultures, the ergot alkaloid biosynthetic process and oxidative stress response processes were upregulated under combined stress conditions, which was not detected in the case of the *A. nidulans* mutant strain, indicating that *gfdB* gene functions interfered with these processes. In the *A. nidulans* Δ*gfdB* strain, the combined stress upregulated the polyol metabolic process, including genes encoding alcohol dehydrogenase, glycerol dehydrogenase and D-arabinitol 2-dehydrogenase. Pyruvate metabolic genes were also upregulated coding for acetyl-CoA hydrolase, lactic acid dehydrogenase and malate synthase. The observed gene expression changes may be exploited in future industrial strain developments aiming at improving the yields of alcohol and polyol fermentations by the aspergilli.

[1] Bodnár et al (2023) Appl Microbiol Biotechnol 107: 2423.
[2] Király et al (2020) Fungal Biol 124: 352.

INFLUENCE OF *CANDIDA ALBICANS* AND *CANDIDA PARAPSILOSIS* INFECTION ON MACROPHAGE POLARIZATION AND PHAGOCYTIC ACTIVITY

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Candida species cause the candidiasis, which can range from superficial mucocutaneous disorders to invasive infection. *C. albicans* is the main causative agent of candidiasis and accounts for more than 70% of the candidiasis. More recently, *C. parapsilosis* showed a higher incidence of candidemia in surgical patients than *C. albicans*. In 2022, the World Health Organization (WHO) reported the fungal priority pathogens list (FPPL). Among the critical and high priority groups are the *C. albicans* and *C. parapsilosis*, respectively. This encourages global efforts responding to the exacerbation and emergence of the pathogens mentioned. Macrophages, essential components of the host's innate immune defense, can be polarized into classically activated (M1) and alternatively activated (M2) macrophages, which exhibit distinct phenotypes depending on specific stimuli and signals in their microenvironment. In this study, the influence of *C. albicans* (SC5314) and *C. parapsilosis* (CLIB 214) in macrophage polarization was determined. Human peripheral blood mononuclear cells (PBMCs) were isolated from a buffy coat and infected with *C. albicans* (host : *Candida* ratios 10 : 1 and 25 : 1) for 24 hours and *C. parapsilosis* (host : *Candida* ratios 1 : 1 and 5 : 1) for 24 and 48 hours. After the infection, PMBC-derived monocytes were immunolabelled using monocyte-specific ($CD68^+$), M1-specific ($CD86^+$), and M2-specific ($CD163^+$) markers. Their polarization was characterized using flow cytometry via fluorescence-activated cell sorting (FACS). Additionally, PBMC-derived monocytes were also differentiated using Granulocyte-macrophage colony-stimulating factor (GM-CSF) and activated using Interferon-gamma (IFN-γ) to determine how IFN-γ-activated M1 macrophages respond to *C. albicans* and *C. parapsilosis* infection through phagocytosis and killing assays. The result showed that the PBMC-derived monocytes infected with *C. albicans* showed no significant differences in their polarization to M1 or M2 macrophages. On the other hand, *C. parapsilosis* infection drives polarization into M2 macrophages. In the phagocytosis and killing assays, IFN-γ-activated M1 macrophages resulted to lower phagocytic and killing activity against *C. albicans*

and *C. parapsilosis*. However, this phenomenon was only significant in the case of *C. parapsilosis*. These findings suggest that *C. parapsilosis* possesses mechanisms that promote M2 polarization of macrophages, potentially contributing to immune evasion and disease progression. The reduced phagocytic and killing activity of IFN-γ-activated M1 macrophages against *C. parapsilosis* may further compromise the immune response and favor the survival and persistence of this pathogen.

THE COMPLEX IMPACT OF VARIOUS FUNGI IN THE AROMATIC PROFILE OF NOBLE ROT GRAPES IN THE TOKAJ WINE REGION

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Sweet wines made from noble rot grapes like Sauternes and Tokaj are considered as one of the most qualitative wines on the market. These wines are also called "botrytized" wines due to the development of *B. cinerea*, giving these wines their unique aromatic profile: citrus fruit, honey, stone fruits and caramel to mention the most recurring ones. Previous research has however shown that there is a complex coexistence of various filamentous fungi and yeast other than *B. cinerea* : *Alternaria alternata*, *Epicoccum nigrum* and *Aureobasidium pullulans*. The impact of these species on the aromatic profile is not yet fully understood. RNA sequencing of noble rot berries was aligned to these species and enriched pathways involved in the synthesis of aromatic compounds such as amino acid-, carbohydrate- and lipid metabolism co-jointly expressed by all filamentous fungi and yeast were identified. This study shows that the aromatic profile of "botrytized" wines is more complex than its name may indicate.

RESOLVING COLISTIN RESISTANCE AND HETERORESISTANCE IN ENTEROBACTERALES

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Colistin, a polymyxin antibiotic, has emerged as a last-resort therapeutic option for treating infections caused by multidrug-resistant Gram-negative bacteria such as *Enterobacter* spp.. These bacteria along with *E. coli* and *Klebsiella pneumoniae*, often harbor carbapenemases, forcing the therapeutic use of colistin. Nevertheless, the continued rise of colistin resistance in healthcare institutions worldwide poses a significant challenge to the successful management of these infections. Furthermore colistin heteroresistance, a phenomenon whereby the presence of subpopulations within a bacterial isolates exhibit varying levels of susceptibility to the antibiotic, further complicates the management of colistin-resistant infections, as these subpopulations with higher resistance may lead to the emergence of fully resistant strains. For bacteria comprising the *Enterobacter cloacae* complex (ECC), taxonomic uncertainty associated with identifying its true members together with the problems relating to routine microbiology susceptibility testing for colistin resistance underestimates both the true prevalence of these bacteria in causing infections and mask the extent of how widespread this resistance is in these bacteria. The development of simple, reliable diagnostic methods that recognize resistance and heteroresistance is therefore urgently needed. The presentation shows that many members of the species *Enterobacter* are inherently colistin-resistant due to L-Ara4N modification of lipid A. By identifying environmental fluctuations in pH as the major determinant of heteroresistance levels in this species and determining those bacterial genes involved in sensing this environmental signal, a simple and robust assay was developed that unambiguously monitors colistin resistance in *Enterobacter* spp. to avoid treatment failures that can be easily implemented in healthcare institutions worldwide. The elements driving clonal heteroresistance are also highly conserved in the order Enterobacterales, such as in the species *Klebsiella*, thereby opening the path for generic tests to accurately determine colistin resistance across a broad spectrum of clinically relevant bacteria.

COMPARATIVE GENOMICS AND TRANSCRIPTOMICS ANALYSES CONFIRM THE DISTINCTIVE MYCOREMEDIATION POTENTIAL OF ARMILLARIOID SPECIES

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Genes involved in mycoremediation were identified by comparative genomic analysis in 10 Armillarioid species and selected groups of white-rot Basidiomycota (14) and soft-rot Ascomycota (12) species where we aimed to confine the distinguishing bioremediation

potential of the Armillarioids. The genomes were explored using phylogenetic principal components analysis (PCA), searching for genes already documented in a biocatalysis/biodegradation database. Results underscored a distinctive, increased potential of aromatics-degrading genes/enzymes in Armillarioids with a specific emphasis on a high copy number and diverse spectrum of benzoate 4-monooxygenase [EC: 1.14.14.92] homologs. Furthermore, other enzymes responsible for degrading various monocyclic aromatics were more significantly represented in the Armillarioids than the other white-rot basidiomycetes and enzymes involved in degrading polycyclic aromatic hydrocarbons (PAHs) were prevailing in Armillarioids and other white-rot species than in soft rot Ascomycetes. Transcriptome profiling of *A. ostoyae* and *A. borealis* isolates confirmed that most of the genes involved in the degradation of benzoate and PAHs were significantly expressed in the fungal mycelia invading wood tissues. Data were consistent with armillarioid species offering a more powerful potential in degrading aromatics. Our results provide a reliable, practical solution for screening the likely fungal candidates for their full biodegradation potential, applicability, and possible specialization based on their genomics data.

FIRST REPORT ON THE PLANKTONIC BACTERIAL COMPOSITION OF THE SALINE LAKES IN CYPRUS

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Saline lakes are extreme environments that can be found in all continents. They have variable chemical composition resulting in different habitats, which shapes biodiversity. There are several saline lakes close to the sea in Cyprus, that serve as resting places for birds, especially for flamingos. Although they are important habitats, there is limited information regarding their chemical and microbiological characteristics. In this study, we compared the physical parameters, chemical and bacteria composition of the sea and their adjacent saline lakes. On site measurement of environmental parameters were complimented with laboratory chemical analysis to determine the nutrient content and concentration of main dissolved ions. Illumina amplicon sequencing was applied to reveal the diversity and taxonomic composition of planktonic prokaryotes. The lakes were hypersaline, had neutral pH and few cm water depth. As the result of this study, the estimated number of bacterial species present in the sea was considerably higher than in the lakes probably driven by the salinity. The contrast between physicochemical properties of the lakes and sea resulted in differences of bacterial taxonomic composition mainly related to phyla Actinobacteria and Proteobacteria.

COULD HHV-6 CONTRIBUTE TO AGEING?

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Many latent infections are prone to reactivate in people of advanced age. Human herpesvirus 6A and 6B are two closely related viruses that infect almost all humans. They cannot be fully treated and remain in latent condition throughout the life of infected individuals, with the possibility of reactivation, especially in immune compromised people. It is a cofactor in AIDS progression, multiple sclerosis, tumor progression, graft rejection, Alzheimer's disease. This drives additional interest towards the research of Human herpesvirus 6A and 6B influence on ageing and concomitant illnesses. Human herpesviruses achieve latency by persisting as a circular episome in the nucleus. Both HHV-6A and B can integrate into human chromosomes and may be vertically transmitted in the germ line. FISH, PCR, sequencing, IPCR, and Gardella gels proved chromosomal integration of HHV-6 (ciHHV-6). Because the HHV-6A genome encodes a perfect TTAGGG telomere repeat array at the right end direct repeat (DRR) and an imperfect TTAGGG repeat at the end of the left end direct repeat (DRL), a working hypothesis that HHV-6A integrates into telomeres via homologous recombination was established. HHV6-A integrates into the telomeres of human peripheral mononuclear cells in vivo and following infection in Jjhan T cells, and HEK-293 cells in vitro. Some cells quickly become latently infected through chromosomal integration, and remain viable. We suppose that HHV-6A/B viruses may have influence on advanced age people's health and life expectancy not only due to their contribution to reactivation and worsening of concomitant illnesses, but also by directly affecting telomeres. Telomeres containing recently integrated HHV-6A can have different lengths. HHV-6 integration into the genome could alter the stability of individual chromosomes and the expression of adjacent subtelomeric genes. Subtelomeric rearrangement of chromosomes has been associated with mental retardation. These findings might also have relevance for telomerase activity. Mammalian and yeast chromosomes express a telomeric repeat-containing noncoding RNA (TERRA). TERRA is proposed to regulate telomerase, as well as many important telomere functions, and TERRA transcription is initiated in the subtelomere. It is possible that integration of HHV-6 could alter the expression of TERRA leading to dysfunction of telomerase. Both seropositivity and antibody titer to HHV-6 negatively correlated with age providing reactivation of the virus. As long as HHV-6B is a virus with a ubiquitous presence, there are many possibilities of being exposed and it could be more likely supposed that antibody titers would increase with age. Therefore, the finding that this was not the case is of great interest and deserves farther research.

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REVEALING THE STRUCTURE OF PHARMACEUTICAL COMPOUND-DEGRADING MICROBIAL COMMUNITIES ENRICHED FROM CONTAMINATED RIVER SEDIMENT

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The increasing volume of production and consumption of pharmaceuticals has predict a widespread distribution in aquatic systems and they have become one of the most detected micro-contaminants. This newly reported group could detect not only in wastewater treatment plants (WWTPs), but also in surface waters, groundwater and drinking water. Additionally, these compounds have limited elimination rate in WWTPs, which phenomenon has caused their continuous presence in aquatic environments, which could foresee long-term exposure to wild-living organism and might induce harmful responses. One of the most represented group of these pharmaceuticals are the non-steroidal anti-inflammatory drugs (NSAIDs) e.g. ibuprofen (IBU) and diclofenac (DIC). Discovering of new perspectives and enhancing wastewater treatment processes are important step for protecting our aquatic ecosystems. For above reasons utilizing of decontaminating properties of bacteria could be a promising way in improving of the efficiency of WWTPs as regards these compounds. To establish selective enrichments a river sediment sample was used originated from Zagyva River, which is a well-known pollution load site due to WWTPs effluents. In the triplicate enrichments, IBU or DIC was applied as sole carbon and energy source at a concentration of 100mg l^{-1}. Diversity of enrichment communities was analyzed by Illumina 16S rRNA gene amplicon sequencing in the third month. In the IBU-degrading enrichments, the three most dominant classes were Gammaproteobacteria (56.08%), Alphaproteobacteria (21.19%) and Actinobacteria (13.54%) The most abundant community members belonged to the genera *Pandoraea* (23.24%), *Rhodanobacter* (13.96%), *Castellaniella* (10.25%), *Afipia* (5.7%), *Hyphomicrobium* (4.21%), *Cupriavidus* (3.69%), *Rhizobium* (3.66%), *Rhodococcus* (3.49%), *Microbacterium* (3.3%), *Sphingomonas* (3.02%), *Mycobacterium/Mycolicibacterium* (2.91%), *Achromobacter* (2.57%), *Brucella* (2.2%) and *Leifsonia* (1.98%). In the case of the DIC-degrading enrichment cultures the predominant class was the Gammaproteobacteria (71.67%) with high abundance of the genus *Pseudomonas* (42.25%), *Rhodanobacter* (8.09%), *Castellaniella* (4.5%), *Pandoraea* (4.26%), *Achromobacter* (3.4%), *Nitrosomonas* (2.09%) and *Cupriavidus* (2.01%), followed by Alphaproteobacteria (25.19%) represented mainly by the genera *Afipia* (10.72%), *Labrys* (4.07%), *Sphingopyxis* (2.82%) and *Hyphomicrobium* (2.17%). After three consecutive subcultivations, thirteen pure bacterial strains were isolated from the selective enrichments, which may lead us to find new IBU- and DIC-degrading bacteria, with which we can perform biodegradation efficiency experiments in the future.

STUDYING THE POTENTIAL OF "TRAINED IMMUNITY" IN HUMAN ORAL EPITHELIAL CELLS USING *CANDIDA* SPECIES

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The term „trained immunity" refers to the immunological memory of the innate immune system. Trained immunity – unlike classical immune memory – lasts up to only a few months, is regulated by epigenetic modifications, and because no specific antibodies are involved in memory formation, the secondary immune response may be activated not only against pathogen inducing the first immune response but also against pathogens whose antigens are recognized by the same pattern recognition receptors. The number of fungal infections has been increasing in recent decades. Members of the genus *Candida* often cause these infections. Systemic candidiasis, vulvovaginal candidiasis and oral thrush are different manifestations of such infections. Since oral candidiasis may frequently develop independent of genre or medical interventions (e.g., catheter insertion), in the followings we aimed to study the first line of the human oral niche, thus the response of oral epithelial cells. Based on our research group's previous work, the natural immune responses induced by *C. albicans* and *C. parapsilosis* are fundamentally different as in *C. albicans* initiates a rapid and excessive inflammatory cascade, whereas *C. parapsilosis* induces only a moderate host response. Taking advantage of the difference in the pathobiology of the two species, we aimed to investigate whether trained immunity can be achieved by pre-treatment of human host cells with *C. parapsilosis* in order to allay future *C. albicans* infections. For our experiments, we used the oral epithelial cell line OKF6/TERT-2, - derived from healthy individual - as the host, and two distinctive strains of *C. albicans* (SC5314 and WO-1) and *C. parapsilosis* (GA1 and CLIB214) as pathogens, to examine species- as well as strain-dependency. OKF6/TERT-2 cells were pre-treated for 24h with live *C. parapsilosis* cells, and then fungal cells were eliminated using the antifungal agent nourseothricin. After that, we stimulated host cells with nourseothricin-resistant *C. albicans* cells on the fifth day for 24h and examined the extent of host cell damage. Our results indicate that the preincubation with the less pathogenic species, *C. parapsilosis* has the potential to decrease host cell damage during infection with the more pathogenic and more frequent cause of oral candidiasis, *C. albicans*, hence suggest a potential preventative effect.

PHYLOGENETIC ANALYSIS OF A NOVEL HEPATITIS A VIRUS IB STRAIN SPREADING IN HUNGARY IN 2022

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Hepatitis A virus (HAV) is one of the leading causes of acute viral hepatitis worldwide. In developed countries, most infections are related to travel, consumption of contaminated food or person-to-person transmission among members of high-risk groups. In December 2021 an increase in the incidence of HAV infections was detected in Hungary. In 2022, a total of 579 serologically confirmed cases of HAV were reported, significantly higher than in the last five years. Many of the patients identified themselves as MSM, suggesting sexual transmission played an important role in spreading the infection. Serum samples from 224 patients were tested for the presence of HAV RNA by RT-PCR specific to the VP1/2A region. PCR products were sequenced and a phylogenetic analysis was performed including previous HAV sequences from Hungary and reference strains from GenBank. Whole genome sequencing of the outbreak strain was also performed on the Illumina MiSeq platform. A total of 181 samples were PCR positive and in 171 patients, a novel HAV subtype IB strain was found. Most of the 171 patients were male (81.9%), their median age was 34.5 and 63.7% were hospitalized. Variants, each with a single nucleotide difference from the main outbreak strain (P1) were detected in 15 patients. Ten patients were infected with strains unrelated to the outbreak. In Hungary, subtype IB strains caused the majority of HAV infections in 2022, while until 2020 subtype IA had been dominant. The novel IB strain had not been detected in Hungary before December 2021 and no identical sequence was found in GenBank. It was most closely related to an isolate from Egypt. Since its first detection, the P1 strain and its variants spread to at least eight other countries in Europe, identified as the cause of person-to-person or foodborne outbreaks. All variants will be closely monitored by ECDC.

ASYMPTOMATIC CARRIAGE OF PATHOGENIC BACTERIA

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Pathogenic bacteria might colonise the mucosal membranes of our body for shorter or longer periods, without causing any disease. However, carrier persons can be the source of infection for other, susceptible individuals, therefore this phenomenon has a deep clinical impact. Pneumococcal conjugate vaccines (PCVs) mean a huge selective pressure on the species, leading to massive serotype rearrangement. By screening nearly 3500 children, attending day care centres, as the major carriers of pneumococci, we could follow this process well. Whereas in the first years still the old ‘pediatric’ types dominated, new, non-PCV7 types took over soon. Among these, 19A was a dangerous one, showing high resistance and invasive potential. The introduction of PCV-13, with six additional serotypes, could supress it successfully. Ever since then, new, non-PCV types are emerging like 15B/C, 11A, 23A/B. Of note, higher valency vaccines (PCV-15 and (PCV-20) will be released soon, covering these types. In parallel to the pneumococci, we have also surveyed *Staphylococcus aureus* carriage both among children and university students. The average carriage rate was 29%, and luckily only a very few MRSAs were found. In general, carried isolates show much less antibiotic resistance compared to the clinical ones. Later the carriage studies were extended to include *Haemophilus influenzae* and *Moraxella catarrhalis*. Both species are major respiratory pathogens, causing recurrent otitis media among others. For *H. influenzae*, most of the strains were non-typeable and no type b was detected. *Moraxella* carriage was >50% in small children. 76% of the children carried at least one species, 26% two species and 12% three species. Interestingly, prevalence of *S. aureus* showed an inverse relation with the other three bacteria. In contrary to the other pathogens, *Neisseria meningitidis* often infects young adults, hence here we surveyed secondary school and university students. The peak age for carriage was 17-18 years, and the average carriage rate was 33%. Unlike among the invasive isolates, here we found mostly non-typeable strains, a few type B and C, and a singly type Y. It is important to know that the vaccine against meningococcus is not yet obligatory in Hungary. As there is a veterinarian in our workgroup, we also involved animals in the carriage studies.Whereas *S. aureus* is the dominant species in humans, it is *S. pseudintermedius* in dogs. By screening 102 dogs and their 84 owners, carriage of identical isolates could be detected by WGS, providing evidence for the potential exchange of both bacteria or just resistance and/or virulence genes among them. Staphylococci are present not only in pets or domestic animals, but also in wildlife. We have screened 200 hedgehogs and found several *S. aureus* isolates, including the first *mecC*-MRSA from Hungary.

WHAT MAKES SEROTYPE 8 PNEUMOCOCCI THAT SUCCESSFUL?

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The pneumococcal conjugate vaccines (PCV) represent a huge selection pressure on pneumococci, resulting in a massive serotype rearrangement. In the last few years, serotype 8 (a non-vaccine type) has occupied the leading position, being responsible for >17% of all invasive diseases (IPD) in 2018, according the latest available ECDC data. For instance in Galicia, Spain, its prevalence has increased by

8-fold between 2011 and 2021. In Hungary, serotype 8 has been second with 5 - 6% in the recent years among IPD cases. In addition, its serotype-specific case fatality rate has been the second highest ever since 2015, after that of serotype 3. Our aim was to try to find some explanations for this unexpected success. First, serotype 8 was proven to have a high invasive potential. In a Swedish study, it showed the second highest invasive potential (after 7F) with a high odds ratio. To support this, our work group has found a very low incidence of serotype 8 (<0.1%) among carried isolates, when screening almost 3,500 children attending DCCs. Serotype 8 isolates seem to be highly clonal and the spread of a dominant clone can contribute to its increasing prevalence. According the results of The Global Pneumococcal Sequencing Consortium, published in 2019, the vast majority of the pneumococci belonged to CC53. The members of this clone are fully sensitive to all relevant antibiotics. However, the emergence and spread of another clonal linage, ST63 was reported from Spain already before 2012, displaying resistance to erythromycin, clindamycin, tetracycline and ciprofloxacin. This clone was due to a recombination event between an 8-ST53 strain (donor) and the multidrug-resistant 15A-ST63 clone (recipient). A work group at the Yale University compared the growth capabilities of isolates belonging to 53 different serotypes. They showed that although serotype 8 did not reach a high maximum density, but it had a rather short lag phase, i.e. started growing sooner compared to most other serotypes. Furthermore, serotype 8 showed similar growth capability under aerobic and anaerobic conditions. This could partly explain its success in causing invasive infections, as the conditions in the blood could be virtually anaerobic. In a study [1] mice were co-infected intranasally with pneumococci of different serotypes. Remarkably, always the same relative prevalence evolved. They concluded that the metabolic cost of capsule synthesis and surface charge contributed largely to serotype-specific fitness of the strains. The capsular polysaccharide (CPS) locus of serotype 8 pneumococci is the second smallest after serotype 3 and the glucuronic acid in its CPS structure might be responsible for the negative charge, which has a protective potential against the negatively charged phagocytic cells. Considering this rapid emergence of serotype 8 pneumococci, it is comforting that it will be part of the soon available new PCV-20 vaccine.

[1] Trzciński et al (2015) mBio 6: e00902-15

MICROBIAL ECOLOGY OF CONTAMINATED ENVIRONMENTS

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Microbial assemblages play an important role in determining the fate of contaminants in the environment, supporting the functioning of biogeochemical cycles and many ecosystem services. The omics approaches have recently provided new insights on microbial ecology regarding organic and inorganic pollutants for both chronic and acute contaminations. The investigations on coastal hydrocarbon contaminated environments, combining in situ analyses and experimental ecology approaches, allowed us to propose a scenario for the adaptation of microbial communities to the hydrocarbon contamination, which represent valuable information to understand the biodegradation of hydrocarbon compounds. Similarly, the microbial characterization of metal(loid)s contaminated sites offered the opportunity to collect important information on the factors driving the microbial assemblages, providing new insights for understanding the assembly rules of microbial communities. The obtained knowledge offers the possibility to define a relevant set of microbial indicators for assessing the effects of human activities on ecosystems. Such information might constitute accurate biomonitoring and assessment tools for the management of microbial resources, which are required for the development of effective restoration strategies.

SCREENING OF MICROALGAL STRAINS SELECTED FROM FRESHWATER GREEN MICROALGAE COLLECTION FOR ANTIBACTERIAL ACTIVITY AND TESTING OF ARTIFICIALLY INFECTED FRUIT FLOWERS

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We performed the biological screening of organic solvent extracts of single-cell freshwater green microalgal cultures. The microalgae collection of Albitech Biotechnology Ltd. consists of its own isolates. The collected samples were diluted and streaked on a solidified enrichment medium. The axenic cultures were identified in the Department of Microbiology of Eötvös Loránd University. Identification was carried out using molecular biological methods based on the 18S rRNA gene. These microalgae strains received a strain code and they are maintained in active and cryopreserved forms. The purpose of our study was to assess whether the selected microalgae produce metabolites that may inhibit the growth of widely occurring human facultative pathogens. There are many microalgae, e.g. *Chlorella* sp., *Scenedesmus* sp., and macroalgae, e.g. *Ulva rigida* with proven antibacterial effects presumably associated with polyphenols, alkaloids, terpenes, polysaccharides, fatty acids, sterols, lactones, and proteins. Debro and colleagues were among the first to study the antibacterial effects of many freshwater algae against *S. aureus* and *E. coli*. We examined the antibacterial effect of the selected algal extracts against the following facultative pathogenic bacterial strains: *E. coli* NCAIM B.01992, *S. aureus* NCAIM B.01055, *P. aeruginosa* NCAIM B.01057 and NCAIM B.01975. Two different organic solvents - ethanol and diethyl-ether - were used to create the extracts from the lyophilised biomass. The antibacterial effect of the extracts was determined using the agar gel diffusion method. The minimum inhibitory concentration was measured by broth microdilution assay in the 24-well cell culture plate. As a positive control, we used antibiotics to

compare the results semi-quantitatively. The *E. coli* strain was the least susceptible to treatments and S. aureus was the most sensitive. In summary, we have confirmed the antibacterial effects of four single-cell freshwater microalgae strains against facultative pathogenic bacteria. In collaboration with the MATE Institute of Horticultural Science, Research Centre for Fruitgrowing, we artificially infected different fruit tree flowers under laboratory conditions and tried to reduce the level of infection with antibacterial algal extracts. Fruit pathogens are one of the most difficult diseases to control, and their control is still not solved. In recent years, alternative and biological control methods have become more important in the fight against the disease. One potential source of biological control agents is microalgae, which are highly adaptable to a range of metabolites that they produce to survive in extreme conditions. The extract of the algal strain N63 was statistically significantly effective on infected fruit flowers.

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FIRST REPORT OF MYCOPARASITISM IN *DIAPORTHE* SPECIES ASSOCIATED WITH GRAPEVINE TRUNK DISEASES

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The ascomycetous genus *Diaporthe* (anamorph: *Phomopsis*) is a large taxon of filamentous fungi, with widespread occurrence and with more than 860 recorded names. While there are few reports on their parasitism on animal or human hosts, most of the species are strongly associated with plants as saprobes, endophytes, or pathogens. Phytopathogenic species have a wide host range, they can infect herbaceous crops as well as woody plants. On grapevine, *Diaporthe* species are responsible for the development of cane and leaf spots, and they can also damage the woody tissues causing trunk diseases. In the present study, the parasitism of several *Diaporhte* spp. isolates towards the phytopathogenic fungus *Botrytis cinerea* were demonstrated. To the best of our knowledge, this is the first report mycoparasitism in the *Diaporthe* genus. A total of 19 *Diaporthe* spp. fungi were isolated from woody tissues of trunk disease-affected grapevines with Italian and Hungarian origins. The preliminary identification of the isolates according to ITS (internal transcribed spacer), TUB (β-tubulin), and TEF (translation elongation factor 1-α) sequences suggest, that the isolates are belonging to *D. ampelina*, *D. eres*, and *D. vacuae* species. Confrontation tests were carried out by co-inoculating the growing mycelia of the *Diaporthe* spp. isolates and three *B. cinerea* strains on top of a cellophane disk, placed on water-agar. The cultures were incubated at 25°C in the dark, until the mycelia of the two fungi contacted. Interaction zones were sampled by cutting the cellophane and mounting in distilled water for microscopic examination. In the case of all *Diaporthe* spp. strains, the attachment of hyphae to the *B. cinerea* host, the formation of papille-like structures on its surface, and massive colonization of host hyphae can be observed. Host mycelia interacting with the parasites were usually dead and even the cell walls showed a high degree of degradation. The *Diaporthe* spp. hyphae did not penetrate the host, however developed invaginations on its surface in some cases. The coiling of the parasite hyphae around host cells was also observed in a few cases when young mycelia established contact. Staining and fluorescent visualization of acidic organelles by acridine orange indicated the recruitment of lysosomes in the parasite hyphae interacting with the host, suggesting intense nutrient uptake.

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AN EFFECTIVE METHOD FOR EXTRACTING HIGH-QUALITY RNA FROM GRAPEVINE RICH IN SECONDARY METABOLITES

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Grapevine (*Vitis vinifera* L.) is one of the most important economic crops in the world. Because of this importance, one finds widespread molecular genetic research on this species, an important element of which is high quality RNA. However, in woody plants, isolation of this molecule is often rather difficult, as the tissues of the various organs may contain significant amounts of secondary metabolites (polysaccharides and polyphenols). These compounds can be removed only with great difficulty during extraction. Our aim was to describe an accurate and usable method for RNA isolation from old leaf tissue starting with a small sample volume (up to 50mg) to make RNA extraction possible even when younger, developing leaves and shoots are not available. Unlike most related studies, we have also tried to provide a highly detailed methodology to avoid lengthy fine-tuning procedures and thus, to save time and money. The advantages of isolation from leaves include the fast and easy collection of samples and the possibility to study both molecular-genetic changes in physiological processes and plant-pathogen interactions. Our version of the so called CTAB method proved to be efficient in extracting grapevine RNA, which is indicated by the A260 : A280 and A260 : A230 ratios measured between 2.05 and 2.06 and between 2.06 and 2.09 using an UV-VIS spectrophotometer (NanoDropTM 2000, Thermo Fischer Scientific, Massachusetts, USA). The integrity analysis of the RNA was carried out using Agilent RNA 6000 Nano LabChip® (Agilent 2100 Bioanalyzer Califronia, USA). The RIN value (RNA Integrity Number) of the samples was between 7.7 and 8.1, which indicated that small degree of RNA degradation occurred during extraction. The efficiency of the RNA isolation was determined by transcriptional analyses of six housekeeping genes (*cysp*, *ysl8*, *actin*,

sand, *ef1-α*, *gapdh*) using RT-qPCR, which was carried out in three biological and two technical replicates applying SYBER Green technology. The presence of a single peak in the melt curve and amplification curve analyses and low standard errors between the Ct values indicated the specificity of the primers to bind to the cDNA, with no interference of PCR inhibitors.

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PLANKTONIC BACTERIAL COMMUNITIES OF A LARGE STEPPE LAKE, LAKE VELENCE (HUNGARY)

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The shallow soda lakes of the Eurasian steppe (and within this, in the Carpathian Basin) represent unique aquatic habitats and are particularly sensitive to sudden weather and long-term environmental changes. A recent example of this was the drastic drop in the water level and the fish die-off in Lake Velence in 2021. Lake Velence is a large shallow steppe lake, has a surface area of 25km^2 and a large open water and a reed-covered protected area. The aim of this study was to reveal the differences in the physicochemical parameters and planktonic bacterial community composition of these habitats, which was supplemented with a cultivation based-study using newly designed media. The environmental parameters recorded during the sampling were similar to the data of previous years, and showed remarkable distinction of the open water and reed zone samples. In the latter, higher bacterial species numbers were detected with Illumina amplicon sequencing, probably because the reed-covered area has higher nutrient content and provides diverse microhabitats for aquatic bacteria. We used two different media for the isolation, and among the cultured heterotrophic bacteria, candidates of new species were identified, and their detailed characterization has been started.

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INVESTIGATION OF MICROAEROBIC BENZENE-DEGRADING BACTERIAL COMMUNITIES BY A STABLE ISOTOPE PROBING (SIP) APPROACH

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The contamination of soil and underground water with petroleum hydrocarbon compound is among most prevalent problem in environment. One prominent class of pollutants are monoaromatic hydrocarbons such as BTEX (benzene, toluene, ethylbenzene and xylene), which significantly damage the ecosystem or even the human health by contamination of groundwater and drinking water. Benzene is among the most abundant chemical produced worldwide by the petrochemical industry. The global demand for benzene totals nearly 50 million metric tons per year, so this aromatic hydrocarbon is one of the most observed contaminations in soil and subsurface sites along with other BTEXs. However, expose to benzene may increase the risk of blood disorders and is known to be carcinogenic. Bioremediation is a process in which microbes are used to eliminate petroleum hydrocarbons, proving the most successful in solving problems of a widespread contamination. Aromatic hydrocarbons decompose most rapidly and completely under aerobic conditions due to the presence of microorganisms, which decrease the concentration of dissolved oxygen during their metabolic processes, so the available oxygen has a key role in the biodegradation. Therefore, the subsurface environment is generally hypoxic and benzene, para- and ortho-xylene are persistent compounds under anaerobic conditions. Consequently, exploration of the bacterial communities of aromatic hydrocarbon contaminated, hypoxic environments has a current importance. In the present study, by DNA-based stable isotope probing (SIP), the most relevant benzene degraders were directly identified in microbial communities of aerobic and microaerobic sediment slurry microcosms, degrading solely 13C6-benzene. Groundwater and sediment samples of microcosms originated from the "Siklós" BTEX-contaminated area. The screening of labeled DNA fractions suggested that distinctly different benzene-degrading bacterial communities were formed in aerobic and microaerobic microcosms after only one week of incubation. Aerobic microcosms were dominated by members of the genus *Thaurea, Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and *Pseudomonas*. In contrast *Azovibrio* and *Malikia* were the most common benzene-degrading genera in microaerobic sediment slurry microcosms.

THE BENEFICIAL ROLE OF A PLANT PATHOGEN: THE TRANSCRIPTOMICS OF GRAPEVINE NOBLE ROT CAUSED BY *BOTRYTIS CINEREA*

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Botrytis cinerea has more than 1,200 host plants and is one of the most important plant pathogen in the grape production. It can lead to the formation of noble rot under certain environmental conditions, which results a unique metabolic profile, changes the physical texture and chemical composition. The functional genes involved in this process are poorly characterized. We generated metatranscriptomic data from "Furmint" grape variety, from three months, representing the four phases of noble rot, from healthy to completely desiccated berries. Weighted gene co-expression network analysis (WGCNA) was performed to associate textural parameters with *B. cinerea* functional genes. The clustered genes were significantly enriched characterizing the carbohydrate and protein metabolism of the fungi involved in the degradation of the berry skin. We identified genes that allow *B. cinerea* to dominate and proliferate during noble rot, including sulphate metabolism genes and genes involved in the synthesis of antimicrobials and wine aroma precursors.

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GRAPE BERRIES FUNGAL COMMUNITY IS STRONGLY RELATED TO THE GRAPE BERRIES' TEXTURABLE CHANGES DURING THE NOBLE ROT PROCESS

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The grapevine pathogen, *Botrytis cinerea*, is known for causing grey rot in berries. However, under specific microclimatic conditions, *Botrytis* infection can lead to noble rot. One remarkable example of this natural occurrence is the production of aszú wines in the Tokaj wine region of Hungary. These wines are crafted using the noble rot process. In our study, we examined the textural characteristics and microorganisms present in grape berries throughout various noble rot phases, ranging from phase 1 (healthy) to phase 4 (noble rot). Our analysis revealed how the mechanical properties of the berries, specifically the berry skin break force (Fsk) and energy (Wsk), changed throughout the noble rot process. These texture parameters exhibited a decreasing trend from phase 1 to 3 and then increased in phase 4, reflecting the practical experiences associated with the process, such as maturity, over-maturity, botrytisation, and withering. Additionally, we observed a continuous decrease in berry hardness (BH), while the elastic modulus (Esk) displayed a drop-like effect (E-drop) between phase 1 and phase 2. According to the DNA metabarcoding analyses to identify the most abundant fungal species of the noble rot process was found strong relationship between the most abundant fungal species - *Aureobasidium pullulans*, *Alternaria alternata*, *Botrytis cinerea*, *Epicoccum nigrum* and *Rhodotorula graminis* - and the texturable changes of the grape berries. Thus, beside the *B. cinerea* other fungi community of the grape berries can derives massive secretion of plant-cell-wall-degrading enzymes.

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BACTERIA DEGRADING XENOESTROGENES (ISOLATION, IDENTIFICATION AND TESTING)

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The study of naturally occurring organic micropollutants (e.g. pharmaceuticals, pharmaceutical residues) is complicated due to their adsorption and accumulation properties and their low concentrations in the environment, although they can cause serious health problems. These pollutants include also endocrine disruptors, which can alter the normal functioning of the endocrine system in humans. The present work aimed the selective isolation and identification of bacteria from a wastewater effluent and test their capability for endocrine disruptor degradation (bisphenol A, nonylphenol, estradiol). Based on literature data we analysed the bacterial genome for groups of genes that may be involved in the degradation of aromatic compounds. Moreover, the antibiotic resistance of the cultivated bacteria was also tested. The bacteria identified all belong to the Gammaproteobacteria class of Proteobacteria phylum (40 bacterial strains of 22 different species), most of them belonged to the family Enterobacteriaceae e.g. *Klebsiella*, *Citrobacter*, *Enterobacter*, *Escherichia* genera, but also the representatives of the *Acinetobacter* and *Pseudomonas* genera appeared. Most of the bacteria were found to be facultative pathogens. The bacterial strains proved to be most sensitive to antibiotics that inhibit cell wall biosynthesis (for meropenem 86%, and for imipenem 97% of strains were sensitive). Analysis of the aromatic metabolic genes resulted 14 metabolic pathways that may be involved in the degradation of the endocrine disruptors we have tested. Among these, the biphenyl degradation pathway appeared to be the most dominant, genes for this pathway were found in 86% of the species. All the isolated strains degraded the investigated endocrine disruptors at different levels. Among them, the members of the *Klebsiella* genus (*Klebsiella grimontii*, *Klebsiella michiganensis*, *Klebsiella pneumoniae*, *Klebsiella quasipneumoniae*, *Klebsiella quasivariicola*, *Klebsiella variicola*) were

dominant in number and in degrading efficiency. These microorganisms are able to use the studied endocrine disruptor molecules, however, antibiotic resistance poses a risk for the environment.

THE 9TH HUMAN POLYOMAVIRUS

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Human polyomavirus 9 (HPyV9) was described as a novel polyomavirus genome from the blood and urine of a kidney transplant patient. The virus was not isolated in cell cultures, and no association with any disease was revealed. Based on the seroprevalence data published by some research groups, HPyV9 is circulating in the human population, but the seroprevalence is lower compared to other human polyomaviruses. The viral DNA was detected rarely in clinical samples of healthy individuals, while higher DNA prevalence was found in immunocompromised patients. The transmission route, the portal of entry is not known, nor it is clarified whether it established persistence, latent infection. The aim of our study was to assess antibodies against HPyV9 in different age groups. We also studied the DNA prevalence of this virus in different samples from the respiratory tract (swab samples, middle ear discharge samples, healthy and cancerous tissue samples). To develop an ELISA method we expressed HPyV9 capsid antigen in bacteria. Briefly, codon optimized VP1 sequence was cloned into pTriEx™-4 Neo vector. The protein was purified by immobilized Ni^{2+} ion affinity chromatography. After checking the quality and measuring the quantity of the antigen, we developed and optimized an indirect ELISA method for the measurements of the anti-HPyV9 VP1 antibodies. Nucleic acid was isolated from respiratory swab samples, adenoids, tonsils and lung tissue samples, and then a quantitative real-time PCR was performed to detect HPyV9 DNA. Seroprevalence study was performed with 1,038 serum samples: 320 samples from children and 718 samples from adults. We detected that seropositivity against HPyV9 is increasing with age, but stable in adults, and the overall adulthood seroreactivity is <40%. HPyV9 DNA was detected in a tonsil sample of a child with low viral load, but not any of the other samples were positive for viral DNA

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ASSESSING THE EFFECT OF ROOTSTOCK GENOTYPE ON BERRY AND LEAF FUNGAL COMMUNITY COMPOSITION AND DIVERSITY IN *VITIS VINIFERA* L. cv KÉKFRANKOS

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Grapevine is one of the most valuable crops globally and it is significantly impacted by environmental factors. The use of rootstocks to reduce this impact on growth and health of the vine is a natural and essential solution to ensure sustainability. The same environmental factors that affect plant physiology may also influence plant-associated microbiome, including pathogenic, symbiotic, and commensal fungi that could directly affect plant health. Therefore, understanding factors that determine the structure and function of plant microbiome is pivotal. We conducted fungal DNA metabarcoding of berry and leaf samples collected from the Nagy-Eged Hill in Northern Hungary, and then statistically compared community composition, diversity and relative abundance of selected fungal taxonomic groups and functional groups among vines grafted on different rootstocks. The results illustrated that species from the genera *Alternaria*, *Aureobasidium*, and *Vishniacozyma* were dominant in both berries and leaves. The richness and abundance the analyzed groups differed significantly among the plant compartments and less so among the rootstocks. PerMANOVA analysis indicated a significant effect of rootstock genotype only on the generalists saprotroph community structure where it explained 17.88% of variance ($P = 0.006$) in leaves. These findings suggest that plant compartments may exert stronger influence on community structure. Further analysis exploring the relationship between changes in elemental profile and community composition may provide more insight.

GENES INVOLVED IN AZOLE RESISTANCE OF *MUCOR LUSITANICUS* AND THEIR REGULATION

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Certain members of the order Mucorales, as opportunistic human pathogens, are capable of causing an often fatal infection in immunosuppressed patients, known as mucormycosis. The increasing resistance to certain antifungal agents used in the clinic, including azoles, complicates treatment of this infection. The mechanism of action of azoles is based on the inhibition of the 14-α-demethylase enzyme encoded by the *erg11* gene. Inhibition of the enzyme results in the accumulation of toxic sterols in the cell membrane. In addition to mutations or duplications in the *erg11* gene, azole resistance may also be caused by the function of active efflux pumps, known as multidrug resistance proteins (MDR). The ABC and MFS transporters that give rise to this phenomenon are embedded in the plasma membrane and deliver antifungal agents and other toxic substances outside the cell. In addition, the activity of genetic elements and transcription factors (PDREs, Zn_2Cys_6, and AtrR) that regulate the above-mentioned processes may also play a role in the development of the azole resistance. Recent studies report that genes encoding ergosterol biosynthesis and ABC transporters show coupled regulation in yeasts and filamentous fungi. Based on the studies the literature suggests that a highly complex system, the PDR network, underlies the resistance of pathogenic fungi to azole, involving active transporters and genes involved in ergosterol biosynthesis, as well as other genes involved in stress response, regulators, and transcription factors that influence their function. In the *Mucor* genome, nine putative PDR coding genes were identified. By examining the transcription of these genes after different azole treatments, we found that they responded very differently to the treatments. *pdr1* gene showing increased activity against all tested azoles. We have started to generate single and double deletion mutants using CRISPR-Cas9 system from these genes.
Our results suggest that there may be a functional link between the identified *pdr* genes and that these genes play a different role in resistance to different azole compounds. Based on the results of the RNAseq analysis and homologous searches, several genes encoding putative transcription factors were found in the *Mucor* genome database. Among these genes, we would like to select, as a first priority, those that may encode factors carrying fungal-specific transcription domains that are also typically reported in the literature to carry transcription factors involved in stress response or antifungal resistance development. Our aim is to identify transcription factors that play a role in the regulation of genes encoding ABC transporters.

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FUNCTIONAL ANALYSIS OF THE DELETION AND OVEREXPRESSION MUTANTS OF THE TRANSCRIPTION FACTOR (AN7872) REGULATING THE AN7884 SECONDARY METABOLITE GENE CLUSTER

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Silent secondary metabolite gene clusters, whose products can be good candidates for medical purposes as bioactive agents, can be activated under environmental including oxidative stress in fungi. For example, in *Aspergillus nidulans* a non-ribosomal peptide synthase gene cluster including the synthase (AN7884) and its transcription factor (AN7872) activated under menadione sodium bisulfite treatment. In this study, we generated and phenotypically examined the gene deletion (Δ) and overexpression mutants (OE) of AN7872. Based on stress sensitivity studies with agar plate assays, the Δ*an7872* gene deletion strain was more sensitive to oxidative stress inducing agents like diamide, tert-butyl hydroperoxide (tBOOH) compared to the control strain. Surprisingly the menadion sodium bisulfite sensitivity of the mutant and control strain was comparable. Under heavy metal stress treatment elicited by $CdCl_2$ the colony of the OE mutant showed abnormal morphology and smaller size. In the presence of the cell wall stress-generating agent Congo red and osmotic stress inducing NaCl as well as sorbitol OE mutant displayed resistant phenotype compared to the control strain. Secondary metabolite production of the mutants was also monitored in different nutrient media. For example, cultures cultivated in *Aspergillus* minimal medium supplemented with 2% maltose and 1% mycological peptone at 25°C, shaking at 200rpm for 2 days affected the siderophore production of the mutants. Ferricrocin concentration of the cultures significantly increased, while triacetylfusarinine C production significantly decreased in the OE mutant. Static cultivation without shaking at 25°C for 14 days, in Czapek-Dox medium resulted in increased asperthecin production in the Δ mutant. The effect of the mutations to the production of the precursor of aflatoxin biosynthesis, i.e. the mycotoxin sterigmatocystin was also tested. Based on the HPLC analysis the sterigmatocystin concentration increased in the Δ mutant, while decreased in the OE mutant.

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ENRICHMENT OF BIOACTIVE PHENOLICS FROM OAT HULL SAMPLES BY ENZYMES OF MUCOROMYCOTA FUNGI

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Phenolic compounds are important plant secondary metabolites. They can exert different beneficial effects on the human body due to their antioxidant, antimicrobial, anti-diabetic and anti-inflammatory properties. Majority of plant phenolics can be found in carbohydrate-ester and carbohydrate-glycoside bounds in the plant cell wall resulting limited bioavailability. Treatment with hydrolytic enzymes (e.g., cellulase and lipase) can be an ecofriendly strategy to liberate these bound phenolics. Oat has gained attention in the recent years due to its high content of health-beneficial compounds. The hull part (a by-product of oat processing) has been characterized to have higher soluble and bound phenolic content than the groats. In this study, cocktails with cellulolytic and lipolytic activities from the zygomycetes *Rhizomucor miehei*, *Gilbertella persicaria* and *Mucor corticolus* were applied to enrich phenolics from two different oat hull samples. The enzyme cocktails were produced in wheat-bran based solid-state fermentation systems and were partially purified by gel filtration before use. During enzyme treatments, 1g ground hull was treated with 10mL of enzyme cocktail, and the mixtures were incubated for 7 hours. Samples were taken at predefined intervals, then, total phenolic content (TPC), total flavonoid content and antioxidant activity measurements were carried out. For both hull samples, the TPC increased markedly until the 3rd hour of incubation, which was followed by an increase in the antioxidant capacity as well. Bound phenolic content of the black oat hull sample was high and this residue showed elevated antioxidant activity after the enzyme treatments. In conclusion, the cellulase/lipase treatment using zygomycetes enzymes had a positive effect on the release of phenolic antioxidants from oat hull samples.

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TISSUE MIMICKING SPHEROIDS IN SARS-COV-2 STUDIES: NEEDS AND CHALLENGES

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The need for intracellular chemotherapy has been recognized for many years, the challenge is to design the delivery vehicles carrying an antiviral agent in a form that can be internalized by host cells and then released into these cells in an active form. Based on previous results our goal is to define new peptide carriers for antiviral compounds. To elucidate the in vitro penetration ability of these peptides using 3D spheroid cultures for mimicking tissue environment in vitro is a promising approach. We have also demonstrated that agarose-based spheroid system is a simple assay platform for comparative penetration studies to evaluate carriers and antiviral drug candidates. Performing experiments simultaneously on multiple 3D cell cultures requires, among others, the availability of suitably comparable spheroids. In our study for spheroid production agarose microwell chambers (also referred as 3D Petri dishes) was employed using the so-called "micromolding" technique. Agarose does not support cell-substrate adhesion and therefore it is cell-cell adhesion that drives spheroid formation in the microwells. A typical spheroid diameter that can be achieved with sufficient reproducibility in a microwell is typically 200µm. Agarose is transparent therefore, spheroids in the microwells can be visualized and observed using an inverted microscope with phase-contrast, bright-field, or fluorescence applications. As the agarose gel is permeable for all components of cell culture media, simultaneous treatment of large number of spheroids is possible using a single microwell chamber placed e.g., in a standard 35mm culture dish or in the well of a 12-well plate.

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CHARACTERIZATION OF THE SUPPLEMENTATION OF *ASPERGILLUS NIDULANS gfdB* IN OSMOPHILIC ASPERGILLI

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Glycerol-3-phosphate dehydrogenase catalyses the conversion of dihydroxyacetone phosphate into glycerol 3-phosphate, which is subsequently transformed into glycerol by a phosphatase. The genome of *Aspergillus nidulans* contains two putative glycerol 3-phosphate dehydrogenases encoded by the genes *gfdA* and *gfdB*, while the genomes of the osmophilic *Aspergillus glaucus* and *Aspergillus wentii* accommodate only the orthologue of the *A. nidulans gfdA* gene. In this work, we studied the effect of the insertion of

A. nidulans gfdB into the genome of *A. glaucus* and *A. wentii*. In *A. glaucus*, the transfer of *gfdB* increased the stress tolerance of the fungus, while in *A. wentii* only minor progress in the stress tolerance including partial reversion of osmophily has been observed. The construction of a dendogram by cluster analysis and multidimensional scaling based on MIC50 values (H_2O_2, menadione sodium bisulfite, $CdCl_2$) and colony diameters measured at selected concentrations (sorbitol, NaCl, Congo red) did not separate the *gfdB* supplemented *A. glaucus* and *A. wentii* from the wild type *A. wentii*, wild type *A. nidulans* and the *A. nidulans* Δ*gfdB* (constructed and characterized in a previous study) strains. Interestingly, *A. nidulans ΔgfdB* and *A. wentii* wild type strain also lacking *gfdB* showed closer relation to each other than to wild type *A. nidulans*. Furthermore, supplementation of *A. nidulans gfdB* into *A. glaucus* and *A. wentii* increased the evolutionary distance between these species. Our observation shed light on the complexity and species-specific effect of the stress response network of the aspergilli, which should be taken into account during the development of stress tolerant industrial strains.

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VARIATION IN THE TAXONOMIC COMPOSITION OF MICROBIOTA BY TYPE OF WATER BODY AND HABITAT IN THE RIVER DANUBE

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Rivers play a fundamental role in providing drinking water and ecosystem services in urbanized regions. In the case of large rivers such as the Danube, the main branch, together with its side arms and oxbows, form interconnected ecosystems. The water quality of these different water bodies is greatly influenced by various municipal, industrial, and agricultural human activities. In addition to the planktonic microbial communities, the epilithon of the riverbed and the epiphyton of the littoral zone vegetation also contribute to the self-purification processes of the river water by transforming and degrading organic pollutants of various origin. Managing water quality of rivers requires exploring and comparing the composition of microbiota in different and interrelated water types and habitats (plankton vs. biofilm) and the environmental factors that influence them. For research, water and biofilm samples were collected at the beginning of the vegetation period (May) from (a) the main branch upstream (north) and downstream (south) of the capital, (b) a regulated side arm, (c) an oxbow, and (d) a separated oxbow lake. Among physical and chemical parameters, temperature, pH, electrical conductivity, dissolved oxygen, orthophosphate, total organic carbon, and nitrate of the water samples were measured. The taxonomic diversity of bacterial communities was determined using 16s rRNA gene-based amplicon sequencing method on Illumina MiSeq platform. Representatives of the phyla Proteobacteria and Bacteroidota dominated both the water and biofilm samples, while the abundance of the phylum Actinobacteriota was high only in the water samples. Additional characteristic community members included the phyla Verrucomicrobiota, Acidobacteria and Cyanobacteria. Based on the taxonomic diversity and composition of the microbiota, the habitat types (water vs. biofilm) were more separated from each other than the different types of water bodies. The differences in the composition of planktonic microbial communities increased in parallel with the degree of separation of the water bodies from the main branch. Some taxonomic groups and physico-chemical parameters characteristic of different water bodies well correlated with each other.

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DETECTION OF THE EXPRESSION OF 18 GENES EXPECTED TO PARTICIPATE IN METRONIDAZOLE RESISTANCE BY RT-qPCR OF *BACTEROIDES FRAGILIS* STRAINS WITH OR WITHOUT *nim* GENES AND VARIOUS METRONIDAZOLE MIC

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Nim genes are regarded as mediators of metronidazole resistance in *B. fragilis* group strains. However, some data request that additional or independent factors are also needed in the process of this resistance mechanism. E.g., the *nim* gene expression is determined by the upstream insertion sequence elements but the metronidazole MICs are independent from that, and without haemin supplementation metronidazole resistance of *B. fragilis* strains is abolished. Based on differential RNASeq and proteomic data of nim-positive (n = 8) and negative (n = 7) *B. fragilis* strains and with or without metronidazole resistance induction we examined the expression of 18 selected genes from various cellular pathways by R PCR. We also recorded metronidazole MICs and analysed the obtained data by bioinformatic methods and also drew gene interaction networks. In the nim-positive group the expressions of *cytB* (cytochrome), *mdh* (malate dehydrogenase), *pgk* (phospoho-glycerate kinase), *relA* (stringent response regulator) were lower and *nanH* (sialidase) expression was higher compared to *nim*-negative ones by means of variance analysis. By correlating the expression of the studied genes with metronidazole MICs the same set of genes was obtained; *cytB*, *mdh*, *pgk* and *relA* correlation was negative, while *nanH* correlated positively with the metronidazole MICs. For the *nim*-positive and negative subset of strains, however, we could not detect significant

correlations This was may-be, because of the low number of strains. We could also detect gene correlation networks since the cross-correlations of the studied genes were sometimes very high ($r>0.7$, $p<0.001$). From our data we concluded that *cytB*, *mdh*, *nanH*, *pgk* and *relA* could be considered as interacting partners or participants of interacting pathways in *nim*-mediated metronidazole resistance.

EVALUATING THE EFFECT OF PEPTAIBOLS IN AGRICULTURAL SYSTEMS

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The members of genus *Trichoderma* are mostly soil-inhabiting filamentous fungi with beneficial properties often used in agricultural systems. The species *T. longibrachiatum* f. *bissettii* has promising biocontrol abilities against plant pathogens; however, it can also infect immunocompromised humans. It is used in tropical countries because of its thermotolerance and exceptional biocontrol abilities. The last few summers of Hungary resulted in drought and elevated temperatures, forcing farmers to use more thermotolerant biocontrol agents, like in tropical countries. However, in order to avoid the usage of potentially infectious agents, it is important to investigate other ways of biocontrol. *Trichoderma* species also produce useful secondary metabolites, including peptaibols, which are antimicrobial peptides especially effective against Gram-positive bacteria and filamentous fungi. These metabolites are linear, consist of 6 - 20 amino acid residues and contain non-proteinogenic amino acids (isovaline and α-aminoisobutyric acid), as well as an amino-hydroxyl group on their C-terminus and an acetylated N-terminus. These peptides can form ion channels through lipid membranes. Their bioactivity is mainly the result of this attribute; however, they are less effective against cells with certain cell wall types. The aim of this study was to observe the benefits of peptaibols on tomato plants, as peptaibol extracts might also trigger the plant's induced systemic resistance and have a positive effect on plant growth and the amount and quality of the fruit. Purified peptaibol extracts were prepared from *T. longibrachiatum* SZMC 12546 and *T. reesei* SZMC 22616 and tomato seeds were treated with different concentrations of peptaibol solutions. The germination rate, growth parameters, photosynthetic pigment level in the leaves, crop yield and the presence of plant pathogens were monitored. After cultivation, fruits were collected and dry mass, soluble dry mass, total polyphenol, total antioxidant, acid, glucose and fructose levels of tomato fruits were measured. Germination proved to be faster after the treatment with peptaibols at the optimum concentration of 0.2mg ml^{-1}. Growth parameters also showed promising results at optimum concentrations. Interestingly, more tomatoes could be harvested from the plants pre-treated with 0.2mg ml^{-1} peptaibols from *T. longibrachiatum* SZMC 12546. The dry mass, soluble dry mass, polyphenol, glucose and fructose contents of fruits were higher than in the control group. Infected fruits were less present on plants treated with 0.2mg ml^{-1} peptaibol solution from *T. longibrachiatum* SZMC 12546 and 0.5mg ml^{-1} peptaibol solution from *T. reesei* SZMC 22611 compared to the control group where fruits were infected with *Fusarium equiseti*, *F. subglutinans*, *Rhizopus arrhizus*, *Aspergillus tubingensis* and *Boeremia exigua*. Based on our results, peptaibols seem to have potential as plant growth promoting and antimicrobial peptides in agricultural systems.

INTER-KINGDOM INTERACTIONS WITHIN NATURAL AND SYNTHETIC ALGAL-BACTERIAL COMMUNITIES

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Past empirical research has demonstrated that bacterial interaction might enhance algal biomass production and modify biomolecule composition (including algal EPS and lipid production as well as biohydrogen evolution patterns). To investigate the mechanisms and microalgal functions activated under bacterial associations different bacterial species were co-cultivated with various eukaryotic green microalgae, including *Chlamydomonas reinhardtii* cc124 green algae. Bacterial species were isolated from diverse environments including biogas sludge, soil and commercial biostimulant products. Pairwise algal-bacterial combinations were cultivated for five days in synthetic wastewater. The accumulated biohydrogen was recorded, the specific algal growth rate was determined, co-cultivation specific physiological and morphological alterations were investigated. Successful bacterial candidates were identified by high algal biohydrogen production as well as by increased algal biomass and lipid production. We have analyzed the effects of bacterial phylogenetic relationship and growth rate on algal functions such as biomass yield, nutrient uptake and biomolecule composition. The mechanisms of the interactions were investigated using transcriptome analysis and advanced microscopy techniques.

MUTATIONS IN THE SECOND ALTERNATIVE OXIDASE GENE: A NEW APPROACH TO GROUP *ASPERGILLUS NIGER* STRAINS

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Alternative oxidase is a terminal oxidase in branched mitochondrial electron transport that provides an alternative for the cytochrome-dependent electron flow and bypasses the proton-pumping complexes III and IV. The consequence of the direct transfer of electrons from ubiquinol to oxygen without concomitant proton translocation is the uncoupling of ATP synthesis via oxidative phosphorylation from NADH re-oxidation, to allow carbon catabolism to continue unabated when ATP demand is low. For this reason, it plays an important role in the energetics of overflow metabolism-based bioprocesses such as the *Aspergillus niger* citric acid fermentation. Alternative oxidase (Aox) is near ubiquitous in the fungal kingdom, but coexistence of paralog alternative oxidase genes is rarely described. However, a second *aox* gene (*aoxB*) present in some *Aspergillus niger* isolates coexists in two divergent species in the subgenus Nidulantes, *A. calidoustus* and *A. implicatus*, as well as in *Penicillium swiecickii*. In these four scattered Aspergillaceae, *aoxB* is orientated divergently from *andB*, a rare paralog of an otherwise ubiquitous gene for type-II NADH dehydrogenase, another "alternative" enzyme in the branched respiratory chain. In this study, we demonstrated that the paralogous *aoxB* gene in some 75 genome-sequenced *A. niger* strains features variation at a level not detected for the ubiquitous *aoxA* gene.
Five mutations were identified that affect transcription, function, or terminally modify the gene product. Citric acid producer strain ATCC 1015 has a full-length *aoxB* gene. One mutant allele involves a chromosomal deletion that removes exon 1 and intron 1 from *aoxB* as well as 75% of *andB* and the intergenic region: this allele occurs in the industrial protein producer CBS 513.88. Another allele results from retrotransposon integration. The other three alleles result from a missense mutation of the start codon, a frameshift, and a nonsense mutation. The *A. niger* sensu stricto complex can be subdivided into six taxa according to the *aoxB* allele.

RHIZOSPHERE MICROBIAL COMMUNITIES OF POACEAE SPECIES IN DRY HUNGARIAN GRASSLANDS: SCREENING FOR PLANT GROWTH PROMOTING PROPERTIES

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Drought is a major challenge for plant growth especially in dry grassland areas. Bacteria that stimulate plant growth are often isolated from extreme environments such as arid regions and these bacteria are able to increase the survival of other types of plants during the dry period, not just those from which they have been originated. The objective of this study is to reveal arid open and closed sand steppes rhizosphere bacterial communities. We investigate whether the isolated bacteria with plant growth-stimulating properties can improve the growth of maize under normal and drought stress conditions. The diversity of bacterial community from the rhizosphere and bulk soil samples were investigated by Illumina metagenome sequencing and culturing methods. Additionally 149 strains were isolated from the sandy grasslands and the selected nonpathogenic 48 strains were screened for plant growth promoting (PGP) traits, such as osmotic stress tolerance, indole-3-acetic acid, exopolysaccharide, siderophore, and 1-aminocyclopropane-1-carboxylate deaminase production and phosphate solubilization. The short-term effect of the strains to maize was tested in a phytotron pot experiment, while the study of drought stress conditions is planned to be investigated in an open field experiment. Based on metagenome analysis representatives of the Actinobacteria, Proteobacteria and Acidobacteria were the most abundant in both rhizosphere and soil samples. A short-term phytotron study of the isolates with the best PGP properties shows that the growth of maize plants was mostly enhanced by the *Brevibacillus*, *Priestia*, *and Kocuria* species. Field trials are currently in progress.

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TAXONOMIC AND METABOLIC CHARACTERIZATION OF *ALTERNARIA* SPECIES IN GRAPEVINE (*VITIS VINIFERA*) IN HUNGARY

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Alternaria, member of the grapevine core microbiome, is a cosmopolitan and ecologically diverse fungal genus including saprobes, endophytes, pathogens, and casual agents of postharvest rots, causing substantial economic loss on different agronomic plants. Besides, numerous species are known to produce a variety of secondary metabolites in field crops, which is particularly relevant in plant protection and food safety. According to our previous findings, a significant number of species belonging to the *Alternaria* Sect. Alternaria are common endophytes inhabiting the different grapevine varieties based on ITS sequencing. The objective of this study was to characterize endophytic *Alternaria* strains associated with different cultivars of *V. vinifera* in the Eger wine region, Hungary, isolated from asymptomatic aboveground tissues of grapevine using multi-locus phylogeny and metabolite profiling. As the ITS region alone is

not adequate in the species-level discrimination within the section, molecular phylogenetic analysis was conducted using five further genomic loci (RPB2, Alt a 1, endoPG, OPA10-2, KOG1058). The analysis of secondary metabolite production and metabolite profiling of the isolates were carried out using high performance liquid chromatography (HPLC)-high-resolution mass spectrometry (HRMS). Both multi-locus and single-locus phylogeny revealed that *Alternaria* isolates obtained from grapevine represent two distinct lineages within *Alternaria* sect. Alternaria, considered as *A. alternata* (AA) and *A. arborescens* species group (AASC). Eight *Alternaria*-specific metabolites were identified in all the collected *Alternaria* isolates regardless of the affiliation to the species and lineages, although at different concentrations. Principal component analysis of untargeted MS data showed no significant separation between AA and AASC, indicating that the major source of variation is not attributable to the AA-AASC grouping. However, a PLS-DA model was built with two components, which model successfully discriminated between the groups by chemical profile, suggesting a difference in chemical composition of the two groups. In the light of our results, we might assume that based on their similar metabolite production, and asymptomatic dominant presence in healthy grapevine tissues, strong functional differences are unlikely between AA and AASC.

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CHARACTERIZATION AND HETEROLOGOUS EXPRESSION OF *MUCOR LUSITANICUS* HsbA PROTEINS

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Mucor lusitanicus is an opportunistic, human pathogenic fungus that can cause superficial or invasive infections known as mucormycosis. Previous research by our group identified cell surface proteins, such as hydrophobic surface binding proteins (HsbA), as potential virulence factors. HsbA proteins belong to the galactomannoprotein family as major components of the fungal cell wall that is released during the growth of fungal hyphae. Our previous studies suggested HsbA proteins influence the biofilm formation and virulence in *Mucor lusitanicus*. The aim of our present study is to characterize knockout and overexpression *hsbA* mutants. The real-time quantitative PCR results suggested the collaborative function of individual HsbA proteins in *Mucor*. Overexpression of HsbA proteins resulted in germination defects. Spores of the strain overexpressing the gene *hsbA1* displayed significantly reduced viability and germination capacity compared to those of the control. Overexpression of *hsbA1* and *hsbA2* associated with a significant reduction in sporangiospore formation, indicating that these genes may play a role in the sporulation. In biofilm formation experiments, the MS12 + pAV1 and the knock-out MS12 - Δ*hsbA3* + *pyrG* strains showed reduced biofilm formation ability compared to the control MS12 + *pyrG* strain, while the knock-out MS12 - Δ*hsbA2* + *pyrG* strain showed significantly higher biofilm formation ability. Overexpression of the HsbA proteins led to a reduction in virulence, while their absence resulted in enhanced virulence. These results suggest that the presence of HsbA proteins and/or surface hydrophobicity affect the pathogenicity of *M. lusitanicus*. Furthermore, we aimed to achieve heterologous protein expression of HsbA proteins in *Pichia pastoris*. We constructed expression plasmids with a single gene of interest and the subsequent expression of the protein inside the recipient strain. Expression plasmids for heterologous expression of HsbA proteins in *P. pastoris*, using the pPICZα vector with an inducible promoter were constructed and the transformation process is ongoing.

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TERROIR, SEASON, AND VINTAGE EFFECTS ON GRAPEVINE PATHOBIOME COMPOSITION

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The terroir concept, extensively employed in viticulture and oenology, is based on landscape-scale differences in environmental factors and is often used to explain differences among wines. These edaphic and mesoclimatic variations are likely to impact the microbes associated with plants, thereby influencing plant health. This study investigates the dynamics of plant pathogenic fungi in three distinct microhabitats (soil, woody tissue, and bark) of grapevine cv. Furmint, sampled during late winter and late summer in 2020 and 2021 in three different terroirs in the Tokaj wine region. Illumina NovaSeq sequencing was employed to generate sequence data of the ITS2 region of the ribosomal DNA repeat. Among the 123 plant pathogenic genera identified, *Diplodia*, *Phaeomoniella*, and *Fusarium* exhibited the highest richness in bark, wood, and soil, respectively. Notably, both richness and abundance varied significantly across microhabitats, with grapevine trunk disease (GTD)-causing fungi displaying greater richness and abundance in wood and bark samples, while non-GTD pathogens dominated the soil. Additionally, noteworthy compositional differences were observed among terroirs, seasons, and vintages. Terroir explained 14.5 - 24.7% of the variance in community composition, while season and vintage accounted for 1.8 - 2.98% and 3.7 - 6.4% of the variance, respectively. These findings suggest that environmental filtering at both the microhabitat and

terroir levels contribute to the observed differences. Furthermore, temporal dynamics of fungi may be influenced by weather conditions and fungicide applications. The insights gained from this research contribute to a deeper understanding of the complex interactions between grapevines, their microbial communities, and the environmental factors that shape them.

TICK-BORNE ENCEPHALITIS VIRUS IN HUNGARY – A CASE STUDY OF AN ALIMENTARY INFECTION

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Tick-borne encephalitis virus (TBEV; genus *Flavivirus*, family Flaviviridae) was isolated for the first time in Hungary in 1952 and since 1958, regular microbiological testing of clinically suspected cases has been applied at the National Reference Laboratory for Viral Zoonoses of the National Public Health Center, Hungary. TBEV can be transmitted to humans by ticks-bites as well as by the alimentary route, via the consumption of raw milk or dairy products from infected ruminants such as cattle, sheep, or goats. In recent years, sporadic cases of alimentary infections or local epidemics have been regularly reported in Hungary. The aim of our study was to summarize the epidemiological data of human TBEV infections in Hungary, between 2009 and 2022. We also present a case report of an alimentary infection with a severe clinical course of TBE. Anti-TBEV IgM, IgA, and IgG antibodies were detected by indirect immunofluorescence assay and ELISA tests, and viral nucleic acid was detected by in-house developed reverse transcription real-time PCR assay. Endemic flaviviruses, other than TBEV were also tested in parallel, due to the evaluation of serological cross-reactivity. Between 2009 and 2022, 453 laboratory-confirmed human TBEV infections were reported in Hungary of which, in seven cases, alimentary infection was suspected. In 2022, three cases of alimentary infections were confirmed accumulated within a family. One of the two children developed unusually severe neurologic symptoms, such as fluctuating state of consciousness, and oculomotor dysfunction. Abnormal findings on MRI scans also indicated neurological involvement. Previous West Nile virus infection was suggested by the serological findings, resulting in a more severe clinical course of TBE, due to the antibody-dependent enhancement of infectivity.

GEOMICROBIOLOGICAL STUDY OF MODERN MICROBIALITES IN A THERMAL SPRING (KÖRÖM, HUNGARY)

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Stromatolites are the oldest known life forms; their presence on Earth can be tracked back to ~3.5Bya in carbonate fossils. Recent microbialites are the modern analogues of the prehistoric stromatolites, as the organomineralization processes are still active and detectable. Thick, red and green colored, multilayered biofilms (microbial mats) with unique microbialite deposition patterns have been formed near the 79.2°C outflow of Köröm thermal karst well (Bükk Region, Northern Hungary). Here, a large amount of aragonite and calcite depositions (travertines) can be observed along the outflow water. For the study of the microbial diversity, samples (water, carbonate precipitates along the water canal at various distances from the well outflow, furthermore red and green biofilms developed near the canal) were collected. Light and scanning electron microscopic (SEM), micro-XRD and molecular methods were performed to explore the relationship between the thermophilic microbial diversity and the physico-chemical factors, and in connection with carbonate precipitation. Optical microscopy observations revealed vertical laminar mineral depositions (typical of stromatolites) in the red biofilm layers, in contrast to the green biofilms, which contained clotted mesostructure (typical of thrombolites). Electron microscopic images of the biofilms several centimeters thick showed network structures formed by filamentous microbes, which contained bacteria with different morphologies (cocci and rods with different sizes) and mineral particles and clusters with different shapes (needle and spherulite). Based on the micro-XRD method, the biofilm layers contained mainly calcite beside aragonite, but aragonite dominated the bottom layer of the green biofilm samples. Bacterial diversity of the samples was compared by 16S rRNA gene amplicon sequencing. The results implied that numerous undescribed thermophilic taxa might have contributed to the carbonate mineralization. The biofilms were mainly composed of the phyla Bacteroidota, Proteobacteria and Cyanobacteria but they differed at low taxonomic levels (e.g., *Geitlerinema* PCC-8501 and *Raineya* were characteristic to the green biofilm, while unclassified Oxyphotobacteria, unc. Saprospiraceae and unc. Cytophagales were abundant in the red biofilm samples). The most abundant phylum were the Aquificota (hydrogen-oxidizing *Hydrogenobacter*) in the water samples, while the unclassified Bacteria and Deinococcota in the carbonate deposition samples.

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CANDIDA ALBICANS EXTRACELLULAR VESICLES, THE THREE SIDES OF THE COIN

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Fungal organisms use extracellular vesicles (EVs) as a mechanism of molecular exportation, releasing a diversity of active compounds to the extracellular milieu where they can interact with neighboring cells. Since their first isolation and partial characterization in 2007, fungal EVs have been associated with pathological and physiological processes. Indeed, some of the activities attributed to these compartments were already attested experimentally and are implicated with contrasting effects. For instance, EVs released by *Candida albicans* yeasts can be used as vaccine formulations to prevent systemic candidiasis in immunosuppressed mice. However, the same EVs carry specific virulence factors correlated with deleterious effects and host lethality.
Recently, the ability of *C. albicans* EVs to impact growth and biofilm formation was demonstrated, indicating that fungal EVs could mediate intercellular communication impacting fungal virulence. Through proteomic, lipidomic and transcriptomic approaches we identified specific molecules carried by *C. albicans* EVs and some of the mechanisms involved with their multitude of functions. Mannoproteins, β-1,3-glucans, secreted aspartyl-proteases (SAPs), terpenes (farnesol and dihydrofarnesol) and medium chain fatty acids stand out as EV-compounds correlated with the three "functional" sides of fungal EVs.

METATRANSCRIPTOMIC ANALYSES OF GRAPES REVEAL DIFFERENCES IN EXPRESSED FUNCTIONAL GENES OF FILAMENTOUS AND YEAST FUNGI DURING NOBLE ROT AND GREY ROT

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Botrytis cinerea is a necrotrophic fungus causing grey rot (GR) with crucial economic losses in fruit crops. It can also cause the desired noble rot (NR) in grape berries used to produce botrytized wines. In both states, *B. cinerea* is associated with several other fungi, but the functional role of these is still poorly understood. Metatranscriptomic data was generated from healthy (H), noble rot (NR) and grey rot (GR) grape berries and RNAseq reads were aligned to the most prevalent filamentous fungi and yeasts based on previous culture-based studies. Differential enrichment analyses and pathway enrichment analyses revealed that all fungi and yeasts are most active in NR. Beside *B. cinerea*, several functional genes of other fungi were linked to well-known physico-chemical changes in NR berries and to the production of antagonistic interaction genes. Our study demonstrates the complex interaction dynamics of the grape microbiome.

TRANSCRIPTOMIC ADAPTATION TO SUPEROXIDE STRESS IN Δ*fvatfA* AND Δ*fvmnSOD FUSARIUM VERTICILLOIDES* STRAINS

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The often endophytically, maize pathogenic *Fusarium verticilloides* may cause substantial damage to ear, stalk, and foliage, and contamination of grains with mycotoxins affecting human and animal foods. At the plant-penetration site, it may cause necrosis leading to the release of active oxygen species (AOS) like superoxide anion ($^{\bullet}O_2^-$) and by the fungal part secondary metabolites release involving mycotoxins produced by the pathogen. Their biosynthesis is linked to the fungal ability to protect itself from oxidative damage. Therefore, it is not surprising that the synthesis of secondary metabolites is genetically linked with oxidative stress in filamentous fungi and plants. Genetic regulation elements of secondary metabolites are e.g., the bZIP-type transcription factors which are proven to be oxidative stress-related with a key role in mycotoxin production. Our work aimed to explore metabolic and transcriptomic events that may be regulated by the bZIP-type FvAtfa transcription factor in *F. verticilloides* and their dependency on oxidative stress-responsive pathways. For this purpose, two mutant strains Δ*fvatfA* and Δ*fvmnSOD* were analyzed under superoxide stress conditions triggered by MSB. Analyzing the adaptive responses, we focused on the production and regulation of secondary metabolites using metabolomic and pathway analysis in silico. The comparative genome-wide transcriptomic profiling revealed significant differences in DEGs associated to strain-specific adaptation involving filamentous growth, carotenoid and fumonisin biosynthetic pathways. GO enrichment analysis highlighted a strong down-regulation of genes in RAS-protein signal transduction in both mutants with an outcome of strain-specific adaptation to superoxide stress. These manifested in repressing of Ras family GTPase genes (*rab, ras, arf*), Ras activated genes (*mob1*) and Ras regulatory elements (PP2A) inhibiting downstream genes involved in process of morphogenesis, virulence, cell polarity, vesicle formation, membrane traffic, pathogenesis and fumonisin production-regulation. In order to compensate these constrained processes different mechanisms of Δ*fvatfA* and Δ*fvmnSOD* strains were assumed. Among which Δ*fvmnSOD* mutant positively regulated NOX to

maintain redox balance in order to do not loss pathogenicity, however Δ*fvatfA* mutant with unabated redox enzymes reinforced mitochondrial metabolism to compensate disturbed growth and virulence.

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ANTIBIOTICS OF THE FUTURE ARE PRONE TO BACTERIAL RESISTANCE

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Despite the ongoing development of new antibiotics, the future evolution of bacterial resistance may render them ineffective. Using laboratory evolution, we demonstrate that new antibacterial compounds currently under development are as prone to resistance formation in Gram-negative pathogens as clinically employed antibiotics. Furthermore, the transfer of antibiotic resistance genes from human-related microbiomes has a further contribution to resistance. The molecular mechanisms of resistance overlap with those found in commonly used antibiotics. As a consequence, these mechanisms are already prevalent in natural bacterial pathogens, indicating that resistance can rapidly emerge through the selection of pre-existing bacterial variants. Additionally, resistance to new peptide-based antibiotics enhances bacterial virulence, raising concerns. However, certain combinations of antibiotics and bacterial strains are less prone to developing resistance, emphasizing the potential of narrow-spectrum antibacterial therapies that could remain effective. Our comprehensive framework could be used to assess the future health risks associated with bacterial resistance to new antibiotics.

PRELIMINARY RESULTS ON BIODEGRADATION OF DRUG RESIDUES

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Due to the growing population, an increase in the consumption and simultaneous release and accumulation of pharmaceuticals in the environment can be witnessed worldwide. The most commonly detected pharmaceuticals in the freshwater ecosystems are diclofenac (DIC), ibuprofen (IBU) and carbamazepine (CBZ). It has been proven that at environmentally relevant concentrations of these compounds can exert ecotoxic effects on aquatic organisms. Conventional wastewater treatment plants are inefficient regarding the elimination pharmaceuticals (PhACs). More suitable alternatives or additional units, preferably biological solutions, are required for the more efficient/proper elimination of PhACs from the wastewater. Selective enrichment followed by isolation in order to identify pharmaceuticals-, especially DIC, IBU, and CBZ degrading bacteria was performed. For this purpose, a groundwater biofilm community originating a hydrocarbon-contaminated site was targeted. Selective enrichments for three months were carried out in mineral salts medium (MSM) supplemented with pharmaceuticals (100ppm, separately DIC, IBU and CBZ). A bacterial strain collection containing 31 species-level identified isolates was obtained. At first, using the semi-quantitative resazurin screening assay potential pharmaceutical degraders were identified. Secondly, to get more accurate information about the biodegradation potential of the selected isolates high-pressure liquid chromatography (HPLC) was applied to monitor the degradation. Isolates affiliating with the genera *Stenotrophomonas*, *Rhizobium*, *Nocardioides*, and *Brevundimonas* showed the highest DIC, IBU, and CBZ biodegradation rates, respectively. Cocultures were established and tested for simultaneous DIC, IBU, and CBZ biodegradation (1.5ppm each) in a Bushnell-Haas medium, in a natural water sample and in a wastewater effluent sample. The results indicated that the higher pharmaceutical biodegradation could be recorded in cocultures. In mineral salt solution, the concentration of DIC, IBU, and CBZ was reduced by 72%, 100%, and 30%, respectively. In natural water samples, in the presence of the autochthonous bacterial community, the concentration of DIC and IBU was reduced by 25% and 94%, respectively, and no CBZ biodegradation was recorded. In the experiment using sewage effluent, ibuprofen was completely broken down within two weeks. After three weeks, we experienced a nearly 100% decrease in the concentration of diclofenac in the test solutions and 25% decrease in the concentration of carbamazepine. In the case of the bacterial strain *S. humi* DIC_5, we thoroughly explored the diclofenac-degrading ability with analytical instruments and identified an intermediate product of the biodegradation, nitro-diclofenac. These isolates could be used in the development of biotechnological tools for elimination of PhACs from the wastewater.

MICROBIAL ANALYSIS OF HUNGARIAN FORAGE SAMPLES

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Yeasts are common in forage fermentations, especially in the first phases of fermentation or as spoilage microbes after aerobic exposure. They are also commonly utilized in animal husbandry as probiotics or supplements. The global diversity of the most important industrially used yeast, *Saccharomyces cerevisiae*, is well known owing to large-scale phylogenomic advancements; however, animal forage isolates of the species are poorly known. Here, we aimed to characterize silage and animal feedstuff samples from Hungary in order to assess their microbial (bacterial and yeast) diversity in the first such study from Europe. Furthermore, we aimed to gain insights on *S. cerevisiae* found in such samples. We reviewed yeast occurrences from silage samples across the globe, applied long-read bacterial metabarcoding to 42 Hungarian samples and assessed bacterial communities, and we identified culturable yeast species that in some cases, represented species never before recorded from animal feedstuff. We identified eight *S. cerevisiae* isolates, all from animal feed mixes, while the species proved absent from silages. After fingerprinting, four isolates were subjected to short-read sequencing and comparative and phylogenomic analysis that was also facilitated by the near complete assembly of one of the isolates. Our whole genome sequencing doubled the number of sequenced animal feed isolates of the species, to eight. We showed that three of these belong to the Wine/European clade, while the rest, the four Hungarian samples and an Italian one, are derived from the Mixed origin group, and are related to baker's yeasts. The Hungarian isolates were tetraploid-aneuploid ones very likely originating from the same commercial feedstuff strain as they showed high uniformity. We thus showed that although yeasts can be diverse in local forages, the *S. cerevisiae* in such products originate from manufacturing processes distinct from silage fermentation.

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NKFIH 2018-1.2.1-NKP-2018-00002 – SUMMARY OF A HUNGARIAN MULTIDISCIPLINARY AFLATOXIN PROJECT

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Aflatoxinogenic *Aspergillus* species are spreading northward in Europe as a result of climate change, and this may even represent a new health risk for the population of Hungary, which is located in the continental climate zone. The NKFIH 2018-1.2.1-NKP-2018-00002 National Excellence Program financed by the National Research, Development and Innovation Office of Hungary focused on (i) the assessment of the current aflatoxin M1 (AFM1) exposure of the Hungarian milk consumers, (ii) the elaboration of aflatoxin risk management measures, and (iii) the prevention and mitigation of aflatoxin contaminations in feed and food. Importantly, taking immediate countermeasures are not necessary based on our risk assessment data but continuous monitoring of the feed and food chain is recommended. In addition to the optimization and validation of HPLC and ELISA-based methods to gain reliable data on AFM1 and aflatoxin B1 contaminations, we also assessed bacterial and yeast communities in fermented forages, screened for microorganisms with high mycotoxin tolerance and good mycotoxin eliminating potential, and mapped the effects of various agroecological factors on aflatoxin production by *Aspergillus flavus* in field studies. Furthermore, we have also completed the phylogenomic and comparative genomic analysis as well as toxic secondary metabolite profiling of some *A. flavus* isolates collected in Hungary. It is worth mentioning that we have successfully adapted the methods enabling the genetic modification of *A. flavus*, and the functional characterization of some bZIP-type transcription factors regulating the oxidative stress defense system and/or the biosynthesis of aflatoxins has started.

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MYCOTOXINS IN FOOD CHAIN - CLIMATE EFFECT AND ELIMINATION STUDIES

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In general, based on currently available data, atmospheric concentrations of CO_2 are expected to double or triple (from 350 - 400ppm to 800 - 1,200ppm) in the next 25 - 50 years. Thus, a temperature increase of 2 - 5°C affecting different regions of the world, coupled with high CO_2 levels (800 - 1,200ppm) and more frequent drought episodes, could have a profound impact on mycotoxigenic fungi and mycotoxin production. New and emerging combinations of mycotoxins in food/feed demonstrated the ability of fungi to adapt to

changing conditions and a stable economic and health threat. Our research group - in relation to long-term research programs - took part in extensive studies to find a "green" solution to the mycotoxin contamination problem. During our studies bacterial, yeast and filamentous fungal isolates and strains from culture collection were tested for their mycotoxin resistance and elimination capabilities. Some of these strains were selected and the cell fractions were tested to gain information on cell-free elimination possibilities.

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CHARACTERIZATION OF C-5 STEROL DESATURASE GENE REVEALS ALTERED VIRULENCE ALONG WITH CHANGES IN THE CELL WALL STRUCTURE IN *MUCOR LUSITANICUS*

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Despite most members of Mucorales are saprotrophic, certain species can cause life-threatening and often-fatal systemic infection known as mucormycosis. Mucormycosis has a high mortality rate, up to 90 - 96% depending on the localization of the infection, the causative species and the medical condition of the patient. Recently the infection became more prevalent thanks to the COVID-19 pandemic and global warming. Treatment of mucormycosis is challenging because Mucorales species possess intrinsic resistance to most antifungal agents. The target of amphotericin B is ergosterol, which is the main sterol component of the fungal cell membrane. The role of ERG3 is to convert the episterol into ergosta-5,7,24(28)-trienol. Moreover, another alternative sterol biosynthesis pathway has been revealed in *Saccharomyces cerevisiae* where the ERG3 converts the 14α-methyl-fecosterol into the toxic fungistatic14α-methylergosta-8,24(22)-dienol. Mutations in the C-5 sterol desaturase gene are already proven to affect the virulence in *Candida albicans*. The genome of *Mucor lusitanicus* encodes one C-5 sterol desaturase (*erg3*). In this study, the role of *erg3* gene in stress tolerance and virulence of *Mucor* was analyzed by generating and analyzing knockout mutants, which were achieved by PEG-mediated transformation, combined with CRISPR-Cas9 method. The stress tolerance of knockout mutants against lower temperature, osmotic stress and cell wall stressors has decreased. On the other hand, these mutants had increased growth at higher temperature. The virulence tests revealed that the virulence of these strains decreased upon the knockout of *erg3*. Antifungal susceptibility against azoles, which are the mainly used antifungal agents other than amphotericin B, have not changed. However, the susceptibility is decreased against amphotericin B. This is in close correlation with the sterol composition of the cell membrane of the knockout mutants, which significantly differs from the parental strain. In silico analysis of amphotericin B docking to ergosterol and to ergosterol biosynthesis intermediates showed that amphotericin B can bind to the intermediates with the same affinity and energy as to the ergosterol.

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HUMAN PAPILLOMAVIRUS E7 PROTEINS ASSOCIATE WITH MYPT1

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Prophylactic vaccines have the potential to have a major impact on the global burden of human papillomavirus (HPV)-associated cancers; however, they have no therapeutic potential. There is still a pressing need to develop better forms of therapeutic interventions and understand the mechanisms by which these viruses cause malignancy. A hallmark feature of HPV attributable cancers is the high-level and continued expression of viral oncoproteins. These HPV proteins, particularly E7, play a significant role in induction of malignancy by targeting critical cell control pathways. Using proteomic analysis, we identified MYPT1, the targeting subunit of myosin phosphatase, as a novel interactor of HPV-16 E7. This creates the possibility that MYPT1 and, through this connection, myosin phosphatase can play a role in the life cycle of the virus and in the development of HPV-associated tumors. We aimed to characterize this association during our experiments. First, we determined the expression levels of MYPT1 protein in several HPV positive and negative cell lines. Next, we investigated the effect of HPV E7 proteins of different virus genotypes (HPV-11, HPV-16, HPV-18, HPV-31) on the steady state expression level of MYPT1, and performed HPV E6/E7 specific siRNA treatment in HPV-18 positive HeLa cells to investigate the effect of gene silencing on MYPT1 protein expression. Moreover, we confirmed the interaction between the HPV E7 and MYPT1 by pull-down method. Protein expression levels were detected by using Western blot. We show that the presence of HPV E7 proteins leads to reduced protein levels of MYPT1, thereby possibly affecting its function. E7 oncoproteins of high-risk HPV types had prominent effect on MYPT1 protein expression. Moreover, we observed a very strong connection between MYPT1 and HPV-16 E7 indicating MYPT1 being important for the function of high-risk E7 to enhance cell proliferation and induce malignancy.

COMPARATIVE BIOFILM ANALYSIS OF *SALMONELLA* SEROVARS AND COHABITANT *ESCHERICHIA COLI* STRAINS

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In the Hungarian broiler flocks, there has been a change of *Salmonella* serovars in the early 2000s, characterized by the drastic reduction of *S.* Enteritidis and the emergence of multiresistant (MDR) strains of *S.* Infantis. We supposed that the biofilm forming activity and the presence of associated (cohabitant) strains of MDR *E. coli* are among the key drivers of rise and global spread of *S.* Infantis in broiler flocks. The aim of this study was to compare the biofilm forming ability of *Salmonella* and cohabitant *E. coli* strains, to reveal the relation between the morphological and quantitative parameters of the biofilm. For this study MDR strains of *S.* Infantis (n = 20) and cohabitant *E. coli* (n = 69) from broiler caecum and strains of *S.* Enteritidis (n = 24) from human stool samples were compared. The biofilm morphotype was determined on Congo red agar plates, incubated for 96h at different temperatures (20°C, 28°C and 37°C). The quantitative biofilm assay was performed in 96-well polystyrene plates by using the same incubation conditions. Statistical analysis of the quantitative data was performed in R commander. Results showed that MDR strains of *E. coli* were characterized by a high diversity of biofilm morphotypes, with the predominance of the rdar (red dry and rough) at 20°C and 28°C. At both temperatures, strains of *S.* Enteritidis showed three different biofilm morphologies: bas (brown and smooth) rdar and pdar (pink dry and rough) were detected in 43%, 35% and 22% of the strains. Interestingly *S.* Infantis strains formed smooth surface biofilm (bas) at 28°C while at 20°C the biofilm had rough surface (rdar). All strains formed tiny bas colonies on 37°C. Consistent with the results on morphology, the quantitative biofilm assay showed that the biofilm activity was significantly ($p<0.01$) higher at lower temperatures (20°C and 28°C) than at 37°C. Regarding the biofilm morphology and productivity, we found that the smooth morphotype was associated with a higher biofilm production than the rough type regardless of the species. The biofilm forming activity of *E. coli* changed the most with the temperature while *S.* Infantis showed high biofilm activity at all three temperatures. In this comparison, strains of *S.* Enteritidis strains showed the lowest biofilm production. According to this, the biofilm production ability of MDR *S.* Infantis was at least threefold higher than that of *S.* Enteritidis and twofold higher that of commensal *E. coli* from broilers. Results are in line with our hypothesis, indicating that the increased environmental survival conferred by the biofilm activity could be one explanation to the competitive success and spread of *S.* Infantis against *S.* Enteritidis in Hungarian broiler flocks.

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COMBINED EFFECTS OF WASTEWATER SLUDGE COMPOST AND ARBUSCULAR MYCORRHIZAL FUNGI ON IMPROVEMENT OF SOIL FERTILITY AND RHIZOSPHERIC ACTIVITY OF GIANT REED (*ARUNDO DONAX* L.)

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We examined the effect of low-dose, commercially available wastewater sludge compost (WSC; 15t ha^{-1}) treatment with or without arbuscular mycorrhizal fungal (AMF) inoculation on the nutritional status, heavy metal (HM) concentration and the rhizosphere activity of giant reed (*Arundo donax* L. var. BL clone (Blossom)) plants in a short-term pot experiment. *Funneliformis mosseae* (BEG12; AMF1), *F. geosporum* (BEG11; AMF2), or their combination (AMFmix) were applied as AMF inoculation treatments. We supposed this combined treatment enhances the fertility of a low fertility soil. The physiological and growth parameters of the host plants, the AMF root colonization, and the microbiological enzyme activity of the mycorrhizosphere were examined. Neither the WSC treatment nor the AMF inoculations changed the extent of root colonization. Based on the results of root electrical capacitance and the phosphorus uptake, plant nutritional status was improved by WSC addition, without any negative impacts. AMF treatments increased soil enzyme activities, decreased the concentration of the potentially toxic HMs (Cu, Mn, Pb, Zn) in the roots, but the difference of Cu and Zn was compensated in the shoots. According to the results of MicroResp™ measurements, changed the pattern of the microbial community. Giant reed's efficient regulatory mechanism to adjust optimal/maximal colonization rate, and to select for preferential AMF partners might be responsible of its invasiveness and tolerance to a wide range of environmental conditions.

MEASLES SEROPREVALENCE AMONG HEALTH CARE WORKERS IN HUNGARY

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In 2017, 2018 and 2019, 36, 14 and 23, respectively, (altogether 73) laboratory confirmed measles cases were detected in Hungary. These were imported from countries with ongoing measles outbreaks (Romania, Ukraine), or had epidemiological link to measles patients. The majority of the cases (54 out of 73) were involved in epidemics (in 6 outbreaks in 2017, one in 2018 and 3 in 2019). In one case, the patient had travel history (Thailand) prior to her illness. Due to high vaccination coverage, neither sporadic case(s) of measles nor outbreak(s) were detected since SARS-CoV-2 pandemic in Hungary. One third of the patients (34/73) were health care workers (HCWs). One of them was unvaccinated (he was born before the vaccination era) and had primary measles but the others' clinical manifestations could be ascribed to waning immunity over time (secondary vaccine failure). The outbreaks among HCWs raised the necessity of a serosurvey in this risk group. Altogether 41,357 HCWs were tested for measles antibody in two periods (in 2017 - 18 and in 2021). The first testing period (21,339) focused on hospital and primary health care workers in five counties next to the Romanian and Ukrainian border and in two cities (Budapest and Kaposvár). In the second period, 20,021 HCWs from all over of the country were surveyed. Four age groups of patients were formed according to different vaccination strategies (for the easier comparison, the imported cases were grouped in the same way): 1) not vaccinated [born before 01/01/1968]; 2) vaccinated with 1 dose of measles-containing vaccine (MCV) [born between 01/01/1968 and 31/12/1978]; 3) vaccinated with 2 doses of MCV (born between 01/01/1979 and 31/12/1990) and 4) vaccinated with 2 doses of MMR vaccine (born after 01/01/1991). The surveys revealed that in 17.7% of HCWs, the anti-measles IgG was under the detectable level (seronegative test result); 10.6% had low antibody levels (grey zone test result) against measles. The highest ratio of seropositivity could be detected in the first age group (not vaccinated, naturally infected before the vaccination era), the lowest was in the group No.2 (vaccinated with one dose of MCV) that also correlates with the prevalence of measles in the vaccinated population. Due to the consequent lack of circulation of measles virus in Hungary, its immune boosting effect is missing. In case of individuals vaccinated decades ago, grey zone or seronegative test results may mean weak protection against measles. For HCWs with seronegative or grey zone test results, immunity against measles is recommended to be boosted by re-vaccination

UNIVERSAL PEPTAIBOL LIBRARY

ÁKOS ROZSNYÓI, DÓRA BALÁZS, CHETNA TYAGI, ANDRÁS SZEKERES, TAMÁS MARIK, CSABA VÁGVÖLGYI, LÁSZLÓ KREDICS

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Peptaibols are secondary metabolites, produced mainly by members of the ascomycetous genus *Trichoderma*. These non-ribosomal peptides are bioactive molecules: by linking to each other, they can form transmembrane ion channels and integrate into prokaryotic and eukaryotic cell membranes, which may lead to cell death. These molecules are known since the middle of the 20th century – since then more than 1,500 different peptaibol sequences were discovered from the genus *Trichoderma*. To gather the information about peptaibols the "Peptaibol Database" and the "Comprehensive Peptaibiotics Database" were created in 1997 and 2013, respectively. The latter contained the data of the first Peptaibol Database and all of the updates until December 2012. Today both of these databases are outdated, as both the number of known peptaibols, and the quantity of the information of their bioactive properties have significantly increased. Therefore our aim is to create a new, "Universal Peptaibol Library", which will contain all the data of the previously discovered peptaibols, and provide a chance to other researchers for uploading their recently discovered data. This library was created in MySQL open source database and will be available from a website. Currently we are working on the website, using HTML, PHP, CSS, and JavaScript web development languages. Our goal is to create functions for searching the known peptaibol sequences, to provide access to the publication of their discovery and the source fungi, as well as to access their bioactive, molecular, and mass spectrometric properties from a single website. Furthermore, by using NGLview, a Jupyter/IPython based molecular structure viewer program, we are developing an interactive peptaibol structure viewing function, which is based on the previously published 3D structures of peptaibols. Our goal is to collect all previously discovered and future data about peptaibols at a single website in order to create a tool aiding the research in this field of molecular biology.

IDENTIFICATION OF NOVEL MYCOVIRUSES IN *RHIZOPUS* SPECIES

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The phylum Mucoromycota represents one of the oldest groups of fungi. These zygospore-forming fungi include members of the genus *Rhizopus*, which belongs to the order Mucorales. *Rhizopus* species are widely used as model organisms in microbiological studies. Besides, they are biotechnologically applied for the production of enzymes and organic acids, as well as various Asian fermented foods. Certain species are regarded as opportunistic pathogens causing surface and invasive mucormycosis. Generally, mycoviruses, i.e., viruses found in fungi, have RNA genomes, most frequently dsRNA. Mucoromycota is one of the less explored groups of fungi regarding virus harboring. Until today, no characterized viruses have been reported in *Rhizopus* species. In our previous study, we identified 15

mycoviruses in seven *Rhizopus* strains belonging to four different species, of which nine viruses had a dsRNA and six viruses had a (-)ssRNA genome. The detected dsRNA genomes contains two open reading frames, one encoding a capsid protein and the other an RNA-dependent RNA polymerase. The genome sizes of the six (-)ssRNA viruses are between 7.8 and 8.8kb and each have an open reading frame encoding a large RNA dependent RNA polymerase.
The dsRNA-genome belongs to the Totiviridae family, while the identity of viruses with (-)ssRNA genome is unclear. The presence of VLPs in the host fungus was confirmed for several viral isolates by transmission electron microscopy.

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EFFECTS OF TREATED WASTEWATER DISCHARGE INTO A STREAM ON MICROSCOPIC FUNGAL COMMUNITIES OF MICROPLASTIC SURFACES

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Microplastics (MPs) are emerging pollutants of the aquatic environment and wastewater treatment plants release a significant proportion of this load. Parallel, MPs provide a new niche for microorganisms, called 'plastisphere'. It might facilitate the modification of natural populations and offers a long-term habitat for previously transient microfungi in aquatic ecosystems. Our previous investigations showed significant differences between communities of aquatic and plastispheric biotopes, as well as between communities of different plastic and non-plastic surfaces. In this preliminary study, we investigated the effect of a treated wastewater discharge on the fungal communities of the plastisphere by incubating plastic particles for a month in Zagyva stream at three locations around the wastewater treatment plant effluent inlet of Hatvan: three samples – polypropylene (PP), polyethylene and polyethylene terephthalate – upstream to the inlet, one PP sample exactly at the inlet and one PP sample a hundred meters downstream to the inlet. Fungal communities were detected by amplicon sequencing of the ITS gene region. Sequences were processed by the DADA2 pipeline, identified based on the Unite reference database and characterized by the FungalTraits database. Statistical analyses conducted in R included the comparison of hill diversity profiles, Permanova and constrained analyses of principal coordinates (CAP). In the upstream samples mainly Ascomycota, Rozellomycota and Chytridiomycota, while in other samples Ascomycota, Rozellomycota and Basidiomycota were dominant. The main taxonomic orders of the upstream samples were Rozellomycota Incertae sedis, Pleosporales and Ustilaginales; but in other samples Trichosporonales, Rozellomycota Incertae sedis and Agaricales were prominent. Based on the FungalTraits database, upstream samples contained just a negligible number of animal parasites and opportunistic human parasites, while in other samples these traits were dominant. The latter sample group also contained a high number of soil and wood saprotrophs. In the upstream samples, Hill profiles were not significantly different, but according to Permanova and CAP, community structures of upstream and other samples differed. The presence of the Trichosporonales order and the absence of the Lentitheciaceae family might be good indicators of a close treated wastewater inlet. Interestingly, treated wastewater inlets did not affect fungal alpha diversities of the plastisphere, although based on the environmental load of chlorinated water along with several xenobiotics, we had opposing assumptions. On the other hand, it affected the community structures by potentially discharging a huge load of organic matter and fungi including some opportunistic pathogens. The inlet creates a new, nutrient rich, desirable niche, and provides new members, able to tolerate the extremities of this transformed habitat.

ORGANORHODIUM COMPLEXES OF 8-HYDROXYQUINOLINE DERIVATIVES WITH ANTIBACTERIAL AND ANTITUMOR EFFECT

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In bacteria, the multidrug resistant (MDR) phenotype is the consequence of selection pressure resulting from the widespread and inappropriate use of antimicrobials. Similarly to the antimicrobial drug resistance, the ability of cancer cells to resist a wide spectrum of structurally unrelated anticancer drugs is referred as cancer MDR. Despite the significant advances in the development of more effective and better-tolerated drugs in the last decades, the increasing new cancer cases and high mortality rate still strongly motivate the researchers to design novel anticancer compounds that combine good efficiency and selectivity. Additionally, the weakened immune system (due to cancer and the treatment) is vulnerable to infections; therefore, the combination of different therapeutic agents is often used, and a desirable option is to integrate different functionalities into a single molecule to obtain dual-targeted drugs. 8-Hydroxyquinoline (HQ) derivatives and their metal complexes are well known of their broad range of pharmacological properties, and among them, we can find derivatives, which are able to target multidrug resistant (MDR) cancer. Half-sandwich organometallic compounds are among the most widely studied anticancer complexes of platinum group metals, and numerous HQ complexes formed with Ru(η6-p-cymene) and Rh(η5-C5Me5) have been already reported to display potent anticancer activity in human cancer cell lines. The HQ derivatives and their half-sandwich organometallic complexes are often fairly lipophilic, which is considered as a favorable

feature for the uptake processes, however, the poor water solubility should be increased for better bioavailability. Herein, we present a comparative study on Rh(η5-C5Me5) complexes of 5-chloro-HQ Mannich bases with or without an additional carboxylate group. Stability and structure of the ligands and their complexes in solution were characterized, in addition to their lipophilicity, in vitro cytotoxicity in sensitive and resistant cancer cells, and antibacterial effect on Gram-negative and Gram-positive bacteria. The (homo)proline derivatives containing the carboxylate moiety were found to be more water soluble, and their bioactivity was similar to those without the carboxylate unit. Among the Rh(η5-C5Me5) complexes some showed significant cytotoxic activity, MDR-selectivity, and remarkable antibacterial activity against the methicillin-resistant *Staphylococcus aureus* (MRSA) strain.

EVALUATION OF PEPTIDE CARRIER CANDIDATES ON TISSUE BARRIER MODELS TO TARGET SARS-COV-2 INFECTED HOST CELLS

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Entry into host cells is a strategy widely used by pathogens and the host cells are not only primarily infected, but also act as a 'reservoir' for pathogens that could seed into other tissues, leading to systemic infections. Targeting peptides represent a promising approach to improve uptake and efficiency of antiviral drug candidates. In vitro barrier models are promising screening tools for characterization of the antiviral compounds and peptide carriers to evaluate their penetration profile and cytotoxicity. In this study, we have characterized lung and kidney epithelial monolayers using Transwell inserts as barrier models. Monitoring the transepithelial resistance (TEER) values of the monolayers reveal cell line specific, time course characteristics. The TEER method with calibration data offers a non-destructive procedure to time pharmacological transport measurements, and to evaluate cytotoxicity of the transported antiviral compounds and carrier peptide candidates. Both in kidney and lung barrier cultures peptide uptake by the detector cells was markedly different between the peptides investigated. Thus, this pilot study offers a method to select promising carrier peptides for anti-viral drug delivery.

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INVESTIGATION OF THE BEAUVERICIN PRODUCTION CAPACITY OF *FUSARIUM* STRAINS CHARACTERIZED BY BEAUVERICIN SYNTHETASE GENE SEQUENCE

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According to comprehensive risk assessment food safety studies by the EFSA and the FAO, a significant proportion of the world's grain supply is contaminated with at least one type of mycotoxin, with 60 - 80% of the unprocessed food tested affected by mycotoxin contamination. This might have a relation to the improved sensitivity of analytical methods and the impact of the climate change. In addition to the major mycotoxins, which are possessed with food safety limits, there are also so-called emerging mycotoxins, whose toxicological properties, distribution and frequency of occurrence are in some cases poorly understood. Beauvericin (BEA) is also one of the emerging mycotoxins, although it is a compound with all sorts of useful properties, it can also pose a potential hazard when present in food. We tested 100 *Fusarium* isolates originated from the Carpathian Basin characterized by the gene sequence responsible for beauvericin production, however this presence of a gene sequence does not imply its transcription, in order to test the actual biosynthetic capacity of the strains, small-scale bioreactors were set up for the toxin production experiment and the results were measured by chemical analytical methods. Samples were quantitatively analyzed for the most typical *Fusarium* toxins (DON, FB1, FB2, T2, HT-2, ZEA and BEA) using UHPLC tandem mass spectrometry. All of the tested strains were capable for the BEA production according to the results, the highest obtained BEA value was 3,131mg kg^{-1} synthesized by strain 24/3F.71. In addition to the target compound except for FB1 and FB2, no significant concentrations were observed.

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COMPARATIVE ANALYSIS OF RECEPTOR-BINDING PROTEINS OF BACTERIOPHAGES LYSING *ESCHERICHIA COLI* O157 STRAINS

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Amidst the growing crisis of emerging antibiotic resistant pathogenic bacteria (the 'post-antibiotic era'), the application of bacteriophages (phages) is a promising alternative antibacterial treatment, both as preventive biocontrol and as therapy against ongoing infections. Understanding the mechanisms contributing to the efficient and specific lysis of their target bacteria is an important aim of basic phage research. In the past two decades, numerous phages were described capable of actively lysing Escherichia coli strains of the O157 serogroup, which contains the most virulent foodborne pathogenic strains of the species, representing the Shiga toxin producing (STEC) and enterohemorrhagic *E. coli* (EHEC) pathotypes. The anti-O157 phages, despite infecting similar hosts, are genetically and phylogenetically diverse. Our aim was to find common functional regions in their genomes that could explain their similar host specificity. We compared whole genomes as well as genes putatively encoding tail fiber components and tail tips, collectively referred to as receptor binding proteins (RBP) of 39 lytic coliphages with proven anti-O157 activity published in earlier studies, four of which were of our own isolation characterized earlier. We also investigated the structure of the proteins in question, to identify common elements and motifs. We found that while on the whole protein sequence level, the RBPs follow the phylogenetical grouping of the phages, there were regions where RBPs of different families showed >30% similarity, which is above the 'twilight zone' of protein sequence similarity, which could account for the O157 serogroup specificity. Our results can provide useful insights for future applications and possibly targeted modification of phages for the elimination and detection of *E. coli* O157 strains.

IN VITRO INTERACTIONS BETWEEN *ERWINIA BILLINGIAE* AND THE ESCA PATHOGENIC FUNGUS *PHAEOMONIELLA CHLAMYDOSPORA*

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Esca belongs to grapevine trunk diseases (GTDs) the group of devastating vascular fungal infections of grapevine. The - mostly ascomycetous - causal agents infect the woody tissues of the host causing local necrosis in the xylem, with additional foliar symptoms caused by secreted phytotoxins. In their severe form, GTDs may lead to the sudden death of the host, causing severe losses to the grapevine and wine industry. The GTDs are regarded as complex syndromes resulting from the complicated interactions between the host, environmental factors, several pathogenic species, as well as non-pathogenic members of the grapevine microbiome. Results presented in this study suggest the possible positive impact of the bacterial species *Erwinia billingiae* on the development of Esca disease, through interaction with the Esca pioneer pathogen *Phaeomoniella chlamydospora*. One *E. billingiae* strain was isolated from an asymptomatic grapevine in the vineyard of Eszterházy Károly Catholic University and identified by sequencing the partial 16S rDNA gene. Confrontation tests with a *P. chlamydospora* isolate revealed the chemotaxis of the bacterial strain toward the fungus. The two microorganisms were able to form mixed colonies with a biofilm-like structure where both the fungal and bacterial cells were viable, indicated by acridine orange staining and fluorescent microscopic examinations. Moreover, the mixed cultures showed greatly elevated levels of cellulase and pectinase activities compared to colonies grown from single species (and the same inoculum density) suggested by experiments on solid media indicative of the enzymes. Besides the action of extracellular digestive enzymes, non-enzymatic reactions also play a role in the virulence of *P. chlamydospora* by decomposing grapevine phenolic compounds through a Fenton-like reaction. This ability of the single- and co-inoculated cultures of *E. billingiae* and *P. chlamydospora* was also tested by measuring the decomposition of crystal violet dye in the presence of culture filtrates. Co-inoculation resulted in a higher rate of decomposition of the phenolic dye compared to cultures inoculated with single species. Polysaccharide contents were also measured in the same culture filtrates showing a drastic increase in the mixed cultures. Results suggest that *E. billingiae* interacts with the Esca pathogen *P. chlamydospora* resulting in the increased production of several virulence factors like cellulases, pectinases, and polysaccharides as well as the increased rate of non-enzymatic degradation of phenolic compounds. The occasional establishment of this interaction in the host may partially explain the latent nature of Esca disease.

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IN SITU ANALYSES OF THE EFFECTS OF TREATED WASTEWATER ON BACTERIAL COMMUNITY COLONIZING LIMNETIC PLASTICS

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Various research studies are focusing on plastic-associated or 'plastispheric' bacterial communities. Useful microbes, like plastic degraders can be transported on plastic surfaces, as well as problematic ones like pathogens or antibiotic resistant strains, in aquatic environments. Most of the European rivers and streams are under the biological effect of wastewater treatment plant's (WWTP) effluents. These treated wastewaters transfer microbes, which could also colonize plastic surfaces. Later these microplastic particles can transport them downstream, in a longer distance. Seasonal changes can have effect on the development of the bacterial community of these biofilms, as well as the quality of the wastewater effluent and the natural freshwater. Thus, in our study in situ plastic colonizers – constructed by our research team - were used to follow the development of bacterial biofilms on different polymers and during three aspects (spring, summer, autumn) of 2023. Group of six colonizers contained different types of polymers submerged under the water for three months period close to Hungarian WWTPs. Upstream colonizers were put to the natural background, another group was directly in the wastewater effluent and downstream was also sampled. Plastispheric community developed in biofilm was determined by 16S amplicon sequencing. Detailed analytical measurement was also performed to follow the changes in water quality. Composition of plastispheric communities was compared to the analytical and weather data to find regulative environmental circumstances.

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STUDYING THE INTERACTION BETWEEN ORAL PATHOGENIC BACTERIA AND *CANDIDA* SPECIES IN AN INDIRECT MANNER: INTERKINGDOM COMMUNICATION AT THE LEVEL OF EXTRACELLLULAR VESICLES

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More than 700 microbes, such as bacteria, viruses, fungi, known as the oral microbiota, colonize the human oral cavity. Because of different environmental effects, such as smoking or infections, the microbial composition may change, which can result in dysbiosis that may lead to diseases, such as oral candidiasis. Oral candidiasis is most commonly caused by *Candida albicans*, which can alter the bacterial diversity and interact with other *Candida* species in the oral cavity. To examine the nature of such fungal - bacterial and fungal - fungal interactions at the level of extracellular vesicles, we used *C. albicans* SC5314, *C. parapsilosis* CLIB214 and *C. tropicalis* MYA3404 strains, along with *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* as pathogenic bacterial counterparts. We optimized the fungal and bacterial EV isolation protocol from liquid and solid media. The characterization of the EVs by transmission electron microscopy and NanoSight showed round shaped particles with diameters between 30 and 1,000nm. We examined the effects of EVs released by *C. parapsilosis* and the yeast and hyphae form of *C. albicans* on the growth and biofilm formation efficiency of *S. aureus*, *P. aeruginosa* and *E. faecalis* and vica versa. As a result, we found that EVs from *S. aureus*, *E. faecalis* and *P. aeruginosa* altered the growth and biofilm formation efficiency of *C. albicans* and *C. parapsilosis* in a species dependent manner. Using confocal microscopy, we showed that the bacterial EV treatment reduced the thickness of *C. albicans* biofilm. These results suggest the presence of an active interaction between fungal and bacterial cells at the level of EVs. We also investigated the effect of *C. albicans* derived EVs on the biofilm formation efficiency of *C. tropicalis* and *C. parapsilosis*. The EV treatment significantly reduced the biofilm formation efficiency of *C. parapsilosis*. From this, we can conclude that the *C. parapsilosis* biofilm is less stable as a result of EV treatment, and the adhesion stability decreases.

GLOMALIN-RELATED SOIL PROTEIN AS A POTENTIAL INDICATOR OF SOIL HEALTH

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The optimization of agricultural land use management contributes to both yield and soil security. Soil organic matter (SOM) stock fundamentally determines soil fertility, it affects aggregates, water storage, chemical properties, and protective functions of soil and it has an important role in the regulation of many atmospheric constituents. Glomalin produced by arbuscular mycorrhizal (AM) fungi forms a significant part of soil organic carbon. Glomalin has an essential role in building the soil structure and protecting soil carbon in aggregates. However, intensive fertilization and tillage harm AMF diversity and functions just like glomalin production. The effect of soil management on glomalin (Easily Extracted Glomalin-Related Soil Protein; EE-GRSP) has been examined at sites of three long-term field experiments: (1) NPK fertilization experiment with and without farmyard manure treatments; (2) conventional and organic farming fields (Martonvásár; Hungary) and (3) no-tillage, moldboard ploughing and deep cultivation (Józsefmajor; Hungary) treatments were tested. The soil disturbance had the most significant effect on soil EE-GRSP. EE-GRSP showed close correlations with the soil humus

and nitrogen content, the dissolved organic carbon, and the macroaggregate stability. It decreased by increasing pH. The EE-GRSP ranged from 0.20mg g^{-1} soil to 0.77mg g^{-1} soil in different long-term experiments. The highest GRSP contents were found in the no-tillage system and N-fertilized plots. The synthesis of our data could result in a land use effect assessment considering the quantity of soil glomalin. We concluded that GRSP has the potential to reveal qualitative changes in SOM connected with increasing reactive N forms. Glomalin is not just a C storage, but it is also a very important soil health indicator.

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TRANSPORT KINETICS OF ANTIVIRAL COMPOUNDS PASSING THROUGH A TRANSWELL BARRIER MODEL -- MATHEMATICAL ANALYSIS AND AUTOMATED SAMPLING

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In the preclinical phase of drug development, it is necessary to determine how the pharmacological compound can pass through the biological barriers surrounding the target tissue. In vitro barrier models provide a reliable, low-cost, high-throughput solution for screening substances at an early stage of the drug development process, thus reducing, and in some cases replacing, more complex and costly animal studies. The transport properties of an in vitro 3D barrier model were determined using a model antiviral drug. The drug was delivered into the apical chamber of the transwell device, and automated liquid sampling and subsequent spectroscopic analysis determined the concentration of the drug passing through the barrier layer. The measurement system replaces the media in the basolateral compartment every 30 minutes and stores the collected samples for further analysis. The total duration of a typical experiment is 6 hours during which period more than half of the compound loaded into the apical compartment passes through the barrier into the basolateral compartment, binds to the filter membrane, accumulates in the cells, or is metabolized.
Comparison of the time-dependent concentration profiles obtained from both the cellular barrier and membranes saturated with serum proteins reveals the extent the cell layer functions as a diffusion barrier to the antiviral compound. Due to the large number of collected samples, a detailed mathematical model of the transwell diffusive currents can be fitted to the measured concentration profiles. Based on the fitted parameters, one can determine the diffusivity of the drug in the cell layer (approximately one order of magnitude smaller than in the medium), the affinity of the drug binding to the cell membrane as well as the rate by which the cells metabolize the compound. This novel sampling and quantitative analysis approach goes beyond the standard permeability coefficient obtained from transwell inserts and thus offers more detailed pharmacokinetic characterization of the transwell barrier model.

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A CASE OF NECROTIZING FASCIITIS AND SEVERE SEPSIS DUE TO ST23 HYPERVIRULENT *KLEBSIELLA PNEUMONIAE*

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Monomicrobial *K. pneumonia* necrotizing fasciitis is a rare, but potentially fatal clinical entity mostly reported from East Asia, and commonly associated with K1 or K2 capsular serotypes harbouring numerous virulence factors such as yersiniabactin or colibactin. In this case, a cirrhotic patient was admitted to our clinical center with septic shock, icterus, leg swelling and pain in the gluteal region. Computed tomography examination revealed the possibility of necrotizing fasciitis in the gluteal area. The patient's condition deteriorated rapidly, becoming comatose; thus, he was transferred to the orthopedic surgery ward for surgical debridement, while meropenem-clindamycin combination-based therapy was initiated. As an early means for identifying the causative agent, BIOFIRE® Joint Infection Panel and Gram staining were performed from the wound exudate obtained from necrectomy, while culturing was also started. Surprisingly, Gram-stained smear showed high number of Gram-negative rods, while the multiplex PCR detected *K. pneumoniae* DNA in the sample, which was negative for common carbapenemases (KPC, VIM, IMP, NDM, OXA-48) and CTX-M beta-lactamases. Despite surgical intervention and apparently adequate therapy, the patient succumbed to the infection two days after admission. Aerobic and anaerobic cultures only yielded string-test positive *K. pneumoniae*, which was susceptible to all commonly tested antibiotics except for ampicillin-sulbactam and amoxicillin-clavulanic acid, confirming the results of Biofire. Next-Generation sequencing of the isolate revealed that it belongs to ST23 based on the MLST profile and harboured the virulence factors commonly associated with hypervirulent *K. pneumoniae*. Despite the adequate antimicrobial therapy and surgical intervention, *K. pneumoniae* necrotizing fasciitis has a poor prognosis with a high mortality rate (~60%). Clinicians should be aware of such cases in patients with typical predisposing factors.

BIOFIRE® Joint Infection Panel may be a valuable diagnostic tool in terms of identifying pathogenic microorganisms and the initialization of adequate therapy in clinical situations like the presented case. The emergence of carbapenem-resistant K1 capsular type hypervirulent *K. pneumoniae* ST23 in the Western hemisphere draws a worrisome picture with limited therapeutic options.

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THE ROLE OF THE SIT1 SIDEROPHORE TRANSPORTER IN *MUCOR LUSITANICUS*

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Mucoromycota fungi includes several opportunistic pathogen species (i.e. *Mucor lusitanicus*, *Lichtheimia corymbifera* and *Rhizopus oryzae*) that can cause a life-threatening systemic infection known as mucormycosis. The incidence of mucormycosis shows an upward trend in recent years. This infection poses a particular threat to immunocompromised individuals with untreated diabetes mellitus, immunosuppressive drug- or corticosteroid treatment recipients, patients undergoing deferoxamine treatment and people with elevated free iron concentrations in the serum. Iron is crucial for the host and the pathogen as it serves as a cofactor, particularly in oxidation-reduction processes. Microorganisms apply three strategies to obtain iron from their environment: acidification of the environment, reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), and secretion of siderophores (soluble iron-chelating molecules). The utilization of hydroxamate siderophores as xenosiderophores, including deferoxamine, can potentially contribute to the development of mucormycosis. Phylogenetic analysis of fungal genomic sequences showed that *Mucor* encodes six siderophore transporter proteins, among them one Arn3/Sit1 (Siderophore-iron (ferrioxamine) : H^+ symporter). The main goal of this study was to investigate the *sit1* siderophore transporter gene and its role in the iron uptake system, virulence and germination in *Mucor lusitanicus*. *sit1/arn3* siderophore transporter gene was characterized by generating knockout mutants using the CRISPR-Cas9 technique. The deletion of the *sit1* gene resulted significant changes in germination and growth ability under the experimental conditions. Furthermore, the mutant strain showed significantly reduced virulence in the *Galleria mellonella* infection model, suggesting that the *sit1* gene plays a crucial role in the pathogenicity of the fungus. Colony diameter was measured in different kinds of media, including YNB, Blood, and CAS agar with (CASFe) or without iron (CAS-Fe). On YNB, Blood and CAS-Fe agars. The growth ability of the MS12-Δ*sit1* mutant did not show significant changes compared to the control strain however, there was a significant difference on CASFe agar, indicating the importance of the *sit1* gene in iron-dependent growth conditions. The relative transcription levels of other components of the iron uptake system were examined using qRT-PCR analysis. The knockout of the *sit1* gene led to an increase in the transcriptional activity of three genes involved in iron uptake: an iron reductase gene (*fre*), a high-affinity iron permease gene (*fet*), and a copper-dependent Fe^{2+} oxidase gene (*fet3*). The absence of the *sit1* gene may trigger compensatory mechanisms in the expression of these genes to enhance iron acquisition.

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INVESTIGATION OF DIVERSITY AND HOST SPECTRUM OF CORONAVIRUSES

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Early recognition of coronavirus infections is of particular importance for both human and animal health care. Due to the genomic diversity, the overall detection of coronaviruses is challenging. Although metagenomic approach provides information on the genetic composition of microorganisms in the absence of prior sequence data, sample preparation and the proportion of the genetic material of variable organisms influence the analysis. Although polymerase chain reaction and sequencing are proven tools for diagnostics, designation of a universal detection system for coronaviruses is difficult due to the numerous virus variants. In this study, we aim to develop a universal diagnostic system specific for coronaviruses. The workflow would ensure the sensitivity and specificity of the assay by extensive processing of available sequence data and, if necessary, by using multiple oligonucleotides.
The amplified products are suitable for direct analysis on next generation sequencing platform. Samples from domestic, companion and wild animals (mammals and birds), as well as wastewater are processed for surveillance purposes. Based on the results we attempt to determine the whole genome sequence of novel coronaviruses. The pan-coronavirus detection system could get a picture of the coronaviruses occurring in the investigated animals and environment, thus about the potential host spectrum.

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EFFICIENCY OF COLD PLASMA TREATMENT AGAINST *SALMONELLA TYPHYMURIUM* AND *SALMONELLA ENTERICA*

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Efficiency of cold plasma against different types of bacteria, molds and viruses has been investigated for years. Currently, cold plasma generators are widely used in hospitals, industrial facilities, restaurants, aeroplanes, etc. to sterilize room air and different surfaces. The disinfection - applying this non-chemical treatment - is fast and effective without side effect. In our study, the bactericidal effect of cold plasma treatment was tested against *Salmonella typhymurium* and *Salmonella enterica* (10^6cfu ml^{-1}). The experimental setup investigated the surface decontamination efficiency of this treatment with relatively short time and large cubature mimicking the requirements of certain industrial processes. A cold plasma generator (airflow: 100m^3 h^{-1}, input voltage: 230V, ventilator: 18W) has been placed in a 40m^3 test-room. Bacteria suspensions (200µl) were dropped into sterile plastic plates (6 x 2cm) and located in five different positions in the experimental room. After 90 minutes treatment, suspensions were collected back, and the rate of destruction was determined. Based on our results, despite the high capacity of the cold plasma generator used sample positioning substantially effects the survival rate of bacteria in different samples. Considering investigated *Salmonella* strains, *S. typhimurium* proved to be more sensitive than *S. enterica*.

THE EFFECT OF *CANDIDA*-DERIVED EXTRACELLULAR VESICLES AND CANDIDALYSIN TO THE PROGRESSION OF ORAL SQUAMOUS CELL CARCINOMA

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Oral cancer is a serious health issue worldwide. In the European Union, especially in Central Europe, including Hungary has the highest incidence of oral cavity tumors. 90% of the mentioned tumor type is oral squamous cell carcinoma (OSCC). In the case of OSCC, oral candidiasis often develops due to the applied tumor therapy and the changed immunological microenvironment. A previous study of our laboratory has shown that, in case of OSCC, the number and diversity of colonizing yeasts in the oral cavity increases significantly, compared to healthy individuals. Furthermore, the presence of *Candida albicans*, which is the causative agent of oral candidiasis, contributes to tumor progression by increasing the production of oncometabolites by tumor cells, the activity of secreted matrix metalloproteinases (MMPs) and signaling pathways involved in tumor progression in vitro and in vivo. An important virulence factor of *C. albicans* is candidalysin, which is a pore-forming toxin encoded by the *ece1* gene. Because of the pore forming effect of this toxin, it can cause the damage of the epithelial cells thus, it can increase the activity various signaling pathways, which can role in the initiation and the progression of OSCC. The interaction between fungi and tumor cells can occur directly through different receptors as well as indirectly through the molecules and particles secreted by them. During our work, we aimed to investigate the effect of candidalysin and extracellular vesicles (EVs) produced by *Candida* cells to the progression of OSCC. During our experiments, we used ECE1 deletion mutant *C. albicans* strain that do not produce candidalysin and EVs isolated from *C. albicans* culture to treat HSC-2 human OSCC cells. After the treatment, we examined the activity and migration of MMPs secreted by tumor cells, as well as the gene expression changes that occur as a result of the treatment after the fungus and EV treatment. Our results showed that the presence of candidalysin is necessary to the *Candida* mediated tumor progression, because after the ECE1 deletion mutant strain treatment we could not detect any significant changes in the gene expression or migration of the tumor cells or in the activity of secreted MMPs. The *Candida* derived EV treatment caused changes in the migration of the cells and in the activity of the secreted MMPs too. Thus, we hypothesize that *Candida* derived EVs could contain candidalysin. Based on our results, we can conclude that EVs produced by *Candida* species and candidalysin also play an important role in tumor progression induced by the fungus.

MACHINE-LEARNING-GUIDED MULTI-OMICS INVESTIGATION OF INDUSTRIAL-SCALE BIOGAS PLANTS REVEALS INTER-KINGDOM INTERACTIONS AND STABILITY OF METHANOGENS

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Multi-omics analysis is a valuable technique for identifying and studying interactions between different kingdoms of organisms. One example is the investigation of bacterial and archaeal members within complex microbial communities involved in biogas production. In this study, we examined the microbiomes of three large-scale biogas digesters that were fed different substrates. To conduct this analysis, we employed a machine-learning guided genome-centric metagenomics framework, along with metatranscriptome data. These data allowed us to gain insight into the relationship between the predominant methanogenic communities and their symbiotic bacterial partners. In total, we identified 297 high quality, unique metagenome-assembled genomes (nrMAGs). Furthermore, when we analyzed the assembled profiles of the 16S rRNA gene from these nrMAGs, we observed that the Firmicutes phylum had the highest copy number, while representatives from the archaeal domain had the lowest. As we further investigated the three anaerobic microbial communities, we found distinct changes over time, but each community remained specific to its respective biogas plant. Interestingly, the relative abundance of different microorganisms, as indicated by the metagenome data, did not necessarily align with their corresponding metatranscriptome activity data. Notably, Archaea exhibited significantly higher activity than what would be expected based on their abundance alone. We also identified 51 nrMAGs that were present in all three biogas plant microbiomes, albeit with varying abundances. The core microbiome demonstrated a correlation with key fermentation parameters, but no single parameter emerged as the primary driver of community composition. Various mechanisms for interspecies hydrogen/electron transfer were attributed to the hydrogenotrophic methanogens in the biogas plants utilizing agricultural biomass and wastewater. Analysis of the metatranscriptome data revealed that the pathways associated with methanogenesis were the most active among all the major metabolic pathways examined.

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HACKING: A NOVEL POLYCISTRONIC SYSTEM FOR THE MULTIPLEXED, PRECALIBRATED EXPRESSION OF SECONDARY METABOLITE BIOSYNTHETIC PATHWAYS IN FUNGI

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We discovered a 9bp nucleotide sequence that enables the efficient translation of more than one protein from a polycistronic mRNA in yeasts and filamentous fungi. Coupling polycistronic expression to multiplexed, markerless, CRISPR/Cas9-based genome editing, we developed a strategy termed “HACKing” (Highly efficient and Accessible system by CracKing genes into the genome) for the assembly of multigene pathways in these organisms. HACKing allows the expression level of each enzyme to be pre-calibrated by linking their translation to those of host proteins with predetermined abundances under the desired fermentation conditions. We validated HACKing by rapidly constructing highly efficient *S. cerevisiae* cell factories that express 13 biosynthetic genes, and produce model endogenous ($1{,}090.41 \pm 80.92$mg L^{-1} squalene) or heterologous (1.04 ± 0.02mg L^{-1} mogrol) terpenoid products in shake flask fermentations. Thus, HACKing addresses the need of synthetic biology for predictability, simplicity, scalability, and speed upon fungal pathway engineering for valuable products, including natural product secondary metabolites.