ABSTRACTS

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HEAVY METAL(LOID) BIOMINERALIZATION THROUGH MICROBIAL ACTIVITYT

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Urban environmental pollution by various toxic compounds is a real concern around the world that endangers the well-being of the ecosystems. Soils act as a deposit for the non-degradable organic or inorganic contaminants that strongly influence the action of soil microorganisms. Most of the heavy metal(loid)s are toxic even in minor amounts for higher organisms damaging cell structure and deteriorating metabolic processes. Current physicochemical remediation technologies contain various disadvantages, besides being extremely expensive. However, the use of microorganisms in bioremediation is one of the cost-effective and environmentally friendly methods where soil microorganisms can detoxify contaminants by adsorption, precipitation, or by their transformation ability. In this study, bacterial heavy metal(loid)-bearing precipitation produced by the bacterial strain *Cupriavidus campinensis* S14E4C was examined and proved as promising for the removal of environmental contaminants. The detailed analysis showed that the strain is able to tolerate ca. 15,000 mg/L Cd, 3,000 mg/L Pb, 1,200 mg/L Hg and 600 mg/L As concentrations in vitro. It was observed that exposure to the heavy metal(loid)s changed the cell morphology and different sized intra and extracellular depositions were observed by TEM (transmission electron microscopy) and Raman spectroscopy analysis. The comprehensive analysis proved the biomineralization of cadmium sulfate by forming carbonate (otavite) through the microbial activity that helped the strain to survive in the metal stress condition. The possible resistance mechanisms were identified by genomic analysis of the strain that indicated a number of plasmid-mediated metal resistance genes and gene clusters (e.g. *czc*, *cad*) against cadmium species. It is obvious that the strain is capable to transform the toxic compounds which is one of the feasible, effective, and eco-friendly approaches for metal remediation.

MYCOTOXIN RESISTANCE AND ELIMINATION CAPABILITY OF *KLEBSIELLA PNEUMONIAE*

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Klebsiella species are facultative anaerobic, Gram-negative, non-mobile, rod-shaped bacteria capable of fixing nitrogen. *Klebsiella* species are opportunistic bacteria found in the environment, in the gastrointestinal tract of animals, contaminate meat and dairy products, and contribute to diseases. From a medical point of view, *Klebsiella pneumoniae* is the most common species that can cause serious hospital infections. Still, it is also found in carbohydrate-rich sewage, surface water, soil, plant products, fresh vegetables, sugar cane, and cereals [1, 2]. *Klebsiella pneumoniae* from fermented maize silage was isolated and identified by MALDI-TOF (Bruker) and 16S RNA sequence. As eliminating mycotoxins contaminate agricultural raw materials is considered an important task, mycotoxin resistance and elimination capability of the isolate was investigated. No changes in growth were detected in microtiter plate experiments applying mycotoxins at different concentrations [aflatoxin B1 (24-100 μg/L), deoxynivalenol (700-1,000 μg/L), and zearalenone (100-500 μg/L)]. The mycotoxin elimination capacity of *Klebsiella pneumoniae* was tested on living cells and cell preparations under 1-hour incubation by addition of aflatoxin B1, deoxynivalenol, and zearalenone toxins. The mycotoxin content of the supernatants was measured using HPLC. The mycotoxin elimination studies have shown that zearalenone was eliminated by the viable cells (100%), the purified cell wall (100%), the S-layer protein fraction (100%), and the cell debris (100%). In comparison, a lower decrease of the toxin was found with the purified peptidoglycan (16%) and the teichoic acid (13%) fractions of the bacterium. Elimination of aflatoxin B1 mycotoxin was observed with the viable cell (17%), the purified peptidoglycan (15%), the purified cell wall (18%), the teichoic acid (20%), and cell debris (27%) fractions at a low rate. In the case of deoxynivalenol, no mycotoxin elimination was observed. Application of the cell wall fractions in mycotoxin elimination should be studied further on.

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[1] Gundogan (2014) Klebsiella. Encyclopedia of Food Microbiology 2:283-388, Elsevier.
[2] Van Dommelen and Vanderleyden (2007) In Bothe et al (eds) Biology of the Nitrogen Cycle 12:179-192, Elsevier

FUNGAL ROOT ENDOPHYTES FROM NORTHERN KAZAKHSTAN – NOVEL LINEAGES AND DOMINANT CORE MEMBERS

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Endophytes are microorganisms that colonize plants without any visible symptoms of disease infection and might produce a plethora of secondary metabolites to prevent plants from various phytopathogens. Root colonizing fungal endophytes, e.g. the so called dark septate endophytes (DSE) are world-wide common in both agronomical areas and natural habitats and can enhance the growth of host plants and provide resistance to plant diseases, drought stress and enhance plant performance. Hence, they may play important role in ecosystem functioning and can have major importance in agricultural applications. Our knowledge on DSE communities is limited in Northern Kazakhstan, one of the main agricultural areas for production of the gramineous plant species in the country. In this study, we aimed to gain information on the DSE community of different plant species agricultural and non-agricultural areas and identify and describe the potential novel lineages in the steppe zone of Northern Kazakhstan. Roots were collected from mainly *gramineous* species such as barley, wheat and *Stipa capillata*. The sampling was carried out in 2018-2019. After isolation fungi on PDA the molecular identification was carried out by the amplification and sequencing of the internal transcribed spacer (ITS) region of the nrDNA. In case of *Fusarium* isolates and novel lineages further loci were also sequenced and used for multilocus phylogenetic analyses. Altogether, 556 isolates were collected from roots of different agricultural and non-agricultural plants in Northern Kazakhstan. The isolates represented various fungal genera in mainly Pezizomycotina. Several lineages were found to represent potentially novel taxa such as *Laburnicola* and *Murispora* species. The most common and widespread genus was *Fusarium* represented by both known species and novel lineages. Here we present the overall results of the study of these root endophytes.

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ECOPHYSIOLOGICAL CHARACTERIZATION OF *BACILLUS* STRAINS ISOLATED FROM RECYCLED SPENT MUSHROOM COMPOST

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In addition to the production of high quality spawn and compost, the basis of efficient and economic white button mushroom (*Agaricus bisporus*) production is to ensure high quality casing material. In mushroom farms, the compost colonized by the mycelia of the white button mushroom is covered by the casing material, the role of which is primarily to ensure the formation of fruiting bodies and to provide a high water retention capacity. A good quality casing layer is influenced by the composition of the containing microorganisms. Microorganisms present in the spent *Agaricus* compost include bacteria (e.g., *Bacillus, Alcaligenes, Pseudomonas, Microbacterium* strains) and fungi (e.g., *Fusarium, Trichoderma, Lecanicillum, Cladobotryum, Mortierella, Rhodotorula* species) which can be either beneficial or pathogenic. Among the beneficial bacteria are members of the genus *Bacillus* and *Pseudomonas*. Our aim is to develop healthy casing layer alternatives from spent mushroom compost. Microorganisms are isolated from spent mushroom compost samples taken during the natural recomposting process, subjected to species-level molecular identification and deposited in the Szeged Microbiology Collection. A total of 20 *Bacillus* strains were isolated from recycled spent mushroom compost samples and identified. The resulting *B. licheniformis* (9) and *B. velezensis* (3) strains were tested for their ecophysiological properties (temperature-, pH- and water activity-dependence), extracellular enzyme activities and siderophore production. The examined strains showed good growth abilities at 30 and 37°C, as well as at pH 6 and 8, suggesting that they could be able to colonize the recycled mushroom compost, which has a pH around 6.5. Most of *Bacillus* strains showed the best growth at water activity values of 0.991 and 0.980, although *B. licheniformis* (5) showed growth at water activity values of 0.968. The enzyme activities of the strains were measured for lipase, protease, cellulase and chitinase. Three of the examined *B. velezensis* strains showed the best lipase where the substrate was 4-nitrophenyl-palmitate, chymotrypsin-like protease and β-glucosidase activities, while 4 *B. licheniformis* strains proved to be the best N-acetyl-glucosaminidase producers. Two strains of *B. velezensis* also showed siderophore production, suggesting that they may have good competitive abilities for

iron. By studying the properties of the isolated microorganisms that are of high importance for fungal compost colonization, microbial strains that support the recomposting process by converting spent compost into a casing material will be selected. The most promising strains will be tested in industrial recomposting experiments.

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MEMBERS OF THE *TRICHODERMA HARZIANUM* SPECIES COMPLEX AS THE CAUSAL AGENTS OF MUSHROOM GREEN MOULD

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The conditions of mushroom cultivation are highly favuored by a wide range of *Trichoderma* species, however, severe green mould infections are usually attributed to *T. aggressivum*, as well as *T. pleuroti* and *T. pleuroticola* in the case of button mushroom (*Agaricus bisporus*) and oyster mushroom (*Pleurotus ostreatus*), respectively. In the present study, the majority of *Trichoderma* strains originating from the growing facilities of *A. bisporus*, *P. ostreatus* and *Lentinula edodes* (shiitake) from various countries were classified to the *Trichoderma harzianum* species complex (THSC) based on their ITS (internal transcribed spacer) sequences. However, the subsequent multilocus sequence typing (MLST) analysis with the involvement of ITS, *tef1* (translation elongation factor 1-alpha), *cal1* (calmodulin) and *rpb2* (RNA polymerase B subunit II) sequences revealed the presence of numerous species. Exclusively *T. harzianum* sensu stricto was detected in the samples of *A. bisporus*, while various species (*T. atrobrunneum, T. guizhouense, T. simmonsii*) were found to be associated with *P. ostreatus* and *Lentinula edodes*. A clear preference for *P. ostreatus* was observed in the case of *T. afroharzianum*, whereas *T. pollinicola* was recovered only from a *L. edodes* farm. The aggressiveness of the isolates towards their host mushrooms was tested in dual plate assays. The examined *T. harzianum* strains could completely overgrow the colony of *A. bisporus*, while shiitake was devastated by both *T. simmonsii* and *T. pollinicola*. Oyster mushroom was found to be the most susceptible to *T. simmonsii*, but *T. guizhouense* and *T. harzianum* also caused considerable growth inhibition, while some resistance to *T. afroharzianum* could be observed. The effect of the commercial fungicides prochloraz and metrafenone on the mycelial growth of the green mould agents was examined using the macrodiution method. The strains showed tolerance to metrafenone, with minimal inhibitory concentration (MIC) values over 16 mg/L. Prochloraz could prevent the growth of the majority of the examined *Trichoderma* strains (MIC 1-16 mg/L), however, the *T. simmonsii* isolates showed considerable growth even in the presence of the fungicide at the highest tested concentration (16 mg/L). Our findings suggest that in addition to the notorious causal agents of mushroom green mould disease, certain members of the THSC, especially *T. harzianum* and *T. simmonsii* also have the potential to cause green mould disease in the cultivation of different edible mushrooms.

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IN VITRO STUDY OF THE NEOFUNCTIONALIZATION OF THE NICOTINATE HYDROXYLASE HXNS, A PARALOGUE OF THE XANTHINE DEHYDROGENASE HXA

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Duplication of the antecedent of purine hydroxylase I coding gene (*hxA*) during evolution gave rise to the origin and evolution of purine hydroxylase II (encoded by *hxnS*). Although the two enzymes (HxA and HxnS) share common enzyme activity, other enzyme activities diverged and the two enzymes got fallen under the control of distinct pathway-specific transcription factors. HxA is a catabolic enzyme of the purine degradation pathway operating under the regulation of UaY transcription factor and the pathway metabolite uric acid. HxA conducts two consecutive hydroxylation steps in the pathway and converts hypoxanthine (Hx) to xanthine (X) and X to uric acid. HxnS belongs to the nicotinic acid (NA) catabolic pathway and operates under the regulation of HxnR transcription factor and a NA derived catabolite compound. HxnS preserved the Hx hydroxylation ability of the ancestor enzyme, lost the ability to convert Hx to X and gained the ability to accept NA as substrate and to hydroxylate this compound. A systematic in silico comparison of HxA and HxnS orthologues across Pezizomycotina revealed eight conserved HxnS specific amino acid residues, which might be responsible for the HxnS-specific functions. To verify the evolutionary role of these eight amino acids, 26 different single or double point mutation-carrying HxA and HxnS expressing constructions were developed, in which the mutated nucleotides result in an amino acid residue change that corresponds to the cognate residue of the paralogue protein. These mutated genes

were expressed in appropriate *hxAΔ hxnSΔ* and *hxAΔ xanAΔ* recipient strains and the NA, Hx and X utilization ability of the transformants was studied. Here we present the development of the constructions, the N-source utilization ability of the mutant enzyme expressing transformants and the modified HxA and HxnS enzyme activities on polyacrylamide gel.

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INVESTIGATION OF THE ANTIMICROBIAL EFFECT OF ECDYSTEROID COMPOUNDS

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Pathogenic microorganisms can cause different diseases in humans, animals, or even plants. Nowadays, the control of pathogenic bacteria and fungi is based on the use of various antibacterial and antifungal agents of natural, synthetic, and semi-synthetic origin. However, the resistance between bacteria and fungi frequently develops against one or more drugs used in clinical or agricultural practice. As a result, there is continuous pressure to find new inhibitors against bacteria and fungi. Ecdysteroids are natural compounds, which are known primarily as analogs of ecdysone, the hormone that affects the development and metamorphosis of insects. These compounds are also produced by several plants in which they are suggested to play a role in pest control. Ecdysteroids are non-toxic, bioactive compounds in mammals, but so far, very few studies have been performed on their antimicrobial activity. In this study, a total of 13 ecdysteroid derivatives were examined: 20-hydroxyecdysone (20E), 20E 2,3;20:22-diacetonide (20E-DA), 20E 20,22-acetonide (20E-MA), 20E (6E)-oxime (20E-EOx), 20E (6Z)-oxime (20E-ZOx), a 20E lactam analog (20EL), 20E 2-acetate (20E-2-AC), 20E 3-acetate (20E-3-AC), 20E 22-acetate (20E-22-AC), poststerone (POST), poststerone 20-oxime (POST-OX), ajugasterone C (AJC), and calonysterone (F6). Among these, the 20E-EOx, 20E-ZOx, 20El, and POST-OX are nitrogen-containing semi-synthetic 20-hydroxyecdysone (20E) derivatives, while all the others are naturally occurring compounds that have been previously isolated from plant sources. The antimicrobial effect of these compounds has been investigated on various Gram-positive and Gram-negative bacteria and some yeasts (*Candida albicans, Candida tropicalis, Candida krusei, Candida guilliermondii, Candida glabrata, Candida metapsilosis, Candida orthopsilosis, Candida parapsilosis, Cryptococcus neoformans, Lodderomyces elongisporus*) with agar diffusion and microdilution tests. Though earlier it has been reported in the literature for some of these compounds (ono-, tri-, and tetra-acetate derivatives of 20-hydroxyecdysone), our tests did not reveal any antibacterial activity for the investigated ecdysteroids. At the same time, mild antifungal activity of 20E, 20El, 20E-EOx, and 20E-ZOx were detected against *Cryptococcus neoformans*. From these compounds, 20E and 20El had fungistatic, while 20E-EOx and 20E-ZOx had a fungicide effect against this yeast. Further studies are in progress involving additional newly synthesized ecdysteroid analogs.

SCREENING OF THE ANTIBACTERIAL ACTIVITY OF ORGANIC SOLVENT EXTRACTS OF SELECTED GREEN MICROALGAE STRAINS

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We performed the biological screening of organic solvent extracts of single-cell freshwater- and filamentous- green microalgal cultures. Our microalgae strains collection provided the studied strains. 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*., Chlorococcum* sp*., Haematococcus* sp. and macroalgae, e.g. *Ulva rigida, Laminaria japonica, Caulerpa racemosa* with proven antibacterial effects presumably associated with polyphenols, alkaloids, terpenes, polysaccharides, fatty acids, sterols, lactones, proteins and peptides. Debro and colleagues were among the first to study the antibacterial effects of many freshwater algae against *S. aureus, B. subtilis,* and *E. coli*. As shown by the zone of inhibition tests, *Chlorella vulgaris, Desmococcus olivaceous* and *Chlorococcum humicola* extracts were effective against *S. aureu*s. 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 and *P. aeruginosa* NCAIM B.01057. Axenic strains were produced by purification, and the biomass required for the extracts was produced using the optimal culture medium specific to the strain. Four different organic solvents - acetone, ethanol, diethyl-ether and hexane – were used to create the extracts from the lyophilised biomass. The antibacterial effect of the extracts was determined using the agar gel diffusion method. As a positive control, we used bactericide antibiotics to compare the results semi-quantitatively. The *E. coli* strain was the least susceptible to treatments, and *S. aureus* was the

most sensitive. Among the solvents used, diethyl-ether was most suitable for the extraction of bioactive molecules of microalgae strains. In addition, we have observed that the inhibition zones of extracts were comparable in size to the inhibition zones of antibiotics, which suggests strong bacteriostatic or bactericidal effects against test strains *P. aeruginosa* and *S. aureus*. In summary, we have confirmed the antibacterial effects of four single-cell freshwater microalgae strains and one filamentous green microalgae strain against facultative pathogenic bacteria.

ISOLATION AND PARTIAL CHARACTERIZATION OF NOVEL BACTERIOPHAGES AGAINST *PAENIBACILLUS LARVAE* SUBSP. *LARVAE*

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Paenibacillus larvae subsp. *larvae*, a gram-positive bacterium, is the causative agent of the American Foulbrood (AFB) disease of honeybees (*Apis mellifera*), causing significant economic losses worldwide. Because there is no effective, registered product are available against AFB, alternative solutions should have been developed. One promising tool to protect honeybees from AFB could be the application of bacteriophages. Seventeen novel bacteriophages were isolated taken from soil under infected hives. Isolated bacteriophages were subject to partial characterization. Plaque morphology showed clear plaques referring to a possible strict lytic lifecycle. This was also confirmed by genome sequencing, where full genomes of the novel bacteriophages were determined. We present also host range analysis data tested on nine *P. larvae* subsp. *larvae* strains, and the results of laboratory efficacy trials. All tested bacteriophages were effective against the tested host bacterium, which efficacy could be (partially) strengthened when bacteriophages were used in a cocktail. Besides partial characterization, a comparative genomics analysis was performed. After completion of the characterization of the bacteriophages, we will treat hives with the formulated bacteriophage cocktail determining its efficacy in the protection of honeybees against AFB. Our results can contribute to providing an alternative solution against this devastating disease.

XYLENE DEGRADATION BY *PSEUDOMONAS* SP. MAP12 AND *SPHINGOBIUM* SP. AS12 ISOLATED FROM MICROAEROBIC AND AEROBIC XYLENE DEGRADING ENRICHMENTS OF A DECADE OLD HYDROCARBON CONTAMINATED GROUNDWATER

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Xylene is one of the most common organic volatile toxic components among BTEX compounds. Because of their relatively high-water solubility, these compounds are often considered as a threat to the primary drinking water reserves. Hence, it is always important to study the diversity of indigenous xylene degrading community to develop effective strategies for xylene degradation. In hypoxic BTEX-contaminated subsurface environment, it has been observed that some indigenous bacterial community adapted to hypoxic BTEX-degradation. Although a broad diversity of microorganisms can degrade mono-aromatic hydrocarbons in the absence of oxygen, benzene along with p-, and o-xylene are among the least degradable BTEX-compounds under anaerobic conditions. To gain in depth knowledge and to find potential strains that harbor *C23O* genes which have a key role in the hypoxic degradation of xylene isomers, an enrichment was setup using hydrocarbon contaminated groundwater sample from a decade old petroleum contaminated site with xylene as a source of carbon and energy under both aerobic and microaerobic conditions. Study of microbial community of both aerobic and microaerobic enrichments showed that, though in both aerobic and microaerobic enrichments *Pseudomonas* was the most dominant member in the community but distinct differences were also visible. In case of aerobic enrichment *Shingobium* was the main difference creating group whereas, a competition between *Azovibrio* and *Rhodoferax* was noticed in microaerobic enrichments. Using conventional techniques members of genus *Sphingobium* from aerobic and member of *Pseudomonas* from microaerobic enrichments were isolated. Among these isolated strains, a novel *Sphingobium* sp. strain AS12 was found that shares 99.3% similarity with its closest relative *Sphingobium terrigena*. Strain AS12 interestingly, capable of degrading all three isomeric forms (meta-, para-, ortho-xylene) of xylene along with ethylbenzene and toluene aerobically unlike its closest relatives. Likewise, a presumably novel strain of *Pseudomonas* sp. strain MAP12 was also isolated that shares 98.4% similarity with its closest relative *Pseudomonas linyingensis* and able to degrade meta -, para-xylene along with benzene and toluene both aerobically and microaerobically. Moreover, strain MAP 12 harbors

five different types of *C23O* genes indicating its efficient role in hypoxic hydrocarbon degradation in contaminated ecosystem. This study is, by far, the first direct evidence of biodegradation of all xylene isomers by a *Sphingobium* strain and microaerobic degradation of benzene and xylene by a *Pseudomonas* strain.

ROLE OF THE ERGOSTEROL BIOSYNTHESIS GENES IN THE AZOLE RESISTANCE OF *MUCOR CIRCINELLOIDES*

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Mucoromycota fungi include several opportunistic human pathogenic fungal species (e.g. *Mucor circinelloides* and *Rhizopus oryzae*), which can cause fatal systemic infections in immunocompromised patients, called as mucormycosis. There are some risk factors associated with fungal infections, such as diabetic ketoacidosis and immunosuppressive drug- or corticosteroids treatments. Prognosis and treatment suggestions differ for each species, which makes fast and reliable diagnosis essential. Mucoromycota species are resistant to the majority of the routinely used antifungal drugs, such as most azoles. The main target of azoles is the lanosterol demethylase (Cyp51), which play an important role in the ergosterol biosynthesis of fungi. Ergosterol is an important component of the fungal cell membrane. In addition to the Cyp51, other enzymes of the ergosterol biosynthesis pathway may also participate in the azole resistance. The main goal of this study was to investigate the ergosterol biosynthesis pathway and its role in the background of azole resistance of *M. circinelloides*. Transcription of 10 genes (i.e., *erg2, erg3, erg6a, erg6b, erg6c, erg7, erg24a, erg24b, erg25a* and *erg25b*) after posaconazole, amphotericin B (AmpB) and terbinafine treatments was analyzed using quantitative real-time PCR. The relative transcription level of the genes (except of *erg24b*, *erg6b* and *erg6c*) significantly increased after posaconazole treatment, while those of erg6b and erg6c significantly increased after AmpB treatment. Genome of *M. circinelloides* contains three *erg6* gene, which encodes sterol C-24 methyltransferase. Erg6 catalyzes the conversion of zymosterol to fecosterol and it plays a role in the alternative ergosterol biosynthesis pathway in yeast. In *Cryptococcus neoformans*, Erg6 plays role in growth at high temperature and virulence. We have started to create erg6 single and double knockout mutants using a CRISPR-Cas9 system. Growth ability, ergosterol content and sensitivity to azoles of the mutants were examined. The lack of *erg6a* resulted decreased ergosterol content and growth ability and increased sensitivity to azoles.

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MICROAEROBIC ENRICHMENT OF BENZENE DEGRADING BACTERIA AND DESCRIPTION OF *IDEONELLA BENZENIVORANS* SP. NOV.

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The increasing level of petroleum hydrocarbon pollution significantly damage the ecosystem or even the human health by its toxic and carcinogen effects. Simple aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (BTEX) are the most common contaminants of the groundwater and thus the drinking water. Aromatic hydrocarbons decompose most rapidly and completely under aerobic conditions and the bioremediation has proven to be the most success in solving a problem of a widespread contamination. In bioremediation procedures, bacteria are used to eliminate petroleum hydrocarbons as a source of carbon and energy for their metabolic processes. Due to the presence of aerobic microorganisms the concentration of dissolved oxygen in the soil decreases rapidly because the BTEX degraders use mono- and dioxygenases for the hydroxylation and the cleavage of the aromatic ring and these enzymes require molecular oxygen as a co-substrate. The availability of dissolved oxygen has a key role in the biodegradation because benzene, para- and orto-xylene are persistent under anaerobic conditions. The subfamily I.2.C-type of extradiol dioxygenases adapted to low oxygen concentrations resulting a large diversity of catechol 2,3-dioxygenase (*C23O*) gene in these environments. Several members of the Betaproteobacteria within the family Comamonadaceae are known to play an important role in the biodegradation of petroleum hydrocarbons as dominant community members in contaminated subsurface. Furthermore, they showed a possession of diverse *C23O* genes in such environments. Hence, exploration of such bacteria and their functional genes that can participate in biodegradation in hypoxic environment has a current importance, because very few known cultured bacteria are available yet for the purpose. To reveal those genes and bacteria, which have a key role in the hypoxic benzene

biodegradation, parallel microaerobic enrichment cultures, degrading solely benzene, were set up by using groundwater sample of the "Siklós" BTEX-contaminated area. Results of the 16S rDNA amplicon sequencing have shown that the microaerobic benzene enrichment was dominated by the *Rhodoferax* followed by *Acidovorax* and *Pseudomonas* consecutively. In addition, a member of the genus *Ideonella*, belonging to the family Comamonadaceae was detected in all of the microaerobic benzene enrichments, was cultivated successfully. The strain designated as B7T represents a novel species of genus *Ideonella* on the basis of the results obtained from polyphasic characterization for which the name *Ideonella benzenivorans* sp. nov. is proposed. It is able to utilize benzene, toluene and ethylbenzene as sole source of carbon and energy.

POSSIBILITY TO NFAP2 *NEOSARTORYA* (*ASPERGILLUS*) *FISCHERI* ANTIFUNGAL PROTEIN 2 RESISTANCE DEVELOPMENT IN *CANDIDA ALBICANS*

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Recently, the clinical therapy of life-threatening fungal infections caused by different *Candida* species has become more challenging as a consequence of the emerging number of drug-resistant isolates. This fact urges the need for the development and application of fundamentally new and safe antifungal strategies in the clinical treatment. The features of NFAP2, a small molecular weight, cysteine-rich, cationic antifungal protein secreted by *Neosartorya* (*Aspergillus*) *fischeri* NRRL 181 render it to be a promising candidate. NFAP2 has an overall β-barrel topology that is stabilized by intramolecular disulphide bonds which provide a high stability against harsh environmental conditions and proteolysis. NFAP2 effectively inhibits the growth of several antifungal drug-resistant human pathogenic yeasts, and shows no toxic effect on mammalian cells both in vitro and in vivo. One of the requirements in the reliable medical application of NFAP2 as novel antifungal drug is an understanding the potential of fungi to develop resistance mechanisms against it. In the present study we investigate the potential of *Candida albicans* CBS 5982 to develop tolerance or resistance mechanism to NFAP2 in comparison with fluconazole (FLK), a licenced and widely used antifungal drugs to treat *Candida* infections. We addressed this question by a continuous subculturing micro-evolution experiment, which was performed in a low ionic strength medium (LCM) developed for functional investigation of NFAP2. The starting concentration of NFAP2 and FLK was 0.5×MIC (Minimum Inhibitory Concentration), and in each subculturing step the dose of NFAP2 or FLK was doubled to reach 32×MIC, finally. This strategy resulted in mixture of differently adapted cells. To select the cells with stable and evolved resistance/tolerance mechanism, the adapted fungal cultures were subcultured ten times in NFAP2- or FLK-free medium, then the cells will be plated on selective agar plate (final MIC of NFAP2 or FLK in the adaptation step). The susceptibility of *C. albicans* strains to different antifungals was tested in a broth microdilution assay in LCM and RPMI-1640 according to the recommendation of the Clinical and Laboratory Standards Institute document M27-A3. The susceptibility of ancestor and the resistance evolved strains was tested against NFAP2 and different, licensed antifungal drugs to determine whether the observed resistance/tolerance is broad spectrum or specific to NFAP2 or FLK. Summarizing our results, we concluded that *C. albicans* has a limited potential to develop resistance mechanism to NFAP2 as it was able to adapt only the 2×MIC of this antifungal protein; meanwhile it was able to growth in 32×MIC of FLK. Interestingly, adaptation to 2×MIC of NFAP2 resulted in increased susceptibility to FLK; meanwhile the FLK-resistance developed strains showed decreased susceptibility to NFAP2. NFAP2- and FLK-resistance development did not change the susceptibility of *C. albicans* to ketoconazole, itraconazole, terbinafine, amphotericin B and micafungin.

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PRELIMINARY RESULTS OF SOIL MICROBIOME ANALYSIS ON FOREST STANDS IN CENTRAL-HUNGARY

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In this study, we carried out metagenome and biochemical investigation of soil samples derived from different types of forest to reveal the microbial composition and its' relation to metabolizable organic carbon content and different soil parameters of forest topsoils. Soil samples were collected from 10-year-old black locust, newly reforested (2 years old) and middle-aged (80-year-old) oak forest stands in 2018. The soil samples were collected from two depths [0-10 cm

(horizon A) and 10-40 cm (horizon B)] in three repetitions and 5 times between April and October. 16S rRNA gene amplicon sequencing technique was used for the determination of the microbial composition of samples and MicroRespTM technique to reveal the catabolic capacity of the microbiome. Different soil physical and soil chemical parameters were also analysed. Based on the analysis of 16S rRNA sequences, the following bacterial taxa were present with the highest abundance in the soil samples: Acidobacteria (22,8%), Actinobacteria (18,6%), Proteobacteria (18,5%) and Verrucomicrobia (10,9%). Significant differences were revealed between stand-horizon combinations regarding more bacterial taxa, including the highly abundant phyla Proteobacteria and Verrucomicrobia as well. SIMPER test was used for revealing the contribution of taxa to the total variance. This test exposed that the most abundant phyla contribute to the total variance most remarkably (60,41%); next to these taxa the phyla Bacteroidetes (9,51%), Gemmatimonadetes (6,33%), Chloroflexi (4,29%), Nitrospirae (3,25%) represented a relatively high contribution to the total variance. Multiple regression analysis was used to evaluate the relation between the abundance of each bacterial taxa, metabolizable organic carbon sources and soil parameters. Based on this evaluation significant differences in regression were exposed in the case of the following parameters: pH_{H_2O} (Acidobacteria, -1,46; Patescibacteria, 1,3), amino acid content (Verrucomicrobia, -1,72), TOC content (Verrucomicrobia, 2,38), NO_3-N content (Verrucomicrobia, -1,18), TIC content (Firmicutes, 2,09) and Al-Mg content (Firmicutes, -2,45). Furthermore, a nonmetric multidimensional scaling analysis on microbiome composition of the three forest stands' soil samples revealed a higher distance between the two oak stands compared to the dual of middle-aged oak and the black locust stands. Based on this result it is presumable that the rate of canopy closure has a more significant effect on the soil bacterial community composition indirectly than the dominant tree species.

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NATURAL PRODUCTS OF THE ROOT ENDOPHYTIC FUNGUS *DARKSIDEA ALPHA*

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Endophytic fungi, colonizing plants internally and asymptomatically, are considered valuable but unexplored sources of diverse natural products, because of their interactions with plants and other microorganisms via secondary metabolites. During investigations on root endophytic fungal communities of semiarid sandy grasslands in Hungary, we frequently isolated the species *Darksidea alpha* (Pleosporales), a dark septate endophyte, characterized by melanized septate hyphae. Since then, we have been able to isolate this fungus from roots collected in Kazakhstan as well. As the metabolite composition of this common and widespread species has not been determined yet, we aimed to identify natural products of *D. alpha*. Metabolite composition of the cultures was analyzed using high-performance liquid chromatography hyphenated with diode array and high-resolution mass spectrometry detection (HPLC-DAD-HRMS). Chemical structures of the detected compounds were elucidated by HRMS and nuclear magnetic resonance (NMR) spectroscopy. As a result, we identified natural products of *D. alpha* for the first time. These compounds belong to diverse structural groups, such as quinones and terpenes. *D. alpha* proved to be the first natural source of an eremophilane sesquiterpene, only derivatives of which have been found in nature. Furthermore, this fungus proved to be an abundant source of certain compounds also known as plant secondary metabolites, confirming its practical utility in the high-yield production of these compounds. The identification of further natural products of *D. alpha* is in progress, the results will also be presented, similarly to the statistical analyses of the metabolite profiles of the isolates studied.

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PHYLOGENOMIC ANALYSIS ON THE GLOBAL DIVERSITY OF THE MYCOTOXINOGENIC *ASPERGILLUS FLAVUS*

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Aspergillus flavus is a globally important mycotoxigenic fungus, a preliminary post-harvest pest, as well as an opportunistic pathogen of plants and occasionally humans, causing invasive aspergillosis. It can infect different agricultural crops, such as cottonseed, peanut, rice, corn, soybean, grains, and other fodder crops. Mycotoxins, like the ones produced by

this mold, are secondary metabolites produced by fungi that are generally cytotoxic. They are highly diverse in terms of their chemical structure, so the symptoms they cause can be varied. It is well known that Aflatoxin B1 is produced by *A. flavus* and *A. parasitcus*, which is one of the most potent, biologically carcinogenic compounds. Tropical and subtropical environments mainly favor the formation of aflatoxins, however, due to climate change, their appearance is an already recorded problem in Hungary, especially on cereals, which are essential foods. Interestingly, non-aflatoxigenic strains of this fungus are applied as biocontrol agents against toxigenic ones. In this study, we sequenced the genomes of four novel *A. flavus* isolates collected in Hungary using Illumina short read sequencing. Among these we analyzed one toxigenic, one atoxigenic, and two isolates that were found to be non-aflatoxigenic speradine producers. To determine the phylogenomic position of the Hungarian isolates, we performed phylogenomic analysis of altogether 178 isolates of *A. flavus* collected worldwide and sequenced by previous studies. Furthermore, we re-sequenced the type strain of the species with high coverage Illumina and mapped it onto the chromosome-level reference genome of the species, uncovering merely a few high-confidence SNP positions that may indicate subclone-level differences in the type strain cultures, or assembly errors. After mapping the 178 isolates to the reference, we built SNP matrices and conducted maximum likelihood phylogenomics, as well as produced phylogenomic networks in SplitsTree for the nuclear and mitochondrial genomes separately, and for the individual chromosomes separately as well. Comparative genomics methods were applied to study differences among clades identified in the species. We determined the mitochondrial genome copy numbers relative to the nuclear genome and identified putative gene copy number variations across the chromosomes using coverage mapping.

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ACQUIRED TRIAZOLE RESISTANCE INFLUENCES VIRULENCE PROPERTIES OF *C. AURIS* MICROEVOLVED STRAINS IN MOUSE SYSTEMIC INFECTION MODEL

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Recently, *C. auris* become one of the most recognized human pathogenic species in the *Candida* genus. Based on prominent phylogenetic differences among *C. auris* isolates, five clades were differentiated, corresponding to geographical sites of frequent isolation. These clades usually associated with distinct antifungal susceptibility patterns and clinical manifestations. Since its first occurrence, several *C. auris* outbreaks have been reported worldwide, mainly in hospital setting. Nosocomial outbreaks were often caused by isolates displaying decreased susceptibility towards fluconazole, the first-line agent for prophylaxis and therapy. Besides fluconazole, clinical isolates of *C. auris* regularly acquire resistance to other azole type drugs, as well as amphotericin-B and occasionally echinocandins. The physiological effects of acquired antifungal resistance on stress tolerance and virulence of *C. auris* was investigated using fluconazole, posaconazole and voriconazole resistant mutant strains generated by in vitro microevolution method. Alterations in antifungal susceptibility and cross resistance were determined by microdilution method, utilizing azoles (fluconazole, voriconazole, posaconazole), echinocandins (caspofungin, micafungin, anidulafungin) and a polyene (amphotericin B). Abiotic stress tolerance of the evolved azole resistant strains was examined by spotting assay, using osmotic stressors, cell wall perturbing agents and a membrane detergent. Collected data implies that acquisition of triazole resistance significantly effects the fitness of the fungal cells in host modelling conditions. The potential relationship between virulence and development of antifungal resistance was also studied both in vitro and in vivo setting. Phagocytosis of the generated strains by J774.2 mouse macrophage-like cells was measured and analyzed by flow cytometry. In intravenous murine infection model, fungal burden of the triazole evolved strains was determined in spleen, kidney, liver and brain and compared to the fungal burden associated with the initial azole susceptible clinical isolate. Significant differences in virulence of the original and the generated strains was observed suggesting a potential connection between the virulence and antifungal susceptibility of the emerging fungal pathogen, *C. auris*. To determine the genomic changes responsible for the antifungal resistance, generated strains were sequenced and compared to the susceptible isolate. In line with recent literature data, point mutations in TAC1b transcription factor were found in two resistant strains. According to the genome analysis all 0387 originated evolved strain harbored the same loss of function (LOF) mutation in the B9J08_002818 gene. In *C. albicans* ortholog of this gene (*BCY1*) responsible for coding the regulator subunit of the PKA kinase, thus playing key role in fungal cAMP/PKA pathway. This data suggests that cAMP/PKA pathway could be involved in the development of triazole resistance in *C. auris* and indirectly influence the virulence properties of these strains.

ROTAVIRUS A IN RED FOXES AND EUROPEAN JACKALS: HIGH GENETIC DIVERSITY AND THE EVIDENCE OF COMPLEX BACKGROUND OF INTERSPECIES TRANSMISSION EVENTS

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Rotaviruses (RV) are widespread pathogens of public health importance, causing approximately 130,000 deaths each year, mostly in children in developing countries. The importance of RV infections in animals is mainly observed in cattle and pigs since RVs are one of the main causative agents of neonatal diarrhea. On the other hand, the knowledge on rotavirus infections in wildlife is rather limited. Rotaviruses, a segmented dsRNA viruses, are members of the family Reoviridae, genus *Rotavirus* among which there are nine officially accepted species with *Rotavirus A* (RVA) being the most significant. Due to the segmented nature of the genome, genetic reassortment is driving RVA diversification, with the constant emergence of human-animal chimeric strains. The aim of the present study was to elucidate the prevalence, molecular epidemiology and genetic diversity of RVA strains in red foxes (*Vulpes vulpes*) and European jackals (*Canis aureus moreoticus*) in Croatia. To the best of our knowledge, our study is the first one focused on molecular epidemiology and genetic diversity of RVA strains circulating in foxes and jackals. From 2018 to 2021 we collected 533 and 70 fecal samples from red fox and European jackal carcasses hunted for rabies monitoring, respectively. The samples originated from 16 counties located in the Continental and Northern Adriatic Croatia. A VP2 real-time RT-PCR was applied on all samples, with positives being a subject for VP7 and VP4 genotyping. For the latter we employed several primer sets primarily due to the potentially high RVA diversity coupled with possibly lower efficiency of previously designed primers. All positive samples were Sanger sequenced which was followed by phylogenetic analysis using MEGAX software. RVA genotypes were defined by the BLAST search following previously defined cut-offs. The results reveal the RVA prevalence of 15% in foxes and 18.6% in jackals. The circulating RVA strains in foxes show a remarkable genetic diversity with 11 and nine different genotypes G and P, respectively. Among these are one and three tentative novel genotypes G and P, respectively. In jackals the genetic diversity was lower considering smaller sample set, with three genotypes G and four genotypes P found to be circulating. The results imply a complex background of previous interspecies transmission events, bringing a new perspective on the potential influence of foxes and jackals in the RVA epidemiology. Their role as potential reservoirs for the broad range of RVA genotypes, usually considered being typical for domestic animals and humans, could not be excluded. Our results show a remarkable genetic diversity which warrants further research in order to gain a complete perspective on the most prevalent RVA genotypes circulating in these species. The complex background of interspecies transmissions highlighted by the present study emphasizes the need for the continuous application of the One Health concept in rotavirus A research.

BIOCONTROL PROPERTIES OF *BACILLUS VELEZENSIS* AGAINST *TRICHODERMA AGGRESSIVUM* CAUSING GREEN MOULD DISEASE ON WHITE BUTTON MUSHROOM

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White button mushroom (*Agaricus bisporus*) is the most popular cultivated mushroom worldwide, folowed by shiitake (*Lentinula edodes*) and oyster mushroom (*Pleurotus ostreatus*). In industrial mushroom production, a large number of fungi, bacteria, viruses, nematodes can cause infections. The biggest challenge is without doubt the green mould disease caused by *Trichoderma* species, in most cases *T. aggressivum* f. *europaeum*, and *T. aggressivum* f. *aggressivum*. The most common control measures include disinfection of the equipment and growing rooms, and the application of fungicides, particularly prochloraz and metrafenol. Biocontrol is a cost-effective healthy alternative to chemical control. Certain microorganisms, e.g., *Bacillus* species are frequently used as biocontrol agents. Our aim was to isolate and examine bacterial strains from mushroom growing materials, that can be potentially used as biocontrol agents. A total of 85 bacterial strains were isolated and tested against *T. aggressivum* f. *europaeum*, *T. aggressivum* f. *aggressivum*, *Cladobotryum mycophilum, Mycogone perniciosa* and *Lecanicilluim fungicola*. *Bacillus velezensis* strain SZMC 25431 was selected for further examination under simulated *Agaricus* cultivation conditions against *T. aggressivum* in a 1,200 L Weiss Gallenkamp plant growth chamber. The concentration of the bacterial suspensions tested were set to 10^7, 10^6, and 10^5 cells/mL. The mould pathogen used in the experiments was *Trichoderma aggressivum* f. *aggressivum* strain SZMC 23834. Mushroom yield was weighted and the effect of the tratment on the cropping was also measured. According to our observations, the bacterial treatment was effective against the pathogen in all cases, but the best results were achieved at an application contentra-

tion of 10^5 cells/mL. To examine the effect of *B. velezensis* SZMC 25431 on white button mushroom yield under industrial conditions, on-site experiments were designed and carried out in mushroom growing houses of the ÚjChampignons Ltd., Kerecsend, Hungary. Growing houses with the bearing surface of 480 m^2 were selected, and the yields from mushroom houses with chemical treatments only, mushroom houses with B. velezensis treatment only, and mushroom houses with integrated treatment using both chemicals and *B. velezensis* were examined and compared. The bacterial treatment was applied using water tanks applied for everyday irrigation. According to our results, the bacterial treatment increased crop yield by 21.95%, while the *B. velezensis* treatment combined with chemicals resulted in a crop increase by 26%. Our results suggest, that the selected *B. velezensis* strain can be potentially used for biocontrol in *Agaricus* cultivation.

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COVID-19 VS INFLUENZA: PREDICTING THE FUTURE

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Acute respiratory infections (ARIs) are the most common infections in humans of all ages. The disease burden from ARIs is substantial and thus their prevention and treatment are a priority for public health agencies. Moreover, the current COVID-19 pandemic that, so far, has affected more than 195 million and killed more than 4 million people worldwide has returned the focus of human medicine to respiratory infections with great epidemic potential. Until recently, the most feared scenario was that one of the influenza viruses could be responsible for the next major pandemic. But the recent outbreak of the COVID-19 epidemic, which began in China in December 2019 and spread throughout the world during 2020, has brought coronaviruses into focus. The pandemic caused by the new coronavirus, SARS-CoV-2, is still not subsiding and has entered its second year of existence. Over the past winter season, it has completely suppressed the flu, which to our great surprise in the Northern Hemisphere was present only sporadically, with over 90% less incidence rate that was previously common. There are many possible reasons for this: a decreased circulation of influenza viruses in Europe, a decreased pool of patients with influenza in the Southern Hemisphere that were the source of epidemic in the Northern Hemisphere, a decreased intercontinental transport and migration of people and goods, decreased diagnostics of other respiratory pathogens including influenza viruses due to increased focus on SARS-CoV-2 (up to 60%), a widespread adoption of community mitigation measures to reduce the transmission of SARS-CoV-2 also decreased the transmission of other respiratory viruses, increased influenza vaccination rate in the 2020/2021 season, etc. Will this happen again in the next winter season 2021/2022? It's hard to guess. It is possible that the circulation of SARS-CoV-2 virus will suppress the circulation of influenza virus again next winter. However, it is also possible that, due to the decline in immunity among vulnerable population following lack of contact with circulating viruses, influenza epidemics might occur on an even more intense scale. It is also justified to fear a simultaneous circulation and co-infection with SASR-CoV-2 and influenza viruses because some preliminary experience has shown that simultaneous infection with these two viruses can cause a more severe clinical picture and be responsible for higher mortality in infected individuals. It is therefore justified to fear the coming winter season and what the future holds for us. Therefore, high vaccination rates for both influenza and SARS-CoV-2 could highly contribute to the decreased incidence of influenza and COVID-19 and associated morbidity and mortality.

INVESTIGATION ON THE BIOSURFACTANT PRODUCTION WITH *BACILLUS SUBTILIS* DSM10 AND *GEOBACILLUS STEAROTHERMOPHILUS* DSM2313 APPLYING RESPONSE SURFACE METHODOLOGY

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Bacilli are frequently applied microorganisms in biotechnology. They have a broad product range from primary over secondary metabolites up to enzymes. Biosurfactants are also one of the well-known frequently studied product group of Bacilli. *Bacillus* species are capable producing lipopeptide type biosurfactants like surfactin, iturin and fengycin. Biosurfactants are lowering the surface tension of water, they can be used in microbial enhanced oil recovery, pharmaceutical, cosmetics and food industry as emulsifiers or encapsulating agents. In the household industry they can replace the chemical surfactants due to their specific phenomena like, biodegradability, environmentally friendly nature and low toxicity. The effectivity and the efficiency of a biosurfactant can be characterized with the critical micelle concentration and the surface tension decrement. Application against oils can be demonstrated with the emulsification activity. During

our research, several Bacilli were tested for biosurfactant production in shaking flask experiments. The most promising strains, *Bacillus subtilis* DSM10 and *Geobacillus stearothermophilus* DSM2313, were chosen for further investigation. The effect of glucose and NH_4NO_3 concentrations and the initial pH of the media were investigated in central composite designs. Based on these experiments a statistical evaluation was implemented. The possible optimal media compositions for *Bacillus subtilis* DSM10 and *Geobacillus stearothermophilus* DSM2313 were verified in further experiments. The critical micelle concentration and the emulsifier capability of the produced biosurfactants were examined, compared and evaluated.

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SECONDARY METABOLITES OF *CADOPHORA* AND *PERICONIA* GRASS ROOT ENDOPHYTIC FUNGI

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Fungal endophytes, which asymptomatically colonize plant tissues, are capable of producing various secondary metabolites of crucial ecological and economic importance. Dark septate endophytes (DSE) are widespread root colonizing fungi that seem to play a major role in plant survival in (semi)arid environments, while they are also said to be an unexploited source of natural products. Our study aimed to analyse the secondary metabolome of DSE fungi deriving from a Hungarian semiarid grassland by applying analytical and genomic approaches. We predicted in silico the secondary metabolites based on biosynthetic gene clusters identified in the genomes of *Cadophora* sp. (DSE1049) and *Periconia macrospinosa* (DSE2036) and assumed potential functions of enzymes in their synthesis. Compared to the predictions, many secondary metabolites were not detected in standard laboratory conditions. We cultured the fungal strains under different treatments to induce the production of more than fifty predicted compounds. Afterwards, we prepared the HPLC-MS/MS profiles, and we tentatively identified the secondary metabolites and their derivatives by using fragmentation profiles and/or NMR spectroscopy. We also estimated the concentrations of the isolated metabolites and compared their production under the treatments. Moreover, it was possible to elicit the putative biosynthetic pathway from gene cluster identification in the case of some metabolites.

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INVESTIGATION OF THE EFFECT OF PARALLEL PRESENCE OF TRACE METAL IONS IN THE ITACONIC ACID PRODUCTION BY *ASPERGILLUS TERREUS* USING RSM AND CENTRAL COMPOSITE DESIGN

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Itaconic acid is one of the most widely produced organic acids with a wide range of uses. The annual production exceeds 80,000 tons, which is produced exclusively by fermentation. Itaconic acid has broad application range such as the manufacturing of absorbents, phosphate-free detergents, cleaners, and bioactive compounds. The polymerized esters of itaconic acid (IA) are widely used in adhesive and paints / coating industries. Itaconic acid is forecasted as a possible replacement of petroleum-based chemicals like acrylic acid or methyl acrylic acids used presently in the polymer industry. Microbiological itaconic acid production is affected by a number of parameters, e.g. temperature, pH, or the amount of trace metal ions. The individual effect of metal ions on the production of *Aspergillus terreus* itaconic acid has been investigated in several studies, however, the synergic / antagonist effects of those metal ions on the production yield was much less in the focus. In our studies, we applied Central Composite Experimental Design, and multivariate analysis to assess the effect of the simultaneous presence of Fe(II)-, Zn(II)-, Mn(II)- and Cu(II)- ions. These ions seem to be the most affecting factors, and some RSM results were already presented for Cu(II)-, Zn(II)- Fe(II)- and Ca(II) ions [1], but as it was shown, Mn(II) ions are crucial in the inhibition of the production. In the recent work we have included the manganese concentration into the multivariate analysis parameters. Use of the Response Surface Methodology reveals such correlations of the experimental parameters and the production that can be hardly visualized and understand with traditional methods. Especially in parameter spaces possessing higher dimension than 3. In addition, the mathematical optimization leads to significantly

reduced sample numbers without loosing information about the whole system. The desirability function calculation – part of RSM – allows the determination of the optimal metal ion composition regardless if the highest yields or the shortest reaction times are the goal of optimization.

[1] Haijan et al (2017) Asian J Microbiol Biotechnol Env Sci 19:216.

EVALUATING THE FIELD PERFORMANCE OF MULTIPLE SARS-COV-2 ANTIGEN RAPID TESTS USING NASOPHARYNGEAL SWAB SAMPLES

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The SARS-CoV-2 pandemic started December 2019 has been posing significant challenges to the health care system worldwide. As the fourth wave of the pandemic is predicted, early diagnosis of infected patients is crucial to successfully limit the spread of the virus. Although real-time reverse-transcription polymerase chain reaction is the recommended laboratory method to diagnose COVID-19 infection, many factors such as availability of laboratory equipment, reagents and trained personnel affect the use of molecular techniques. To facilitate on-the-spot diagnosis, we aimed to evaluate the on-field performance of ten commercially available SARS-CoV-2 antigen rapid tests and compare with RT-qPCR as reference method. The issue is particularly important due to the recent unanimous agreement by the European Commission Member States on a recommendation setting out a framework for the use of antigen rapid tests that contains a list of the mutually recognized assays and the basis of independent validation protocols. In this study, the Hungarian National Public Health Center provided 1597 antigen rapid tests from ten manufacturers to the National Ambulance Service, the COVID-testing trucks and two hospitals in Budapest treating COVID-19 patients to evaluate the performance of the rapid tests among symptomatic patients using nasopharyngeal swab samples. For strongly positive samples ($Ct < 25$) the sensitivities ranged between 66.7% and 100%, while for positive samples ($Ct < 30$) they gave a maximum sensitivity of 87.5%. The specificity of the tests was adequate ranging between 79% to 100%. It is important to highlight that SARS-Cov-2 rapid tests play important role in early and on-the-spot diagnosis of potentially infected individuals. The results presented here are of high importance to the EU Commission and also helps governmental decision-making regarding the application of the proper rapid tests for screening asymptomatic and testing symptomatic populations.

ADAPTATION TO COMBINATORIAL IRON LIMITATION – OXIDATIVE STRESS IN *ASPERGILLUS* SPECIES

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Fungi have to cope with oxidative stress under iron limited conditions when they grow in the human body. The sensitivity of the strain against this combinatorial iron limitation – oxidative stress can substantially influence the outcome of the infection. Here, the stress tolerance of nine different *Aspergillus* species was compared in a plate assay. Iron limitation and oxidative stresses were induced by H_2O_2 and the iron chelator deferriprone (DFP), respectively. DFP tolerance showed positive correlation with the diameter of conidia. Conidia developed under iron limited condition had less DFP tolerance than those formed on iron rich media. It supports the view that conidia can store substantial amount of iron to support germination upon iron famine. Oxidative stress treatment of mycelia increased the growth inhibitory effect of DFP in all species. Surprisingly, if the same oxidative stress treatment was applied on conidia the growth inhibitory effect of DFP was reduced. It suggests that conidia that have survived the oxidative attack of our immune system have better chance for adaptation to the iron limited condition occurring in the human body than those have successfully hidden from our immune cells.

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SEASONAL BACTERIAL VARIABILITY OF THE LAKE BALATON AND KIS-BALATON WATER PROTECTION SYSTEM

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The Lake Balaton with surface area of 594 km^2 and 3,2 m average depth is the largest central European shallow lake. A total of 51 water course feeds the lake, among them, the largest Zala River drains the 45 % of the catchment area, while contributes with about half of the total phosphorus and nitrogen load of the lake. As a result of high external nutrient loads the lake became hypertrophic in the 70's, therefore strict regulations were introduced. To ameliorate the water quality and retain the nutrients from the lake the Kis-Balaton Water Protection System (KBWPS) was constructed. The aim of the present study was to reveal how this 18 km^2 wetland area influences the dynamics of Balaton microbial community. To discover the spatial and the seasonal heterogeneity of planktonic bacterial community 4 sampling areas and 5 time points were set based on the physicochemical and algae distribution parameters. Illumina 16S rDNA amplicon sequencing was used to assess precisely the bacterial community composition of the chosen samples. The bacterial community at different points of the KBWPS generally differs from each other and from the samples of Keszthely basin (located at Lake Balaton). Samples were dominated by members of Proteobacteria (28-39%), Bacteriodetes (12-16%), Actinobacteria (6-38%), Cyanobacteria (9-27%) and Verrucomicrobia (7-12%). *Fluviibacter phosphoraccumulans* and *Limnohabitans curvus* were the most dominant members of gamma-proteobacteria. The former microorganism was mainly detected at the KBWPS outflow, while the latter was higly abundant both KBWPS inflow and outflow areas. The number of alfa-proteobacterial *Fonsibacter ubiquis* was higher between the two main parts of KBWPS (4T flood gate) and at the Keszthey basin. The phylum Bacteriodetes showed a great versatility since several genera with low abundance were found. Among the cyanobacteria, the dominance of *Aphanizomenon flos-aquae* was observed at the Keszthely basin during the summer, while lower abundance was found at the KBWR area. *Dolichospermum circinale* and *Anabaena sigmoidea* species were detected in variable amount both Lake Balaton and KBWPS samples. Within the Verrucomicrobia an unknown *Luteolibacter* sp. was dominant, this microorganism was the most abundant at the 4T flood gate sampling area during the late summer. Members of the genus *Nanopelagicus* and *Planktophila* were the most detected actinobacterial strains, the former genus were most dominant in the samples of 4T flood gate and Keszthely basin, while *Planktophila* species showed a more homogeneous distribution.

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THE EFFECT OF ARSENIC ON BACTERIAL AND ARCHAEAL COMMUNITIES IN MICROCOSM EXPERIMENTS

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In the present study, a 5 week microcosm experiment (applying drinking water from a water fountain in Dunavarsány, near Budapest) was conducted to assess the effect of arsenic trioxide [As(III)] at various concentrations (200, 400, 800, 1,500, 3,000, 5,000 mg/L). For monitoring the composition of the prokaryotic community, microscopic cell counts (after DAPI staining with epifluorescent microscopy), cultivation [using R2A medium containing 800 mg/L As(III)] as well as amplicon sequencing (NGS) were applied. From microcosms containing high arsenic (3,000 mg/L), *Acidovorax facilis* and *Pseudomonas extremaustralis* strains were isolated. Optical density (OD) measurements revealed significant arsenic resistance in them (surviving even 1,500-2,000 mg/L concentrations). Since arsenic is charaterised by low acute toxicity, compared to the control, the number of cells decreased only in one magnitude. Based on NGS, the structure of the bacterial community significantly changed: the number of Proteobacteria decreased in the samples with high arsenic content. Patescibacteria, Bacteroidetes and Verrucomicrobia could be found in all samples. Bacteria in samples with the highest arsenic content (3,000-5,000 mg/L) belonged to Cyanobacteria, Epsilonbacteraeota and Nitrospirae. The most frequent Archaea belonged to Nanoarchaeota (Woesearchaeia) in each samples (controls and with high arsenic content).

TYPE OF ANION DETERMINES THE SALT TOLERANCE OF BACTERIA IN SALINE LAKES

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Saline lakes are globally widespread, and although most of them are dominated with sodium chloride, aquatic habitats having significant amounts of other dissolved ions also exist. Furthermore, non-NaCl-dominated saline sites could be found relatively frequently in seafloor basins, subsurface environments and are predicted on other planetary bodies. Therefore, the aim of this research was to improve our understanding on the effect of salt anion type on bacteria. Accordingly, we isolated 172 strains from the water of Central and Eastern European saline and soda lakes that differed in ionic composition. The salt composition and concentration preference of the strains was tested using 25 different combinations of media that contained either carbonate, chloride or sulfate as anion in concentration values ranging from 0 to 0.58 mol/L. Different salt type preferences were observed among the bacterial strains that proved their adaptation to the salt type that can be found in their natural habitat. In conclusion, the role of dissolved salts in saline lakes is not limited to triggering osmosis, but the type of anion determines the adaptation and survival of individual microorganisms.

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STUDYING ANTIVIRAL POTENTIAL OF DIFFERENT ORGANIC COMPOUNDS USED FOR FACE MASK MATERIALS

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At present, the world is experiencing the outbreak of atypical pneumonia caused by novel coronavirus, SAR-CoV-2. Due to the lack of the ability to inactivate the viruses, masks are prone to cross-infection and become an additional source of infection after being discarded. If the filtration and antiviral effects can be simultaneously integrated into the mask, it would be more efficient for a longer time and create less difficulty in post-treatment and disposal [1]. Therefore, the development of new protective textile materials with an ability to protect the wearer from viruses and other pathogenic microorganisms and at the same time not impose great environmental issues is needed. It is known from literature that organic antiviral materials inactivate pathogenic microorganisms by the reaction with the surface proteins or nucleic acids, or effect the morphology or proliferation of microbes, via generation of reactive oxygen species (ROS) [1]. Biopolymers, like chitosans are a class of naturally derived materials [2]. Chitosan is a polysaccharide biomaterial found on the shells of crustaceans. This material is biocompatible and used in many medical applications. Chitosan has been shown to display, among other activities, also antiviral and antibiotic characteristics. The cationic nature of chitosan enables the formation of polyelectrolyte complexes with negatively charged biomolecules [3]. In our study we first tested the antiviral activity of different organic solutions on two different bacteriophages phi6 and MS2. Phi6 can be used as model surrogate for SARS-CoV-2, because it is enveloped by a lipid membrane, has spike proteins, and has similar spherical structure and size (~80–100 nm). To broaden the study and applicability of the solution, a non-enveloped bacteriophage MS2 was used. To determine the survival of bacteriophages, we used a double layer agar assay, via incubation of the treated bacteriophage sample with the host bacteria in a semi-solid medium, which is poured over the solid medium. As expected, the inactivation of MS2 was much lower compared to phi6, which could be attributed to more stable nature of non-enveloped bacteriophages. Two of the organic solutions: LMW (low molecular weight) chitosan, HMW (high molecular weight) chitosan showed extremely high inactivation of phi6, with the antiviral activity value (Mv) higher than 6 log. In the case of MS2, only approx.. 0,54 log reduction was determined for those two compounds. Organic compounds with the highest antiviral activity were screen printed on polypropylene face mask materials and antiviral activity was assessed following a modified protocol from standard ISO 18184:2019. The results showed slightly lower inactivation of the bacteriophage phi6, but still above 1,6 log. More optimisation of material preparation and antiviral compounds used needs to be done in the future.

[1] Zhou et al (2020) Adv Fiber Materials 2020:1.
[2] Rakowska et al (2021) Commun Mater 2:53.
[3] Samal et al (2012) Chem Soc Rev 41:7147.

THE COMPOSITIONAL TURNOVER OF GRAPEVINE-ASSOCIATED PLANT PATHOGENIC FUNGAL COMMUNITIES ARE GREATER AMONG INTRAINDIVIDUAL MICROHABITATS THAN AMONG HEALTHY AND ESCA-DISEASED PLANTS

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Fungal diseases, including grapevine trunk diseases (GTD) cause the greatest damage in the wine industry by causing the decline of grapevines and huge yield loss. In the past decades, a number of studies have adressed grapevine-associated pathogens, using mostly culture based techniques. To understand better the nature of grapevine trunk diseases, culture independent methods - DNA-metabarcoding - were used to reveal whether differenences in richness, abundance, and composition of plant pathogenic fungi exist among below- and aboveground microhabitats and among asymptomatic and symptomatic grapevines. Soil, bark, and perennial wood samples were collected from asymptomatic and symptomatic grapevines from the Tokaj wine region. Larger compositional differences in plant pathogenic fungi were found within grapevine plants than among them. GTD-associated fungi were represented mostly in the wood and to a lesser extent in bark, while non-GTD pathogens were dominant in soil. There were no significant differences among healthy and Esca symptomatic grapevines. According to our results, fungal pathogens related to Esca disease are the members of the core microbiome, and likely act as commensal endophytes and/or latent saprothrops, some of which can act as opportunistic pathogens on stressed plants. Enviromental factors may be important for the developement of Esca disease, and studies are needed to investigate the abiotic conditions on fungal compositional dynamics in Esca-affected plants.

COMMUNITY DYNAMICS OF SOIL-BORNE FUNGAL COMMUNITIES ALONG ELEVATION GRADIENTS IN NEOTROPICAL AND PALEOTROPICAL FORESTS

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Fungal diversity in tropical forests remains little known, and opportunities to compare data from similar guilds across diverse tropical forest types at local and global scales are rare. Because of their steep gradients in abiotic and biotic factors, mountains offer an ideal setting to enhance our understanding of mechanisms that underlie species distributions and community assembly. We compared the structure of taxonomically and functionally diverse fungal communities in soils along five elevational gradients in mountains of the Neo- and Paleotropics (northern Argentina, southern Brazil, Panama, Borneo, and Papua New Guinea). The data presented here show that composition of the total fungal community in soil, as well as that of all functional groups, is strongly structured according to elevational forest types in both the Neo- and Paleotropics. Contrary to vascular plants, we did not find substantial differences in total soil fungal richness among the three elevational zones in most sampled regions, which is similar to the lack of latitudinal differences in fungal richness on a global scale. On the other hand, some functional groups of fungi showed significant differences in richness along the elevation gradients. In addition, we observed strong compositional differences among elevational forest types in all functional groups and in all regions, which appears to be driven at least in part by environmental filtering according to contrasting climatic and edaphic conditions, and associated differences in plant communities. Among environmental factors, mean annual temperature and soil pH, appear to have the strongest influence on richness and composition of soil fungal communities, with some shared patterns among neotropical and paleotropical regions. The observed elevational turnover appears to be driven by contrasting environmental preferences among functional and taxonomic groups, with spome phylogenetic niche conservatism, resulting in the replacement of species within each functional guild. For functional groups dependent on symbioses with plants (especially ectomycorrhizal fungi), the distribution of host plants drives richness and community composition, resulting in important differences in elevational patterns between neotropical and paleotropical montane communities. This is the first study comparing community structure of fungi along elevation gradients in the Neotropics and Paleotropics. The pronounced compositional and functional turnover along elevation gradients in all sampled regions implies that tropical montane forest fungi will be sensitive to climate change, resulting in shifts in composition and functionality over time.

ENVIRONMENTAL DNA SEQUENCING PROVIDES INSIGHTS INTO DIVERSITY, DISTRIBUTION AND HABITAT PREFERENCE OF ECTOMYCORRHIZAL FUNGI AMONG DIFFERENT PANNONIAN FOREST TYPES

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Soil microbial communities represent the greatest reservoir of biological diversity known in the world, with thousands to tens of thousands of microbial species found a single gram of soil. Fungi in particular are known to drive plant diversity and productivity and are crucial for ecosystem functioning and resilience towards disturbance. We carried out DNA metabarcoding of fungi from soil samples taken at 62 sites in the Bükk Mountains in northern Hungary to compare richness and community composition of ectomycorrhizal (ECM) fungi among various types of Pannonian forests and to assess the influence of selected environmental variables on their community dynamics. The deep sequence data presented here indicate that both niche (environmental filtering) and neutral (stochastic) processes shape ECM fungal community composition at landscape level. Fungal community composition correlated strongly with edaphic variales, particularly with pH and soil moisture, with many ECM fungi showing preference for specific zonal, topographic or edapic forest types. Several ECM fungal genera showed significant differences in richness among forest types and exhibited strong compositional differences mostly driven by differences in environmental factors. Finally, the data presented here provide an unprecedented insight into the diversity and landscape-level community dynamics of ECM fungi in the Pannonian forests.

TRANSCRIPTIONAL ACTIVITY OF SECONDARY METABOLITE CLUSTER GENES IN CARBON STRESSED *ASPERGILLUS FUMIGATUS* CULTURES

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Aspergillus fumigatus is one of the most important non-*Candida* human pathogen fungi. Its genome contains more than 30 secondary metabolite gene clusters. Many of them proved to be important in the pathogenesis of the fungus: The Sid cluster is responsible for the production of extracellular siderophores needed for efficient adaptation to the iron limited conditions of the human body, the DHN-melanin cluster is responsible for the stress protectant dihydroxynaphthalene-melanin content of conidia, while the Gli and Fum clusters encode genes for the formation of the immunomodulatory gliotoxin and fumagillin mycotoxins, respectively. In the human body, *A. fumigatus* has to cope with several stresses including carbon limitation and carbon starvation stresses. Here, we evaluated genome wide transcription data to describe how carbon stress affects the transcriptional activity of secondary metabolite clusters. Four types of cultures which were incubated on glucose, glucose + peptone, peptone or without any carbon sources were compared. The transcriptional activity of altogether 20 secondary metabolite gene clusters changed in these experiments (carbon stress responsive SMG clusters). The presence of peptone downregulated many of them and its effect was stronger in the absence of glucose (10 SMG clusters showed downregulation) than on glucose (7 SMG clusters showed downregulation). The Gli, Fum and DHN-melanin clusters were downregulated while the Sid cluster was upregulated by peptone irrespectively to the presence of glucose. Surprisingly, glucose upregulated many (altogether 8) carbon stress responsive SMG clusters irrespectively to the presence of peptone including the Sid, Gli and Fum clusters. Eight SMG clusters, with unknown products, however were downregulated by glucose in the absence of peptone. Our results suggest that carbon stress is generally not beneficial for bulk production of versatile secondary metabolites in *A. fumigatus*. Nevertheless, other factors/stresses, like oxidative stress, however can outweigh the effect of carbon stress.

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NICOTINATE DEGRADATION IN A MICROBIAL EUKARYOTE: A NOVEL, COMPLETE PATHWAY EXTANT IN *ASPERGILLUS NIDULANS*

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Nicotinic acid (NA) degradation pathways have been extensively studied in prokaryotes and several strikingly different aerobic and anaerobic pathways had been described. Despite many eukaryotic microbes can utilize NA as sole nitrogen source, no similar work was carried out in any eukaryote. Our research group was the first to describe a regulon composed

from 11 genes that are regulated by a C_2H_2 transcription factor HxnR, the GATA factor AreA and a hitherto not identified catabolic derivative of NA [1, 2]. Here we show for the first time a novel, complete eukaryotic nicotinate catabolic pathway. The elucidation of the individual steps of the pathway required the combinatorial application of reverse genetics and analytics (ultra-high performance liquid chromatography – high-resolution mass spectrometry). We also provide the comparison of prokaryotic pathways to the only extant eukaryotic pathway and demonstrate that the pathway extant in *A. nidulans* is completely different from any other previously analysed pathways in bacteria. While no redundantly functioning enzymes are involved in the prokaryotic routes, three steps of the fungal catabolism use alternative enzymes and the catabolic steps differ from those in prokaryotes and lead to novel intermediate metabolites which have never been detected in any organism (neither in prokaryotic nor in eukaryotic organisms).

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[1] Ámon et al (2017) Open Biol 7:170199.
[2] Bokor et al (2021) bioRxiv 2021.2004.2019.440407.

PURIFICATION OF SURFACTINS FROM THE FERMENT BROTH OF A *BACILLUS SUBTILIS* STRAIN

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Surfactins are cyclic lipopeptide produced mainly by *Bacillus* species, consisting of a peptide ring of seven amino acids and a ß-hydroxy fatty acid of various chain length linked together by a lactone bridge. These compounds have been proven to exhibit various biological effects including antimicrobial and antitumor activities; therefore their therapeutic and environmental applications may be promising. Differences in length of the fatty acid chain and the amino acid sequence lead to the formation of several isoforms, of which more than 30 variants have been described so far. However, there is hardly any information regarding their exact structure and the possible differences in their biological activities. Therefore, the aim of our work was the purification of the different surfactin variants by several separation techniques in order to study relationships between the structure and biological activity of the surfactin variants. In our work, after a comprehensive screening program the *B. subtilis* GBB64 strain was selected for surfactin production. The pre-treatment of the fermentation broth included centrifugation and precipitation steps followed by the purification by normal phase flash chromatography. The collected fractions were analysed by thin layer chromatography and the surfactin containing fractions were combined. Then the separation of different surfactin variants from each other was carried out by reverse phase preparative HPLC. Determination of surfactin variants in the fractions during purification were carried out by HPLC-HESI-MS.

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IDENTIFICATION AND COMMUNITY ANALYSIS OF YEASTS IN HUNGARIAN SILAGE SAMPLES

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Nowadays the performance of global agriculture and associated food systems is drastically endangered due to chemical contamination and infection of pathogens. These losses affect forage crops as well. The most typical fodder crops in Europe are grasses and maize. These forage plants are usually preserved and utilized as silage. The method of producing silage is essentially based on the principle of preservation under anaerobic conditions together with the growth of lactic acid bacteria. These conditions in these products (low pH and anaerobiosis) are unfavorable for the growth of most fungi and other harmful microbes. However, incorrect storage conditions can lead to the growth of anaerobic and microaerobic acid-tolerant fungi and other microorganisms including yeasts. As a result, undesirable compounds, such as mycotoxins, butyric acid, proteolysis products, and bacterial toxins may be present in the silage, negatively impacting animal and human health. Undesirable microorganisms in silage include Enterobacteriacea, *Clostridium* spp. and *Bacillus* spp., filamentous fungi of the genera *Fusarium, Penicillium, Aspergillus*, and ethanol-producing yeasts. Certain filamentous fungi are capable of producing mycotoxins. Contamination of food and feed with mycotoxins is one of the most important factors in food safety. Yeasts are the initiators of aerobic deterioration but some species have an antagonistic effect against pathogenic/mycotoxinogenic fungi or ar able to detoxify mycotoxins. Monitoring the microbiome during the ensilaging process

helps to understand and improve the quality of silage. Therefore, our goal was to isolate and identify yeasts from silage samples and to assess the bacterial community compositions in which they were found. Most of the examined fermented forage did not contain viable yeasts, most probably due to anaerobic conditions, low pH and the different fermentation products (such as lactic acid and acetic acid). All isolated yeasts belonged to the order Saccharomycetales of the Ascomycota. The most commonly isolated species was *Pichia kudriavzevii*. The presence of this species can be observed in all fermented forage, as well as in many alfalfa hay and rapeseed samples. This species has certainly adapted well to both field and fermented forage conditions. Its survival in feeds is due to its high temperature tolerance (up to 44°C), and, it can grow in an anaerobic environment at low pH as well, both in aqueous and solid media. Other yeasts identified in this work were also dimorphic yeast as *Diutina rugosa, Hyphopichia burtonii*, and *Wickerhamomyces anomalus*.

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EFFECTIVE PREVENTION OF OCCUPATIONAL SARS-COV-2 INFECTION AMONG HEALTHCARE WORKERS AT THE UNIVERSITY HOSPITAL FOR INFECTIOUS DISEASES "DR. FRAN MIHALJEVIĆ", ZAGREB DURING THE FIRST WAVE OF THE COVID-19 PANDEMIC

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Because of their often extensive and close contact with infected individuals in the healthcare setting, healthcare workers (HCWs) are at increased risk for occupational acquisition of SARS-CoV-2 infection. Therefore, intense and strict implementation of pre-exposure Infection Prevention and Control (IPC) recommendations and post-exposure management are necessary to prevent nosocomial spread of SARS-CoV-2. The University Hospital for Infectious Diseases "Dr. Fran Mihaljević" in Zagreb was the first hospital to admit patients with COVID-19 in Croatia with the first patient hospitalized on February 25, 2020. This study aimed to evaluate the effect of the prevention of occupational SARS-CoV-2 infection among HCWs at the University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Zagreb during the first wave of the COVID-19 pandemic (between February 24 and May 27, 2020). During the study period, additional preventive measures against SARS-CoV-2 infection were introduced in the Hospital, in accordance with CDC, ECDC and WHO recommendations, with enhanced aerosol precautionary measures. The Hospital also introduced continuous monitoring of the implementation of IPC, daily contact tracing among employees for potential contacts with persons infected with SARS-CoV-2, reporting of risky contacts with obligatory self-isolation, and regular screening with PCR test. In case of symptoms of COVID-19 and/or suspicion of contact with a SARS-CoV-2 infected person, all employees were tested by RT-PCR for SARS-CoV-2 virus infection. For persons with proven contact with a PCR-positive patient, an epidemiological self-isolation measure lasting for 14 (later on 10) days was mandatory. At the end of the study period (May 2020), all employees were offered serological testing for SARS-CoV-2 in order to detect persons who had COVID-19 and no PCR-proven infection or asymptomatic infection. Prior to testing, all employees were interviewed about the possibilities, forms and intensity of occupational exposure, self-isolation measures and possible infection. During the studied period, a total of 3,118 patients with suspected COVID-19 were examined at the Hospital, ranging from 3 to 159 / day (median 28.5), in 297 of whom RT-PCR test confirmed SARS-CoV-2 infection, and 170 of them was hospitalized. At the same time, the Hospital had a total of 705 employees. All employees were monitored for the detection of occupational infection throughout the study period, and serological screening and surveys were applied in 565 (80.1%) employees, of whom 467 (82.6%) were female; the median age was 44 (range 19–66). Regarding formal education levels among tested employees, the highest (university) education was recorded in 116 (20.5%), higher (post-secondary) education in 120 (21.2%), secondary education in 227 (40.2%), and lower basic education in 102 (2.8%) employees. Of these, 395 (69.9%) were healthcare professionals and 170 (30.1%) non-healthcare professionals: 45 (8%) administrative and 125 (22.1%) technical staff. Among those tested, there were 70 (12.4%) medical doctors, 195 (34.5%) nurses/technicians, 99 (17.5%) laboratory workers, 11 (1.9%) engineers in radiology, and 20 (3.5%) other HCWs. Of all subjects, 143 (25.3%) had COVID-19 symptoms at least once during the study period, and 157 (27.8%) were tested due to suspected contact with an infected person. Epidemiological measure of self-isolation due to proven contact with a sick person was imposed on 93 (16.5%) employees. Finally, SARS-CoV-2 infection was detected by RT-PCR in only two employees (0.35%), however, occupational acquisition of COVID-19 could not be confirmed in any of these cases. Subsequent serological testing confirmed the presence of IgG antibodies to SARS-CoV-2 as evidence of previous infection in only one employee; while the other employee who had previously tested positive for PCR, was negative, probably due to a false positive laboratory result. Overall, it is clear that adherence to adequate preventive measures, and timely and adequate post-exposure management can successfully protect HCWs from occupational infection with SARS-CoV-2.

DISCRIMINATION BETWEEN TWO *BACILLUS* SPECIES BASED ON WHOLE-CELL FATTY ACID PROFILES

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Bacillus velezensis and *B. amyloliquefaciens* are two closely-related species. They have similarly shared morphological, physiological, biochemical, phenotypic and phylogenetic characteristics. Thus, separating these two species from one another have proven to be difficult. The efforts to understanding the genetic relationship of two taxa have been considered over the last 15 years. However, until now, the tight relatedness between two taxa have still revealed as a taxonomic trouble. The current study introduced the discrimination between two taxa based on whole-cell fatty acid profiles. Firstly, the classification was determined by the partial sequences of the *gyrA* and *rpoB* genes. After that, classified *B. velezensis* and *B. amyloliquefaciens* strains were considered as samples to develop the identification method based on fatty acid profiles. The method using fatty acids 9-20 carbons in length and automated GC analysis analyzes qualitatively and quantitatively bacterial whole-cell fatty acids as a taxonomic and diagnostic tool operated with the Sherlock CAS software applied to the Shimadzu GC-2010/2030 system. As a result, the 15:0 iso, 15:0 anteiso, 16:0, 17:0 iso and 17:0 anteiso have been primary fatty acid components in both taxa. The 15:0 iso and 15:0 anteiso were predominant components with 30.39 ± 2.53 and 32.13 ± 2.33 (%) in *B. velezensis* and 27.85 ± 1.67 and 31.88 ± 1.98 (%) in *B. amyloliquefaciens*, respectively. Furthermore, the dissimilarity in 16:0, 17:0 iso and 17:0 have drawn also a distinction between them. *B. velezensis* and *B. amyloliquefaciens* together with *B. agaradhaerens, B. pumilus, B. licheniformis* and *B. subtilis* forming one cluster which could be distinguished from other clusters revealed their close relationship. The fatty acid profiles of two taxa can be distinguished into two separated group of species. Furthermore, all samples were identified without misreading. Our study proposes that the applied method based on cellular fatty acid profiles can be applied to distinguish *B. velezensis* and *B. amyloliquefaciens*.

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DEVELOPMENT OF A NOVEL METHOD FOR GENETIC MODIFICATION OF *LICHTHEIMIA CORYMBIFERA*

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Lichtheimia corymbifera is an opportunistic human pathogenic Mucoromycotina species, which can cause primary cutaneous and deep tissue infections in immunocompromised patients. The knowledge about Mucoralean species is less comparing to other fungal pathogens due to limited genetic tools for investigating. Development of genetic transformation methods allows studying properties of chosen genes by targeting, modification or inserting new genetic elements into the genome. Until now, an effective transformation system has not been available for the genetic modification of *L. corymbifera*. CRISPR-Cas9 system is a highly efficient versatile genome-editing tool generating a double strand break, which can be repaired by non-homologous end joining (NHEJ), or homology directed repair (HDR). It has recently been optimized for another Mucoromycotina species, *Mucor circinelloides*. In this study, the plasmid free CRISPR-Cas9 method was used to construct an uracil auxotrophic mutant from *L. corymbifera*, Efficiency of the method was examined by disrupting *pyrG* gene encoding orotidine-5-monophosphate decarboxylase. gRNA and Cas9 nuclease were directly introduced to the fungal protoplasts using PEG-mediated protoplast transformation. After the transformation procedure, the protoplasts were inoculated onto YNB minimal media supplemented with uracil and 1.5 mg/ml fluoroorotic acid. The transformation efficiency was 8 colonies / 10^5 protoplast and the genome editing efficiency was 37.5 %. Molecular analysis of the transformant colonies indicated a three nucleotides gap upstream the PAM sequence in three isolates. The tested mutant strains were mitotically stable. The plasmid free CRISPR-Cas9 system was successfully used to disrupt the *pyrG* gene by NHEJ and our results suggested that the disruption of the *pyrG* gene has no effect on the growth and virulence of fungal strains. Therefore, obtained *pyrG* deletion mutants can be used for further experiments as a parental strain for analyzing the role of various virulence factors.

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VIRULENCE FACTORS AND IN-HOST SELECTION OF PHENOTYPES IN INFECTIOUS PROBIOTIC YEAST ISOLATES

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Saccharomyces yeast probiotics (marketed under the taxonomically invalid name *S. boulardii*) have long been successfully applied in the treatment of several gastrointestinal conditions, including *C. difficile* infection and diarrhea, in microbiome management, and have gained traction in animal husbandry/nourishment. Despite their widespread use, these probiotic yeasts are rare opportunistic pathogens responsible for a high proportion of *Saccharomyces* fungemia and mycosis cases. The potential virulence attributes of *S. 'boulardii'* as well as its interactions with the human immune system have thus been in the focus of various studies. Although we gained valuable insight into the characteristics of these yeasts, no information is available on how their phenotypes and virulence factors may change under the selective pressure exerted by the human host's body upon infection or colonization. To extend observations to yeasts that underwent potential in-host selection, we obtained both commercial and clinical isolates of the probiotic yeasts along with host data and compared the general phenotypic characteristics, cell morphology, virulence factors, epithelial and immunological interactions, and pathogenicity (in *Galleria* larva model) of altogether four probiotic product samples, two mycosis, and four non-mycosis (asymptomatic colonizer) samples of *S. 'boulardii'*. We assessed dozens of characteristics related to major steps of a potential yeast infection, including adhesion, penetration, tissue damage, inflammation, immune evasion, along with antimycotic susceptibility and found that the human isolates of the probiotic have diverse phenotypes. Mycosis and non-mycosis isolates both displayed novel characters when compared to the product isolates, but in the case of most virulence factors and in larval pathogenicity, differences were negligible or, surprisingly, the yeasts from products showed elevated levels. None of the tested isolates inflicted considerable damage to the human epithelial model or could cross it, and none of the isolates bore the hallmarks of adaptations towards immune evasion. Large differences were found in antimycotic susceptibilities among the samples. Our results showed that in-host selection is not directed towards refining virulence factors commonly associated with pathogenic yeast. In other words, the strains in the probiotic products possess characteristics that *ab ovo* enable them to act as pathogens upon permissive conditions, but nevertheless, may change in many ways once they start evolving in the host. These facts call attention on the shortcomings of virulence phenotyping in yeast research. More thorough assessment will thus be needed in potential future probiotics, especially in an era when genetically modified, designer probiotics are gaining momentum.

TUNING GENOMICS FOR HIGHLY HETEROZYGOUS AND POLYPLOID *SACCHAROMYCES* GENOMES: WHERE DID OUR COMMERCIAL AND CLINICAL YEASTS COME FROM?

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Saccharomyces cerevisiae is widely used yeast species subdivided into several clades adapted to human-made environments. These often show a tendency towards larger, polyploid genomes, and many of them show hallmarks if intra- and even interspecific hybridization. The large genome sizes and high heterozygosity, along with frequent and variable genome structure variations are defining features of these clades. But they are still compicated to study using genomic methods that are mostly tested on diploid organisms. To study commercial and related clinical yeasts, we applied ploidy- and chromosomal rearrangement-aware variant calling, machine learning variant scoring algorithms, and suitable internal controls, along with alignment- and assembly-free phylogenomic methods. Using phylogenomic network analysis based on alignment- and assembly-free methods and on ploidy-aware allele calling, we were able to trace back contemporary baking strains to artificial strain crossing and improvement strategies applied in the middle of the last century. We could show that most of the currently available baking yeasts are derived from a single common ancestor that became ubiqui-

tous not due to natural or human selection, but simply due to the fact that global yeast production companies eliminated competitors in the past decades. We show that probiotics, wine and baking yeasts colonize and infect humans frequently. Surprisingly, the domestication-driven genomic characteristics of commercial yeasts have resulted in baking-, probiotic- and wine-yeast-derived human isolates being remarkably different. The former preferably colonize the female genital tract and display large-scale chromosome copy number changes and rearrangements in their mosaic, polyploid genomes. At the same time, wine- and probiotic-derived human isolates lack such an anatomic niche preference and show lower levels of genome structure variation. The genomic adaptations we observe in human isolates derived from commercial strains are in striking contrast with the indigenous yeasts from non-Westernized populations sequenced in previous studies. Our study shows that the pathogenic and colonization potential of *S. cerevisiae* has been drastically altered by domestication and than by business decisions in the recent past, and even if *S. cerevisiae* is a true member of the human microbiome, it is now mostly present in us in the form of non-natural, domesticated clades.

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EFFECTS OF THE ORIGINAL HOSTS: POT EXPERIMENTS OF *PERICONIA MACROSPINOSA* ROOT ENDOPHYTES ORIGINATING FROM WHEAT AND MAIZE MONOCULTURES

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Dark septate endophytic (DSE) fungi represent one of the various groups of microorganisms that live in symbiosis with plants. DSE fungi colonize internal tissues of the roots without causing any immediate, overtly negative effects. *Periconia macrospinosa* is a common and widespread DSE that extensively colonizes gramineous plants on both agricultural and natural habitats. Although *P. macrospinosa* is a well-studied fungus, even on genome level, the interaction between the host plant and *P. macrospinosa* is still not completely understood, partly because of the genetical and functional heterogeneity of the different isolates. The functional heterogeneity of *P. macrospinosa* and other root colonizing dark septate endophytic (DSE) fungi may be derived from a certain degree of adaptation to a host plant. In this study, to broaden our understanding on host-adaptation of DSE fungi, we investigated conspecific isolates of *P. macrospinosa* originating from different hosts. Our aims were to examine the presence of intraspecific genetic diversity of *P. macrospinosa* using nineteen isolates, and test in pot experiment whether host-adaptation could be detected. The 19 *P. macrospinosa* strains were isolated at Martonvásár Agricultural Institute, from 60 yearlong monocultures of two crops: wheat and maize. Seven isolates originated from wheat and 12 isolates were collected from maize roots. In the experimental setup, both host plants were inoculated with all nineteen isolates. After 6 weeks of growth under 14 hours light-10 hours dark cycles, chlorophyll content of the leaves was measured. The roots were washed and collected for microscopy. The root and shoot of the plants were separated and dried out. The dry weight of the roots and shoots were measured. The root samples were stained by WGA-Alexa Fluor 488, and colonization was verified by fluorescent microscopy. For the better understanding of genetic heterogeneity, besides multilocus molecular phylogeny based on the internal transcribed spacer (ITS) region of the nrDNA, partial translation elongation factor 1-α (TEF) gene, large subunit (LSU) and in few cases small subunit (SSU) region of rDNA, inter sample sequence repeat (ISSR) analysis with four primers was also carried out on nineteen isolates.

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CARACTERISATION OF SURVIVAL FACTOR GENES IN *MUCOR CIRCINELLOIDES*

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In recent years, the incidence of mucormycosis (i.e., invasive fungal infections caused by members of the order Mucorales, such as *Mucor circinelloides*) has significantly increased worldwide. Such infections are considered as the third most frequent type of invasive mycoses after candidiasis and aspergillosis in patients with hematological malignancy, haematopoietic stem cell- and solid organ transplantation and diabetes mellitus. Survival factor protein (SVF) plays an important role in the protection of cells from oxidative and other stresses (e.g., cold stress) in *Saccharomyces cerevisiae*. Furthermore, this protein seems to participate in the sphingolipid biosynthesis of the fungal cells. Transcriptomic studies showed the upregulation of the encoding genes in several human pathogenic fungi during the host-pathogen interactions. However, the function and regulation of the SVF proteins and the encoding genes are still unknown in Mucorales fungi.

In the *M. circinelloides* genome, two hypothetical *svf* genes were identified and named as *svf1* and *svf2*. We have studied the expression of the genes after culturing the fungus under different conditions by real-time quantitative reverse transcription PCR. Using the CRISPR/Cas9 technique, single gene disruption mutants were constructed for each gene and we have started the characterization of the resulting strains. Macromorphology and sensitivity to different stressor chemicals (e.g., acetate, H_2O_2, Congo red and Calcofluor white) were tested. Mutants showed altered characteristics compared to the original strain suggesting that the cellular integrity may be damaged in the mutants. Pathogenicity of the mutants was also examined in alternative *Drosophila melanogaster* model and a decreased virulence was detected. The sphingolipid metabolism of the knock-out strains was also investigated with HPLC techniques. Our results showed significant differences in the sphingolipid content of the strains.

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IN-DEPTH ANALYSIS OF AAV-CONTAINING FRACTIONS EXTRACTED FROM A CSCL ULTRACENTRIFUGATION GRADIENT

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In recent years, many types of RNA and DNA viruses have been developed as recombinant viral vectors for the delivery of therapeutic genes to treat a variety of acquired and inherited diseases. Among them, adeno-associated virus (AAV) has shown great potential, what led to the approval of gene therapeutics. Since AAV are also included in the vaccine program of COVID (e.g. AAVCOVID vaccine), the increasing demand for rAAV highlights the urgent need to address the current bottlenecks in production, where the cell harvest always contains a mixture of impurities and viral particles. Unfortunately, not all viral particles produced are effective as they may lack the DNA molecule completely and are therefore also considered as impurities. Current downstream purification (e.g. CsCl gradient ultracentrifugation) may not be sufficient in removal of impurities, what reduces the expected therapeutic effect. In our work, we performed an in-depth comparison of four AAV-containing fractions from the 2nd CsCl ultracentrifugation gradient (named heavy, empty, intermediate, and empty), which surprisingly exhibited similar transduction efficiencies. We implemented a holistic approach and analysed the distribution of AAV particles and contaminants such as unwanted DNA molecules, host cell proteins, unwanted AAV viral particles, and aggregates. Numerous analytical methods (transmission electron microscopy [TEM], cryo-electron microscopy [cryoEM], transduction efficiency assay, digital droplet PCR [ddPCR], quantitative PCR [qPCR], Enzyme-linked immunosorbent assay [ELISA], multi-angle dynamic light scattering [MADLS], multi-angle light scattering combined with size-exclusion chromatography [SEC-MALS], capillary electrophoresis and High-Throughput Sequencing [HTS]) were tested side by side on two different rAAV9 (single-stranded AAV and self-complementary AAV) produced at International Centre for Genetic Engineering and Biotechnology (ICGEB) in Triste, Italy. The results showed that the fractions were relatively similar in terms of relative vector and impurities content, except for the heavy fraction, in which we observed a high proportion of single-stranded host cell DNA and many aggregates. As expected, the optimal fraction containing the highest amount of full AAVs and the lowest amount of impurities was the full fraction. Interestingly, the intermediate fraction, which is normally discarded, also contained a high proportion of full virus particles. Although the empty fraction contained the highest proportion of empty and damaged viral particles, the viral genome titer was relatively high. Furthermore, multiplex ddPCR in combination with TEM, demonstrated that rAAVs classified as full viral particles using TEM may not contain full size vector genomes, suggesting that the distinction between full and partially filled viral particles cannot be correctly assessed using TEM alone. We have noticed some improvements in the classification of viral particles using cryoEM instead of TEM and automatic image analysis with cryoSPARC software instead of manual image analysis. Additionally, fragmentation of vector genomes was observed using capillary electrophoresis performed on the isolated vectors' DNA.

MODIFIED STRESS PROTEIN PROFILE OF *CAMPYLOBACTER JEJUNI* IN INTERACTION WITH FOOD SPOILER

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Foodborne pathogen *Campylobacter jejuni* is the most common cause of acute bacterial gastroenteritis worldwide. The persistence of *C. jejuni* in food processing is mainly associated with biofilms on food manufacturing surfaces, which are the main risk for the spread of *Campylobacter* in the food chain. We therefore hypothesise that biofilms provide an envi-

ronment in which other bacteria also contribute to the survival of *C. jejuni* in food-related environments. Spoilage-causing bacteria *Pseudomonas fragi* are ubiquitous in the environment and are often part of the primary food microbiota. In addition, they colonise surfaces in the food industry where they form biofilms and thus persist. Our aim is to study the protein profile changes in biofilm cells of *C. jejuni* while interacting with *P. fragi* by the following steps: (i) Modification of the atmosphere (aerobic and microaerophilic) and temperature of 30°C of a 48 h mature biofilm. (ii) Optimization and isolation of total proteins, purification and enzymatic degradation with a trypsin/Lys-C mixture. (iii) Identification and quantification of proteins by label-free mass spectrometry and analysis using bioinformatics tools. The total protein profile of *C. jejuni* biofilm cells that interacted with *P. fragi* was evaluated in comparison to *C. jejuni* biofilm cells in monoculture. *C. jejuni* biofilm cells interacting with *P. fragi* under aerobic conditions showed a lower presence of proteins critical for the stress response of *C. jejuni*, such as protein biosynthesis (Tu), peroxidase (AhpC), refolding protein (groEL) and expression of DNA stabilisation protein (HU). This lower stress response in biofilm cells confirms the altered survival mechanism of *C. jejuni* when interacting with the excellent biofilm former *P. fragi*. Future analyses will allow us to outline the proteins involved in biofilm processes with *Pseudomonas*. In summary, our proteomic research reveals the dynamic biofilm interactions that enable the pathogen *C. jejuni* to adapt to new conditions, particularly in biofilm formation.

THE EFFECT OF PHYTOBIOTIC-PREBIOTIC MIXTURE ON INTESTINAL MICROBIOTA OF PIGLETS

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The gut microbiota plays important role in maintaining the health of host animal. Escherichia coli and other members of Enterobacteriaceae (commonly called “coliforms”) are part of the normal intestinal microbiota of humans and animals, but many of them are known as pathogens, therefore coliforms represent a risk in livestock breeding, especially in the case of swine. Antibiotics and other antimicrobial agents have been used as feed supplements to preserve the health of farm animals for decades. In case of swine, zinc oxide is very efficient against post-weaning diarrhoea, although increased use of it can cause the emergence of multi-resistant *E. coli* strains as well as environmental hazards. In 2017 the European Medicines Agency decided to ban the use of zinc in the future, thus, pig breeders need to find new solutions against post-weaning diarrhoea, such as the use of pre/probiotics or herbal extracts. Curcumin, wheat germ, and chicory are favorable natural products for enhancing the growth performance and improving the intestinal condition of the pig. The present study aimed to investigate the effects of the phytobiotic-prebiotic mixtures of these compounds on the growth performance and the intestinal microbiota in weaning piglets. The trial feed supplement was compared with the effect of positive (zinc oxide) and negative (no feed supplement) controls. Treated and control groups consisted of 16-20 animals. All feed supplementations started at weaning (4 weeks of age). To investigate the effects of feed supplements on intestinal microbiome fecal samples were collected and analyzed by conventional microbiological and metagenomic methods. Changes in the total number of coliforms (potential pathogens) and lactic acid bacteria (beneficial microbes) was evaluated on semi-selective and differential agar plates in case of treated and control animals. The abundance of the bacterial communities of fecal samples were estimated using high-throughput sequencing on the Illumina MiSeq platform. Both phytobiotic-prebiotic mixture and ZnO were able to significantly reduce the amount of coliforms after weaning, although ZnO reduced the amount of colifoms more than the trial feed additive, but the amount of potentially beneficial bacteria was also significantly reduced with the use of ZnO. The analysis of metagenomic data also confirmed this result: Enterobacteriaceae and *E. coli* were 1.20% in trial while it was 0.21% in positive control at 6 weeks of age, but Lactobacillaceae were more abundant in trial group (29.98%) than positive (8.67%) and negative (22.45%) controls. Similar tendencies were observed at 12 weeks of age. Our results suggest that the mixtures containing curcumin, wheat germ, and chicory may prove to be suitable alternatives to prevent infection caused by coliforms without disturbing the beneficial microbes of the intestinal microbiome.

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INVESTIGATION OF THE ANTIMICROBIAL EFFECTS OF PROTOFLAVONOID COMPOUNDS

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Treatment against infection of pathogenic bacteria causing different diseases in plants, animals, and humans is based on the use of antibiotics, synthetic antibacterial agents, or derivatives of these compounds. However, the resistance between bacteria can easily develop to one or more drugs resulting in a continuous need to design new inhibitors against resistant or multidrug-resistant bacteria. Various compounds of natural or synthetic origin, including semi-synthetic derivatives of natural products, are investigated as potential antibiotics against pathogens. Protoflavonoids form a special group of flavonoids, possessing a non-aromatic B-ring and a hydroxyl moiety at C-1′. In nature, these compounds can generally be found in ferns, however, synthetic, and semi-synthetic methods are also available to obtain natural and artificial derivatives. Protoflavonoids created scientific interest due to their potent anticancer activities, in which regard, protoapigenone and its analogs represent the yet most deeply studied derivatives due to their strong cytotoxic effect on breast-, ovarian- and prostate tumor cells. Besides the anticancer activities of protoflavones, however, only limited information is available on other, possible biological effects of the compound class. In this study, the antibacterial effect of four protoflavonoids, protoapigenone (PA), tetrahydroprotoapigenone (PAH), and the corresponding 1′-O-butyl ether analogs of these compounds, protoapigenone 1′-O-butyl ether (PABUT), and tetrahydroprotoapigenone 1′-O-butyl ether (PABUTH) were examined. The antibacterial effects of these compounds on various Gram-positive (*Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, Bacillus cereus*), and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, Klebsiella pneumoniae*) were evaluated. In microdilution tests, protoapigenone and its 1′-O-butyl ether analog showed antibacterial effect against Gram-positive bacteria and were ineffective against the tested Gram-negative bacteria. In contrast, saturated B-ring analogs of these compounds (PAH and PABUTH) did not show an inhibitory effect against the tested bacterial strains. Adjuvant antibacterial activities of the examined protoflavonoids with various commercially available antibiotics have also been tested, but no synergistic effects were observed. Our results suggest that the presence of the p-quinol dienone molecular moiety in the B-ring of protoflavonoids, consisting of the 2′-3′ and 5 ′-6′ double bonds, might appear as a prerequisite for the antibacterial effects of these derivatives. Further studies are underway involving additional newly synthesized protoflavonoid derivatives.

THE FUNGUS *KALMUSIA LONGISPORA* IS ASSOCIATED WITH GRAPEVINE TRUNK DISEASES

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Grapevine trunk diseases (GTDs) are devastating fungal infections of the grapevine. The associated pathogens colonize the woody tissues of trunks and develop necroses which - alongside symptom expression on green parts- can lead to the death of the host plant. To this date, more than 200 filamentous fungal species are linked to this disease group. Our study extends the list of these pathogens with the otherwise poorly characterized fungus *Kalmusia longispora*. One strain (T15142) of this fungus was isolated from a GTD-affected trunk in 2015 and its pathogenicity was compared with two strains from the CBS-KNAW collection (CBS 824.84 and CBS 582.83) isolated from *Triticum aestivum* and *Arceuthobium pusillum*. Artificial infections were carried out on green shoots, canes in the field, and on potted cutting of grapevine. The highest pathogenicity was observed in the case of T15142, while moderate development of symptoms was seen in the case of CBS 824.84. The strain CBS 582.83 showed low pathogenicity, especially lignified tissues. Despite their varying virulence, all strains can be reisolated frequently from all types of infected plants. This suggests, that the low virulence of CBS 582.83 may result from the lack of virulence factors responsible for the damaging of the host, rather than the poor growth capacity of the strain in planta. To test this hypothesis, digestive exoenzymes were detected on indicative media and quantified by spectrophotometric methods in liquid cultures. While no amylase activity was detected, all three strains proved to be equally good producers of cellulases and secreted a low amount of pectinases. The levels of laccase activities are seemingly correlated with the pathogenicity of the strains. CBS 824.84 and T15142 strains produced 30 to 45 times more laccases than CBS 582.83. Since these enzymes are responsible for lignin decomposition in woody tissues, their low level in the case of CBS 582.83 may explain the low pathogenicity of the strain. The observed differences in the patho-

genicity of the strains may be due to their previous adaptation to different hosts, or the long-term preservation of the two strains obtained from a collection. All the above results suggest that *K. longispora* is a considerable vascular pathogen of grapevine and its possible role in the symptom development of GTDs should be taken into account.

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DESCRIPTION OF FIVE NOVEL MYCOVIRUSES BELONGING TO THE TOTIVIRIDAE FAMILY IN FOUR DIFFERENT *MUCOR HIEMALIS* STRAIN

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Up to this day, around 250 mycovirus genome sequence can be found in the NCBI (National Center for Biotechnology Information) database and, due to the new bioinformatical approaches, this number is continuously increasing. A vast majority of the described mycoviruses have linear double-stranded RNA (dsRNA) genomes and are typically symptomless (cryptic), which only manifests in the phenotype of the fungi under certain circumstances. They have a unique characteristic relative to transmission route, since they can spread only intracellularly. Five genera belong to the Totiviridae family, among them, fungal viruses are generally found the *Totivirus* and the *Victorivirus*. Recently, more and more mycoviruses are identified from the so-called early-diverging lineages of fungi including Mucoromycota; however, we still have less information about them compared to the subkingdom Dikarya. Previously, we have screened 123 *Mucor* strains for the presence of dsRNA molecules without further molecular studies. In this work, we describe novel dsRNA viruses detected in four different *Mucor hiemalis* strains with next-generation sequencing (NGS). We have done the molecular characterization of five dsRNA viruses, namely, *Mucor hiemalis virus 1a* (MhV1a) from the strain WRL CN(M) 122; *Mucor hiemalis virus 1b* (MhV1b) from NRRL 3624; *Mucor hiemalis virus 2* (MhV2) from NRRL 3616; and *Mucor hiemalis virus 3* (MhV3) and *Mucor hiemalis virus* (MhV4) from NRRL 3617. The identified genomes contain two open reading frames (ORF) encoding the coat protein (CP) and the RNA dependent RNA polymerase (RdRp). In MhV1a and MhV1b, it is predicted to be translated as a fusion protein via a -1 ribosomal frameshift, while in MhV4 via a rare +1 (or-2) ribosomal frameshift. In MhV2 and MhV3, the presence of specific UAAUG penta-nucleotide motif point to the fact for coupled translation termination and reinitialization. Based on the phylogeny inferred from the RdRp sequences, MhV1, MhV2 and MhV3 belong to the genus *Victorivirus*, while MhV4 belong to the *Totivirus*. The VLPs detected in the *Mucor* strains are from 75 to 185 nm in diameter. Northern blotting was used to identify the dsRNA molecules containing the virus genes.

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HMBA REGULATES NORMAL EXPRESSION OF THE ENDOCHITINASE CHIA

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B-type high mobility group box domain proteins (HMGB) have a role in the modulation of chromatin organisation. In our research group we study the function of the HMGB proteins in the Ascomycota haploid filamentous fungus *Aspergillus nidulans* which is one of the most studied filamentous fungal model organism. Beside linker histones (H1 and H5), HMGB proteins plays architectural role in chromatin organisation and their ability to bind to linker DNA together with their ability to establish protein-protein interactions with other chromatin associated proteins makes the HMGB proteins as important functional chromatin component necessary for the proper induction or repression of gene expressions. Three architectural HMGB proteins, namely HmbA, HmbB and HmbC were identified in *Aspergillus nidulans*. We have many information about HmbB, but the function of the other two, HmbA and HmbC is unknown yet. The presented study reveals the important role of HmbA in the establishment of mycelial morphology and normal growt rate. We created a *hmbAΔ* deletion mutant strain which grows much slower on all tested media in comparison to the wild type strain and it has an abnormal micromorphology. While the apical growth of wild type strain is continuous, chitin is accumulated at the hyphal tips, the apical growth of *hmbAΔ* mutant is discontinous. The polar growth is stopped and after a time the elongation is re-started by emerging a new tip, the stalled tip remaining behind. This aberrant growth-process is deduced on the basis of the observation that the mid-sections of the elongated hyphae show widened round structures with high chitin deposition at their perimeters. We monitored the expression level of genes involved in chitin biosynthesis and chi-

tin hydrolysis and found that the endochitinase encoding *chiA* gene is significantly downregulated in the *hmbAΔ* mutant compared to the *hmbA*+ control. We hypothesised that downregulation of *chiA* contributes to the discontinous growth phenotype. To test this hypothesis we overexpressed the *chiA* gene in a *hmbAΔ* strain. The overexpressed *chiA* remediated the abnormal accumulation of chitin, and the micromorphology of the mycelia was restored.

DICHOTOMOPILUS FINLANDICUS SP. NOV.: A NEW *CHAETOMIUM*-LIKE SPECIES FROM EUROPEAN INDOOR ENVIRONMENTS

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The genus *Chaetomium* is one of the most common fungal taxa world-wide, present in various substrates and geographical regions. *Chaetomium*-like species occur in indoor environments where they can degrade cellulose-based construction materials, thereby the structure of the building is damaged. In addition, many species of this genus can have adverse effects on human health. This study aimed to identify *Chaetomium*-like strains isolated from indoor environments in Hungary and Finland, investigate their morphology, study their physiological properties, measure their extracellular carbohydrate-degrading enzyme activities. *Chaetomium*-like strains were isolated from schools, offices and flats in Hungary and Finland. Fragments of the internal transcribed spacer (ITS) region of the ribosomal RNA gene cluster, the translation elongation factor 1α (*tef1α*), and β-tubulin (*tub2*) genes, as well as the second largest subunit of RNA polymerase II (*rpb2*) were sequenced, and phylogenetic study was performed by maximum likelihood analysis. The morphology of the *Chaetomium* strains were performed by using stereo-, optical- and scanning electron microscopy. Growth of the isolates was examined at different temperatures (4, 15, 20, 25, 30, 37, 35, 40 and 45°C), while their extracellular enzyme production was determined spectrophotometrically. Thirty-one *Chaetomium* sp. strains (15 from Hungary and 16 from Finland) were examined during the study. The most common species was *Ch. globosum* in both countries. In Hungary, 13 strains were identified as *Ch. globosum*, 1 as *Ch. interruptum*, and 1 as *Ch. cochliodes*, while in Finland, 10 strains proved to be *Ch. globosum*, 2 strains were *Ch. cochliodes*, 2 were *Ch. rectangulare*, and 2 isolates (SZMC 26527, SZMC 26529) proved to be representatives of a yet undescribed phylogenetic species from the closely related genus *Dichotomopilus*, which we formally described as the new species *Dichotomopilus finlandicus*. The optimal growth temperature of all isolates was between 25–30°C. The *Ch. globosum* strains grew at temperatures between 15-40°C, they were unable to grow at 4°C, and only three Hungarian *Ch. globosum* isolates grew at 40°C. One *Ch. rectangulare* strain grew at 4°C. The studied *Ch. cochliodes, Ch. interruptum* and *Ch. rectangulare* isolates showed no growth at 37°C. Strains of the new species *D. finlandicus* showed similar growth at all temperatures tested, the optimum growth temperature for both strains was around 30°C, and both could also grow at 37°C. The enzymatic activity of the *Chaetomium*-like strains proved to be diverse, and no correlation was found with either the isolation site or the growing substrate.

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BIODEGRADATION OF POLYLACTIC ACID BASED BIOPLASTIC BY BACTERIA STRAINS

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For the comfort of modern living, the use of plastics has become essential (clothing, construction, medicine, packaging, food industry), and the production of plastic has preceded the production of any other material. Due to beneficiary properties of plastic (strength, stiffness, etc.) the availability is versatile and easy, although after usage the natural degradation of plastic is very slow, or partly undegradable, causing serious pollution to the environment. It is our own interest to maintain a healthy habitat and reduce the consumption of plastic. According to a regulation adopted by the European Parliament in 2019 (Directive 2019/904), the use of petroleum-based plastics in the European Union should be phased out from 2021 to the use of alternative biodegradable plastics in order to reduce pollution. Such an alternate and environmentally friendly solution can be to switch to the use of bioplastic made of biological resources (renewable energy sources) and biodegradable materials, like the PLA (polylactic acid) from lactic acid with poliesterification in industrial conditions, which is a heat tolerant bioplastic. It is crucial to get to know the background of the biological degradation process besides the knowledge of preparations of production process. Because of the strain selection pre-experiments are necessary for

testing the degradation of polylactic acid, which examines the applied bacteria's (which are *Bacillus* and *Rhodococcus* strains) PLA utilization on solid medium. Another important step is to map the enzyme set of the applied microorganisms especially because there are references in the scientific literature about the degradation of natural polymers are coherent with proteolytic-protease and lipolytic- cutinase enzyme activity properties of certain microorganisms. The examination of the microbiological degradation of PLA operates on submerged environment for 60 days, it can be followed by the released lactic acid and the mass change by the help of previously selected microorganism strains. During the monitored 60 days of experiment, the protease and cutinase enzyme production peaked at highest on the 7th day with the bacterial consortium which reached 25% mass decrease. Hopefully, by selecting new bacteria strains or consortium suitable for biodegradation, then increasing the efficiency of these strains and expanding the knowledge gained on the subject, it will provide a promising basis for developing and increasing the biodegradability of polylactic acid-based bioplastics.

A NATURAL APPROACH AGAINST THE ACTIVITY OF FOOD SPOILAGE YEASTS: FOCUSING ON PLANT PHENOLICS

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Phenolic compounds are important in the defense mechanisms of plants and can be extracted from antioxidative fruits and vegetables through physical, chemical and biological methods. These natural compounds are potent antimicrobial agents against a host of microorganisms, however, the activity on spoilage yeasts is rarely studied. In this study, we investigated the activity of selected phenolics, i.e., hydroxybenzoic acid, hydroxycinnamic acid, stilbene, flavonoid and phenolic aldehyde compounds, against the planktonic growth, biofilm formation and adhesion of *Pichia anomala, Saccharomyces cerevisiae, Schizosaccharomyces pombe* and *Debaryomyces hansenii*. These yeasts can be spoilers in fruit juices, wines and dairy products. Results showed significant anti-yeast properties for most phenolics studied. Cinnamic acid and vanillin exhibited the highest antimicrobial activity with minimum inhibitory concentration (MIC) values ranging from 500 µg/mL to 2 mg/mL. For instance, cinnamic acid had more than 90% growth inhibition in all the food spoilage yeasts at 500 µg/mL. And vanillin had more than 90% growth inhibition at 1 mg/mL in *D. hansenii* and *P. anomala*. Quercetin, (–)-epicatechin, resveratrol, 4-hydroxybenzaldehyde and p-coumaric acid were also efficient growth inhibitors for certain yeasts with a MIC of 2 mg/mL. Growth inhibitory effect of some studied phenolics was also investigated by combining them in a microplate assay. In *S. pombe*, for instance, checkerboard methodology showed 16-, and 4-fold reductions in MIC of (–)-epicatechin and cinnamic acid, respectively, in the presence of vanillin. The *D. hansenii, P. anomala* and *S. pombe* biofilms were sensitive to the phenolics, while the *S. cerevisiae* biofilm was quite resistant to the activity of the phenolics. Fluorescence microscopy analysis revealed the disruption of the biofilm matrix by the phenolics. The highest antiadhesion activity was registered for cinnamic acid with inhibition effects between 48% and 91%. Concerning molecular targets, in silico studies revealed a high binding potential for some phenolics to certain proteins in *S. cerevisiae*; many binding sites are located on conserved sequence motifs. The active phenolics can be valuable natural compounds against food-contaminating yeasts in future preservative technologies.

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EMERGING AND RE-EMERGING INFECTIOUS DISEASES AS THE BIGGEST CHALLENGE OF PUBLIC HEALTH

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Globalization, climate change, and socio-economic changes are contributing to the emergence of new and re-emerging infectious diseases that pose threat to the human and animal population. Identification of these microorganisms as quickly and accurately as possible has utmost importance for public health. According to the law, the National Public Health Center (NPHC) is responsible for the diagnostic of the Risk Group 3/4 pathogens. Over the last decade in the NPHC's National Biosafety Laboratory the traditional and molecular diagnostic background of the emerging pathogens has been set up, further developed, and tested in international external quality assurances. Crimean-Congo hemorrhagic fever (CCHF) is an emerging tick-borne disease that is endemic in the Balkan region of Europe. CCHF is spreading northwards following expanding distribution of its main vector *Hyalomma marginatum* which is present in Hungary. First comprehensive country-wide pilot surveillance focusing on both the human and animal population in Hungary was set up and seroprevalence

was assessed. Overall 2,700 serum samples from healthy blood donors and 1,711 serum samples obtained from free-range cattle were screened for anti-CCHF IgG presence. Ten seropositive blood donors were found in the central, southern, and western regions of Hungary. Only 2 cattle serum samples showed reactivity, both from the southern part of the country. The Ebola outbreaks in Africa posed significant challenges for outbreak response teams to ensure adequate conditions of specimens for reliable laboratory testing. Viability during mid-term storage of the Ebola virus in different clinical specimens was determined. Ebola virus (EBOV) was the most stable in EDTA-whole blood and plasma samples, whereas rapid decay of infectivity was observed in simulated capillary blood, urine, and semen samples, especially at higher temperatures. Both temperature and clinical specimen type have significant effects on virus viability, whereas donor differences were not observed. The virus isolation is an essential tool to assess the presence of infectious virus in different body fluids and to estimate the risk of infection posed on the population. When comparing the results of the *in vitro* (VeroE6) and *in vivo* animal (suckling mice and SCID mice) virus isolation we found the animal model is more sensitive. Only 12.44% of the samples could be isolated on VeroE6 cell cultures, while 23.08% SCID mice were EBOV positive and 15.91% suckling mice groups were positive. The disease caused by SARS-CoV-2 has impacted the world beyond the limits of public health. Continuous virus isolation, characterization and maintenance of SARS-CoV-2 strain collection support the Hungarian vaccine development. SARS-CoV-2 genomic surveillance coordinated by NPHC's is a critical public health function with the primary objective to inform national decisions around public health and social measures, diagnostics, therapeutics, and vaccination. Variant surveillance consists of targeted and randomized sample selection and use different molecular tools including real-time RT-PCR, digital droplet RT-PCR and next generation sequencing methods. All variant of concern were detected. To facilitate on-the-spot diagnosis, on-field performance of 10 commercially available SARS-CoV-2 antigen rapid tests was compared with RT-qPCR as reference method. At strong RT-qPCR positive samples (Ct < 25) majority of the tests showed good sensitivity and specificity. The NPHC as a reference laboratory center has a leading role in Hungary on the field of emerging infectious diseases.

CHARACTERIZATION OF A *SPORENDONEMA CASEI* ISOLATE, A RARE FUNGAL CONTAMINANT OF CHEESE

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Sporendonema casei is a rarely encountered fungal species, despite its first description was nearly two hundred years ago by Desmazières in 1827. It is known from cheese and a dried meat product Culatello, forming characteristic red spots on their surface. This species is mainly considered as a contaminant on food products, but it is also used as a ripening starter culture in the production of some types of hard cheese. Previously, *S. casei* was available as starter culture used for example in the production of Cantal. It was thought that this species adapted to the habitat of foods which are preserved by salting and drying, furthermore, high in fat and protein. However, recently it was also isolated from a marine-derived sediment sample and described as a producer of some anthraquinone derivatives showing anticoagulant, cytotoxic and promising antibacterial activities. Previously, we have isolated a *S. casei* strain from a Transylvanian hard cow cheese. Based on the presence of this species on certain foods, the few available representatives in culture collections and its Janus-face nature of being used in starter cultures, at the same time described as potential producer of toxic metabolites, we started to characterize the isolated strain of *S. casei*. Our results showed its halotolerant nature and potential of extracellular lipase-production makes it suitable for colonization of food habitats as cheese and dried meat products. Its interaction with some other fungal species as well as its metabolite production were also investigated.

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DEVELOPMENT OF A FERMENTATION GROWTH TECHNOLOGY FOR A *MESORHIZOBIUM CICERI* CHICKPEA SYMBIONT STRAIN

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Chickpea (*Cicer arietinum* L.) is a nutrient-rich dry legume plant. Its dry crop (called also chickpea) is one of the cheapest sources of protein for food and feed. Global production of chickpea increased from 7.68 million tonnes to 17.22 million tonnes in 2018. Its sowing area is also increasing in Hungary, where it is a priority crop under the National Feed Protein Programme. Chickpea contains several essential amino acids such as lysine, methionine, threonine, valine, isoleucine and leucine. In terms of nutrient composition, it contains 60-65% carbohydrate, 6% fat and 12-31% protein. In addition, it is an excellent source of various soluble and insoluble fibres, vitamins (rich in B vitamins) and minerals (such as potas-

sium and phosphorus). Besides its beneficial nutritional properties, it also contributes to soil fertility through biological nitrogen fixation. The symbiotic nitrogen-fixing rhizobium of chickpea plant is *Mesorhizobium ciceri*. Our aim was to develop a culture medium and fermentation technology on which *M. ciceri* could grow faster and have a higher titer, than on the commonly used YEM (yeast extract-mannitol) medium, which is unsuitable for commercial-scale production. As a starting point, we have mapped the carbon source utilization pattern of the strain in a high-throughput, microtiter plate experimental setup. Then, after narrowing down the potential carbon sources, we performed growing experiments in agitated flask cultures: we investigated the effect of the phosphate salts and fermentation time on the cell number obtained, in addition to the most utilizable carbon sources. Subsequently, using agitated flask cultures, the effect of four complex nitrogen sources -such as meat peptone, soybean peptone, corn syrup and yeast extract - on the cell count was also investigated. Finally, the optimal concentrations of the most suitable carbon or nitrogen sources for the production of biomass were determined by a response-surface method in a centrally arranged experimental design. The results of the experiments showed that the quality of the complex nitrogen source had no significant effect on the growth of the strain. The optimum culture medium contains 9.2 g/L D-mannitol and 3.4 g/L soybean peptone, on which the outstanding 1.13×10^{10} CFU/mL titer was achieved after 41 h of growth.

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CHARACTERIZATION OF THE PLEIOTROPIC DRUG RESISTANCE TRANSPORTERS IN THE AZOLE RESISTANCE OF *MUCOR CIRCINELLOIDES*

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Mucormycosis is a life-threatening systemic infection caused by certain members of the filamentous fungal order Mucorales (*Rhizopus oryzae, Lichtheimia corymbifera*, and *Mucor circinelloides*). Mucormycosis is associated with a high mortality rate, which can be nearly 100% depending on the underlying condition of the patient. Treatment of mucormycosis is a great challenge because Mucormycotina species are intrinsically resistant to most of the routinely used azoles. Members of the ATP binding cassette transporter superfamily, especially the pleiotropic drug resistance (PDR) transporter subfamily, can play role in the drug efflux and drug resistance. In the *Mucor* genome database, eight putative *pdr* genes were found. The relative transcript level of the *pdr* genes was measured after azole treatment using quantitative real-time PCR. We have started to create and characterize single (growth ability, susceptibility to different antifungal agents) and double knock-out mutant from pdr genes using a CRISPR-Cas9 system. *Pdr* genes responded differently to the azoles but, only *pdr1* showed significantly increased relative transcript levels in response to all azoles. From the six tested azoles, only itraconazole had the widest effect on *pdr* expression upregulating five of the eight genes. Deletion of *pdr1* caused significantly increased transcript level of *pdr2* and *pdr6*, while the lack of *pdr6* resulted significantly increased transcript level of *pdr1, pdr7*, and *pdr8*. Disruption of *pdr1* and *pdr2* resulted increased sensitivity to posaconazole, ravuconazole, and isavuconazole. Our result suggested that the regulation of *pdr* genes is highly interconnected and certain *pdr* genes may compensate the lack of the deleted genes. The azole resistance of *Mucor* cannot be completely explained by the activity of PDR proteins, other proteins, regulatory processes might be involved.

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LIGHTING THE DARK – TAXONOMIC AND METABOLIC DIVERSITY OF THE WORLDWIDE COMMON GRASS ROOT ASSOCIATED FUNGAL GENUS *DARKSIDEA*

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Dark septate endophytes (DSE) represent a group of fungal root colonizers that live internally and asymptomatically in the below-ground tissues. DSE fungi are worldwide common and frequent in arid, semiarid and also temperate grasslands ecosystems of Asia, Europe and North America. Previous findings suggest that there are core members of those

communities common to disparate regions worldwide. One of these dominant DSE groups is represented by *Darksidea* species. This complex genus belongs to Pleosporales (Ascomycota) and has been described recently comprising at least six species to date. However numerous distinct lineages represent further potential novel congeneric species. Based on the variant colony morphology of the *Darksidea* isolates, their genetic diversity and complexity, functional differences such as the production of different and variant secondary metabolites are hypothesized. In this work our aims were (i) to carry out a comprehensive study on phylogeny and metabolite diversity of the genus *Darksidea* using isolates originated from wide scale, and (ii) to find potential correlations between the arrangement of congeneric isolates by multilocus phylogeny and metabolite profiling. Classical morphological examination and molecular identification of more than one hundred *Darksidea* isolates from different countries such as Hungary, Kazakhstan, Mongolia, and the USA, representing all of the known species including the undescribed lineages has been carried out. For molecular multilocus phylogeny four loci: ITS (internal transcribed spacer region of nrDNA), LSU (partial 28S region of nrDNA), TEF (partial translation-elongation factor 1-α gene) and TUB (partial β-tubulin gene) were amplified and sequenced. The metabolite profiling has been carried out using high performance liquid chromatography (HPLC)-tandem mass spectrometry (MS/MS). Chemical structures of the detected compounds were also elucidated by nuclear magnetic resonance (NMR) spectroscopy. In conclusion, we found at least six further novel species and high metabolic diversity within *Darksidea*. Several common and species-specific natural products of the distinct lineages have been identified.

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FUNCTIONAL ANALYSIS OF BZIP TRANSCRIPTION FACTORS IN *ASPERGILLUS NIDULANS*

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Regulation of signal transduction pathways is critical for the maintenance of cellular homeostasis and development in fungi. Transcription factors are key elements of this regulatory network. For example the b-ZIP type transcription factor, AtfA is essential in the stress defense system of filamentous fungi. It also coordinates the production of secondary metabolites as well as fungal development for instance in *Aspergillus nidulans, Neurospora crassa* and *Fusarium verticillioides* or even fungal virulence in *Aspergillus fumigatus*. In *A. nidulans* another b-ZIP type transcription factor, the AtfB may be important in the survival of conidiospores. These two transcription factors can cooperate in the stress defense of *A. nidulans* by forming a heterodimer. To confirm this hypothesis we constructed and phenotypically studied deletion and overexpression mutants of *atfA* and *atfB* in all combination (Δ*atfA,* Δ*atfB,* Δ*atfA*Δ*atfB,* Δ*atfAatfBOE,* Δ*atfBatfAOE, atfAOE, atfBOE* and *atfAOEatfBOE*). The following phenotypes of the mutants were observed in the presence of oxidative stress generating agents: The Δ*atfA* was sensitive to 0.8 mM tBOOH, 2 mM diamide and 0.08 mM menadione. The oxidative stress sensitivity of the Δ*atfB* mutant was comparable to that of the control strain. The Δ*atfA*Δ*atfB* strain turned out to be moderately sensitive to 0.8 mM tBOOH and also highly sensitive to 2 mM diamide. Neither the *atfA* nor the *atfB* overexpression compensated the negative effect of tBOOH in the deletion mutants. However, the overexpression of atfB protected the fungus against tBOOH stress. Neither *atfA* nor *atfB* overexpression increased the tolerance of the fungus to menadione, but atfB overexpression alone resulted in similar menadione sensitivity as in the control strain. The stress sensitivity of the *atfAOEatfBOE* mutant and the control strain were comparable in the presence of 2 mM diamide, 0.8 mM tBOOH as well as 0.08 mM menadione. We are also planning bimolecular fluorescence complementation experiments (BiFC) for the confirmation of the possible AtfA-AtfB heterodimer formation in vivo.

A SELECTIVE PROCEDURE FOR DNA AND PROTEIN ISOLATION FROM MARINE MACROPHYTE SURFACES

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To successfully apply methods such as 16S rRNA sequencing, metagenomics, and metaproteomics on microbial communities inhabiting surfaces of marine macrophytes, microbial DNA and proteins need to be selectively isolated with a minimum of disrupting the host tissue. Methods for separating microbial cells from the host such as shaking, scraping

and ultrasonication have been shown to be insufficient. These methods do not provide a complete cell detachment and in the case of ultrasonication may also cause host tissue disruption. To circumvent these problems, two protocols for a selective extraction of DNA and proteins from epiphytic communities of two marine macrophytes, the seagrass *Cymodocea nodosa* and the macroalga *Caulerpa cylindracea*, were adapted. An almost complete removal of the epiphytic community regardless of the sampling season, location, settlement or host species was achieved. The extracted DNA was suitable for metagenomic and 16S rRNA sequencing, while isolated proteins could be identified by mass spectrometry. Low macrophyte DNA and protein presence indicated a high specificity of the protocols towards epiphytic communities. Furthermore, the procedures are based on universally available laboratory chemicals making them widely applicable. In conclusion, the adapted protocols ensure an almost complete removal of the surface associated microbial community, are highly selective for microbes inhabiting macrophyte surfaces and provide DNA and proteins suitable for 16S rRNA sequencing, metagenomics and metaproteomics.

SPATIAL, TEMPORAL AND VEGETATIONAL EFFECT ON BACTERIAL AND ARCHAEAL PLANKTONIC COMMUNITIES

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Samples were taken monthly throughout a year from waters and sediments of two shallow lakes, Lake Fertő and Lake Kolon. Besides cultivation-based techniques (e.g. targeting carbon-utilization using Ecoplates, or transformation nitrogen-forms), DNA-based methods were applied to reveal the taxonomical, ecological and functional diversity and capacity of the prokaryotes inhabiting the studied natural environments. Archaea:Bacteria ratio was determined with quantitative PCR targeting the 16S rRNA gene. Amplicon sequencing, based on the V3-V4 region of the 16S rRNA gene, was applied to determine the composition of the prokaryotic communities. The sediment samples showed the highest most probable number of active cells as well as the capacity to assimilate the highest numbers of the 31 studied carbon-sources. Prokaryotic assemblages of the studied environments were turned out to be dominated by members of the Bacteria domain: archaea were found to be less than 2% in the water columns and fell within the 5-10% range in the sediments. Archaea were so scarce, that in many samples the sequenced archaeal gene copy numbers failed to pass the 2,000 reads/sample threshold, therefore these samples were excluded from the subsequent data processing during the bioinformatic analysis since the biological irrelevance of the domain was confirmed with qPCR. Overall, archaeal diversities of the (sediment, *Phragmites*-dominated and *Utricularia*-associated) samples are approximately half of the bacterial ones, where ace varies around 2,000, Chao-1 and Sobs around 1,000, while inverse Simpson around 50. Certain microbial OTUs showed clear habitat-preferences, e.g. members of clades SAR11 and hgcI preferred the open waters, *Flavobacterium*, *Limnohabitans* preferred the macrophyte-associated sites, *Thiobacillus* the sediments, while a *Hydrogenophaga* and a *Cyanobium* OTU were found almost exclusively in Lake Fertő and Lake Kolon, respectively. The microbial community structures of the lakes are found to be stable through time, noteworthy differences can be observed in both in numbers and ratios of certain OTUs. In the middle of summer the microbial numbers usually peak a record, which is followed by a slow, but steady decline in autumn. Peaks are caused by different bacteria, e.g. in case of the *Utricularia*-dominated inner pond in Lake Kolon, in time of the winter peak, the bacterial community was dominated by *Rhodoferax* and *Geobacter*, while during the mid-summer anoxia *Chlorobium*, *Geobacter* and an uncultured Rhodocyclaceae genus found more favourable conditions.

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ENHANCING METHANE PRODUCTION FROM LIGNOCELLULOSIC BIOMASS PRE-TREATED WITH ANAEROBIC FUNGI

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Degradation of lignocellulose-rich material into biogas is an attractive strategy to face growing energy demands and moderate greenhouse gas emissions from the exploitation of fossil energy resources. Lignocellulosic residues (e.g. crop residues, green waste, mill waste) are highly frequent [1], they are easily accessible, cheap and do not require additional

land to grow on in this way do not trigger "food or fuel" conflicts. This biomass is composed of interwoven cellulose and hemicellulose, coated by anaerobically almost undegradable lignin [2]. Microbial pre-treatment utilizing the fibre degrading potentials of aerobic fungi may be a much cheaper alternative but there are some drawbacks e.g. loss of carbohydrates by respiration and biomass build-up and the requirement of long pretreatment periods [3]. Anaerobic fungi (AF) from the phylum Neocallimastigomycota are natural inhabitants of the digestive tract of herbivorous animals [4], which decompose a big share of the ingested forage. The AF attach to the plant material and crack the fibres mechanically by growth and expansion of their rhizoids or bulbous holdfasts [5]. In addition, AF possess cellulosomes which contain a multitude of lignocellulolytic enzymes. The objective of this study was the application of two newly isolated strains to the hydrolysis phase in order to improve hydrolysis of lignocellulosic biomass. The applied isolates were obtained from animals living on a high fibre diet, namely sheep (*Ovis aries*) and Asian elephant (*Elephas maximus*). The effects on biogas production of anaerobic fungi from both animal species were assessed in two step batch experiments, comprised by a hydrolytic/acidogenic stage, followed by a methane production stage. The assimilated carbohydrates were measured by HPLC. Additionally, gas composition was analized by GC during the methane production stage.

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[1] Williams et al (2016) Advances in biofeedstocks and biofuels, Volume 1 Biofeedstocks and their processing pp. 121.
[2] Rodriguez et al (2017) Renew Sust Energy Rev 68:1193.
[3] Isroi et al (2011) BioResources 6:5224.
[4] Liggenstoffer et al (2010) ISME J 4:1225.
[5] Akin and Borneman (1990) J Dairy Sci 73:3023.

TRANSCRIPTOMIC APPROACHES FOR THE FARNESOL EXPOSURE OF *CANDIDA AURIS*

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The threat of antifungal resistance of Candida auris necessitates bold and innovative therapeutic options. Farnesol is a quorum-sensing molecule with a potential antifungal and/or adjuvant effect; it may be a promising candidate in alternative treatment. To gain further insights into the farnesol-related effect to C. auris, genome-wide gene expression analysis was performed using RNA-Seq. Farnesol exposure resulted in 1,766 differentially expressed genes. Out of them, 447 and 304 genes with at least 1.5-fold increase or decrease in expression, respectively; were selected for further investigations. Genes involved in morphogenesis, biofilm events (maturation and dispersion), gluconeogenesis, iron metabolism, and regulation of RNA biosynthesis showed down-regulation, while genes related to antioxidative defence, transmembrane transport, glyoxylate cycle, fatty acid β-oxidation, and peroxisome process were up-regulated. In addition, farnesol treatment increases the expression of certain efflux pump genes including MDR1, CDR1 and CDR2. Growth was significantly inhibited within two hours of the addition of farnesol regarding CFU changes ($5.8\times10^7\pm1.1\times10^7$ and $1.1\times10^7\pm0.3\times10^7$ for untreated control and farnesol-exposed cells, respectively) ($p<0.001$). In addition, farnesol treatment caused a significant reduction in intracellular iron (152.2±21.1 vs. 116.0±10.0 mg/kg), manganese (67.9±5.1 vs. 18.6±1.8 mg/kg), and zinc (787.8±22.2 vs. 245.8±34.4 mg/kg) ($p<0.05$–0.001) compared to untreated control cells, whereas the level of cooper was significantly increased (274.6±15.7 vs. 828.8±106.4 mg/kg) ($p<0.001$). This is the first study analyzing the global changes in gene transcription in *C. auris* following farnesol exposure, providing important insights into the mechanism of antifungal action of farnesol and the response of *C. auris*, facilitating a better understanding of farnesol-related antifungal activity. In summary, farnesol exposure enhanced the oxidative stress response and up-regulated drug efflux pumps, while reducing zinc and manganese intracellular content as well as iron metabolism. Moreover, cellular metabolism was modulated towards β-oxidation. These results may open new directions in developing alternative therapies against *C. auris*.

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BACTERIOPHAGE-BASED BIOCONTROL AGAINST PLANT PATHOGENIC BACTERIA

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Bacterial plant diseases can cause significant economic losses worldwide. There is an urgent need for the development and application of sustainable biocontrol agents against plant pathogenic bacteria. Bacteriophages are promising antimicrobial agents, as it was demonstrated for five phytopathogenic bacteria. Phages infecting *Acidovorax citrulli*, causing seedling blight and bacterial fruit blotch of cucurbits, were isolated and characterized. Lytic life cycle and some of the biological characteristics of investigated phage strains indicated their potential in the control of watermelon fruit blotch. *Xanthomonas euvesicatoria* causes bacterial spot disease of pepper and tomato. Phage KΦ1 was isolated from the rhizosphere of pepper plants showing symptoms of bacterial spot. Foliar applications of the unformulated KΦ1 phage suspension effectively controlled pepper bacterial spot compared to the standard treatment and the untreated control. *Xanthomonas oryzae* pv. *oryzae* (Xoo) is the most devastating agent of rice, causing its bacterial leaf blight (BLB). We isolated and characterized ten OP2-like bacteriophages. Laboratory efficacy trials strengthened the hypothesis that these bacteriophages can control BLB effectively. A bacteriophage cocktail protected rice plants against Xoo successfully during a field trial. *Xanthomonas arboricola* pv. *juglandis* (Xaj) causes walnut blight disease. Bacteriophages against Xaj were isolated from Italian and Hungarian samples and characterized. Two phages were tested individually for their ability to suppress symptoms in walnut plantlets following bacterial inoculation. A significant reduction in the degree of symptoms was observed following phage application when compared to the control. We formulated a cocktail containing six bacteriophages and implemented field trials by spraying the phage suspension on different *Juglans regia* varieties. The applied bacteriophage cocktail protected walnut trees against Xaj effectively, albeit the efficacy was different depending on which variety was treated. *Erwinia amylovora* causes fire blight disease of Rosaceae plants. 125 bacteriophages were isolated from soil, and aerial samples were taken in Hungary, Romania, and Spain, from which 55 showed lytic plaque morphology. Based on host range analysis conducted on 101 bacterium strains, 27 phages were subject of detailed characterization. The applied bacteriophage cocktail carefully formulated protected apple trees against *E. amylovora* during two field trials. A special permit was issued for marketing the Erwiphage bacteriophage-based biopesticide in 2012. This product was the first bacteriophage-based biocontrol agent against *E. amylovora* and the second one among all bacteriophages-based biopesticide that was available on the market worldwide. Our results support that bacteriophages are potent biocontrol agents against phytopathogenic bacteria.

ENZYME-ASSISTED EXTRACTION OF PHENOLICS FROM *SORGHUM* SAMPLES

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Tons of sorghum samples are produced / year worldwide, and the sorghum grain is rich in many nutrients including bioactive phenolic compounds. These phenolics are important secondary metabolites of the plant, and they participate in defense mechanisms, and pigmentation and adaptation processes. Due to their antioxidant properties, polyphenols can inhibit oxidative processes and scavenge reactive oxygen species in many organisms. In addition, plant phenolics may have antimicrobial, anti-inflammatory and other health-protective effects. However, many of them are conjugated to plant polysaccharides via glycosidic or ester bonds resulting in a less bioavailable form. Treatment with enzymes able to cleave these bonds can be an ecofriendly strategy to obtain free phenolics from plant residues. In this work, we tested an enzymatic approach using cellulolytic and lipolytic cocktails from *Rhizomucor miehei, Gilbertella persicaria* and *Mucor corticolus* to enrich phenolics from sorghum flour and grounded grain residues. Based on previous studies, enzyme cocktails were produced in a wheat bran-based solid-state fermentation system and then were partially purified by gel filtration before use. The obtained enzyme extracts diluted in acetate buffer (pH 6.0) had detectable cellulase, lipase, xylanase, and amylase activities. During enzyme treatments, a mass of 1 g of substrate was treated with 10 mL of enzyme extract, and the reaction mixtures were incubated at 30°C (in case of *Gilbertella* and *Mucor* enzyme cocktails) or 50°C (in case of Rhizomucor enzyme cocktail) for 7 hours. Total phenolic content (TPC), and antioxidant activity, i.e., free radical scavenging activity and ferric reducing antioxidant power, measurements were carried out in the samples taken at

predefined intervals. Results showed an increase in TPC for all enzyme treatments in sorghum flour samples. Treatment with *R. miehei* enzyme extract resulted in the highest TPC, to which its outstanding lipase activity may also have contributed. In addition, our researches confirmed the role of different cellulase activities in phenolic enrichment from sorghum substances. In general, the largest increase in TPC was observed in the 1st hour of the treatment, followed by a decrease in variable intensity, presumably due to the degradation of phenolics. Similarly, TPC of the grounded sorghum grain samples exhibited an increase after a one-hour incubation with the enzyme cocktails. In some samples, the enzyme treatments caused an improved antioxidant activity as well. Anyway, the enzymatic treatment with cellulase/lipase activities showed a positive effect on the release of phenolics from sorghum samples.

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ENZYMATIC PRODUCTION OF BIOACTIVE FATTY ACIDS FROM VEGETABLE AND FISH OILS

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The vegetable and fish oils can contain bioactive fatty acids in high amount. These natural compounds have variety of health benefits such as anti-inflammatory, antihypertensive, antioxidant and anticancer activities. Hydrolysis of the lipid substrate by lipases can be an eco-friendly tool for extraction of the fatty acid content. Lipases catalyze the release of fatty acids from their esters, and the action operated does not need additional chemical and physical treatments. Moreover, lipases can be produced from microorganisms in high amount that makes the process more economically. In this work, enzymatic treatment of olive, rapeseed, linseed, almond, peanut and cognac vegetable oils and menhaden fish oil were performed by *Aspergillus niger, Rhizopus oryzae, Rhizopus niveus, Rhizomucor miehei* and *Candida rugosa* microbial lipases. Firstly, we studied the enzymatic hydrolysis on solid medium containing the appropriate oil and phenol red acid-base indicator. In this assay, the *R. miehei* lipase showed excellent activity against the oils tested. To monitor the fatty acids released during the enzymatic treatment, the lipids were subjected to hydrolysis in liquid environments using *R. miehei* lipase as a biocatalyst. Gas chromatography analysis was used to detect fatty acids in the mixtures. In vegetable oils, concentrations of both unsaturated, i.e., oleic acid (OA), linoleic acid (LA), linolenic acid (ALA) and saturated, i.e., stearic acid and palmitic acid fatty acids demonstrated an increase during the reaction. In menhaden oil, besides saturated and monounsaturated fatty acids, polyunsaturated fatty acids such as hexadecanedioic acid, LA, ALA, stearidonic acid, arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were liberated after the hydrolysis. Enzymatic treatment also caused an increase in the total phenolic content of some samples, indicating the release of phenolic compounds from ester bonds. According to our results, treatment with *R. miehei* lipase may be suitable technology for high-yield enrichment of bioactive fatty acids from natural lipid substrates. After the extraction, the fatty acid compounds can be used as individual additives and/or preservatives in functional foods.

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EFFECT OF *TRICHODERMA* PEPTAIBOLS ON THE YIELD OF CULTIVATED TOMATO

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Trichoderma species are cosmopolitan, free-living filamentous fungi commonly used as biocontrol agents in agricultural fields. They can also promote plant growth and directly attack plant pathogenic fungi via mycoparasitism. Rarely, they may also cause human infections in immunocompromised patients, therefore the species used in agriculture should be carefully selected, or instead of the living fungi, their beneficial secondary metabolites should be considered for application. Peptaibols are secondary metabolites produced by most members of the genus *Trichoderma*. Their bioactivity relies on the ability of forming ion channels in bilayer membranes. They are short peptides (6-20 residues), which include unique amino acids like α-aminoisobutyric acid (Aib), isovaline (Iva), as well as C-terminal 1,2-amino alcohol. Positive effects of *Trichoderma* strains on plants are commonly known from the literature, like their role in plant growth promotion and induction of systemic resistance. Peptaibols have also the potential of plant growth promotion, therefore they could be included in the arsenal to control plant pathogenic fungi affecting agricultural crops. In the present study, an indeterminate

and a determinate type of cultivated tomato plants were used to examine the direct effects of peptaibols from 2 strains of *Trichoderma* on plant growth. *Trichoderma reesei* – a species commonly used in biotechnology due to its ability to produce hydrolytic enzymes degrading cellulose or hemicellulose – and *T. longibrachiatum* f. *bissettii* – a clinical isolate from a bone marrow transplant recipient – were selected for the experiments. After HPLC-ESI-MS investigation of the crude extracts deriving from *T. reesei* SZMC 22616 and *T. longibrachiatum* f. *bissettii* SZMC 12546 cultures, the extracts were purified by preparative HPLC and tested on *Micrococcus luteus* to reveal their bioactivity. Plants were treated with a dilution series from 1 mg/mL to 0.1 mg/mL of the purified peptaibol extracts. The treatment with high peptaibol concentrations proved to be toxic to the germination of tomato seedlings, though, after a certain level of dilution, more intense germination and growth could be observed. Growth parameters and the quantity of photosynthetic pigments extracted from the second leaf of the treated tomato saplings were measured. Consequently, the saplings were cultivated in field experiments, which resulted in increased fruit production in the case of treatments with certain dilutions. Furthermore, lower sensitivity to fungal diseases was also observed in the plants treated with peptaibols. Further studies will be carried out to evaluate the applicability of peptaibol compounds and their direct effects on the plants.

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A COMPOSITE MICROBIAL FORMULATION FOR AGRICULTURE AND ITS PERFORMANCE IN SWEET POTATO CULTIVATION

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Microbiological agents for agricultural purposes gained space during the past few decades, as they enable more ecological and environment-friendly approaches of disease and pest management, fertilization and plant growth promotion than agricultural chemicals. Our aim was to assemble a composite microbial formulation from multiple beneficial microorganisms. In this study, 56 *Bacillus* strains and 56 potentially nitrogen-fixing bacterial strains were isolated from different soil samples collected from agricultural fields in Hungary and Serbia. The strains were identified by sequence analysis of the 16S RNA gene or a fragment of the DNA gyrase alpha subunit (*gyrA*). *Bacillus velezensis* SZMC 24986, an aerobic, Gram-positive, endospore-forming bacterium known to promote plant growth and reported to suppress the growth of microbial pathogens was selected as a biocontrol component of the composite soil inoculant based on its efficient in vitro antagonistic abilities against various plant pathogenic fungi on PDA medium. *Arthrobacter globiformis* SZMC 25081, an aerobic, Gram-positive bacterium was selected as a potential plant growth promoting component of the inoculant due to its ability to grow on nitrogen-free medium, which may be due to its ability to oxidize ammonium into nitrite, nitrate and hydrylamine, thereby providing reliable source of nitrogen that plants need to grow. *Pseudomonas resinovorans* SZMC 25872 was selected as a potential plant growth promoting component due to its ability to promote seed germination and produce indole-3-acetic acid (12.30 µg/mL and 0.54 µg/mL in the presence and absence of tryptophan, respectively). Two *Trichoderma* strains were also selected out of 41 *Trichoderma* isolates from the Hungary-Serbia cross border region, which were identified based on the sequence analysis of a fragment of the *tef1α* gene: strain SZMC 25217 from *Trichoderma rodmanii*, the only member of the Brevicompactum clade of the genus *Trichoderma* that is not producing trichothecene-type toxins was selected as a potential stem degrading component component due to its excellent extracellular cellulolytic, xylanolytic and phosphatase enzyme activities, while *Trichoderma afroharzianum* SZMC 25231 from the Harzianum clade of the genus *Trichoderma* was selected as a biocontrol component due to its good antagonistic abilities against various plant pathogenic fungi. The assembled consortial inoculant was tested in field experiments on sweet potato plants. Treatment with the inoculant could increase the sweet potato yield compared to both the fertilized and non-fertilized controls, however, the rate of increase in yield was different in the various treatments. The results suggested that fertilization or higher basic nutrient levels of soil may decrease the efficiency of the developed microbial inoculant.

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BIOACTIVE PEPTAIBOLS AS POSSIBLE AGENTS OF DISEASE MANAGEMENT AGAINST MULTI-DRUG RESISTANT HUMAN PATHOGENIC BACTERIAL STRAINS

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With growing instances of antibiotic resistance all over the world, the scientific community is more than ever desperate to identify novel, fail-safe ways of treatments for a plethora of devastating diseases. This search is not limited to human pathogens but has also been extended to the problem of agricultural pathogens. A group of peptides, known as peptaibols, are produced as secondary metabolites by filamentous fungi like *Trichoderma* species and show promising future. Peptaibols are short, amphipathic molecules that have long piqued the interests of researchers owing to their antibacterial, antifungal, anti-viral, anti-helminth, and anti-tumor properties, as well as to their abilities to elicit plant defense responses. They show antibiotic properties either by forming voltage-gated channels within biological membranes or by the disruption of membrane integrity. The cell membrane structure of Gram-positive bacteria seems to be especially sensitive to the action of peptaibols. Antimicrobial peptide drugs like daptomycin are already in use as first line of defense against multi-resistant bacteria like methicillin-resistant *S. aureus* (MRSA). We have focused our attention towards peptaibols like Trikoningin KA V and related Koningiopsins identified earlier, that were found to be effective against Gram-positive human pathogenic bacterial species like *Micrococcus luteus, Staphylococcus aureus, Enterococcus luteus*, etc., in vitro. They also showed similar toxicity against multi-resistant bacteria such as MRSA. Furthermore, we used the latest enhanced sampling molecular dynamics (MD) simulation techniques to obtain the three-dimensional structures of these peptaibols, evaluate their mechanism of action and correlate it with their bioactivities.

PRELIMINARY EXPERIENCE WITH THE BL-DETECTOOL: AN INNOVATIVE TOOL FOR RAPID DETECTION OF BROAD-SPECTRUM BETA-LACTAMASES AND CARBAPENEMASES DIRECTLY FROM CLINICAL SAMPLES

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The increasing rate of infections caused by Enterobacteriaceae that produce broad-spectrum β-lactamases (ESBLs) and carbapenemases is a major concern worldwide. AMR-DetecTool or NG Detectool is a Lateral Flow immunoassays system that gives the new possibility for the direct detection of β -lactamases and carbapenemases directly from clinical samples. We performed the tests using the residual materials after the routine microbiological procedure from the clinical samples received in our laboratory. 400-400 blood (positive blood culture), urine and stool (perianal swab) were investigated. Following the easy sample preparation (filtration, concentration, extraction, incubation and loading) the possible presence of CTX-M-ESBLs and carbapenemases (VIM, IMP, NDM, KPC and OXA) has been investigated. Our preliminary study showed excellent biological performances close to 100% in sensitivity and specificity, especially if proper pre-screening is used. The tests are sensitive, specific, and easy to use and could be implemented in any microbiology laboratory. It allows for a dramatic reduction in the treatment decision time upon sample reception from 24 hours (current workflow) to 15-30 minutes.

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SEIZING MULTIPLE OPPORTUNITIES - FUNGAL CO-INFECTIONS IN COVID-19

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Severe COVID-19 can result in acute respiratory distress sysndrome (ARDS), usually with an onset of 8-12 days after initial symptoms. COVID-19 patients that require ICU treatment, mechanical ventilation and/or extracorporeal oxygenation and anti-inflammatory treatment are particularly vulnerable to co-infections. Besides bacteria, invasive fungal infections can aggravate severe COVID-19. Among the relevant fungal co-infection in patients with COVID-19 are invasive aspergillosis (IA, specifically COVID-19 associated pulmonary aspergillosis CAPA), invasive *Candida* infection (IC) and

– mainly in India and neighbouring countries – COVID-19 associated mucormycosis (CAM). Invasive aspergillosis is a known complication of severe respiratory viral infection and has been described in a relevant share of patients treated in ICU for severe influenza (influenza-associated pulmonary aspergillosis, IAPA). This prior knowledge has early on created awareness to look for IA in COVID-19 patients on ICU. Several studies have confirmed that CAPA does occur and expert guidelines on diagnosis and management have become available in the meantime. IC is a major complications of patients treated in ICU in general. Consequently, IC has also been observed in patients treated for severe COVID-19. The situation was aggravated by the recent emergence of *Candida auris*, a species that is frequently resistant to available antifungals and can spread via nosocomial transmission. The occurrence of *C. auris* on COVID-19 ICU units has resulted in hospital outbreaks in several countries. These outbreaks are difficult to contain and pose considerable problems for clinical management, especially during exceedingly high demand for ICU places. Finally, in India, a massive surge in COVID-19 cases has resulted in yet another devastating fungal co-infection. Extraordinarily high numbers of mucormycosis have been observed that often created massive problems for hospital care, including a rise in the need for surgical interventions and a shortage of antifungal drugs. However, current data suggest that COVID-19 associated mucormycosis (CAM) is geographically limited to the Indian subcontinent. Fungal co-infections can be a relevant problem in COVID-19 patients. Experiences with fungal co-infection in COVID-19 from the German National Reference Center NRZMyk will be described and recommendations on diagnostics and clinical management will be summarized.

FOLLOWING TRENDS OF SARS-COV-2 EPIDEMICS AND VARIANTS´ DYNAMICS USING WASTEWATER ANALYSIS: A SLOVENIAN CASE STORY

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During the SARS-CoV-2 pandemic, many research activities and resources have been directed into the development of efficient and informative systems for tracking the trends of the epidemic on different population levels, from countries to individual residential buildings. Besides testing individual patient samples, wastewater-based epidemiology is being employed to estimate the fluctuations in the number of infected individuals in many countries worldwide. The presence of SARS-CoV-2 RNA in feces, and subsequently in wastewater, provides an opportunity to use wastewater for: i) a complementary surveillance of the spread of SARS-CoV-2, and ii) the detection of SARS-CoV-2 variants of concern circulating in the population. In our past studies, we have investigated human and plant pathogens in wastewater, studied wastewater viromes and developed an array of methods for efficient virus concentration, quantification, and shotgun high-throughput sequencing (HTS). With the onset of the current SARS-CoV-2 pandemic, in March 2020, we have used this experience to determine the changing trends of SARS-CoV-2 concentrations in untreated wastewaters in Slovenia. First, we have assessed the stability of inactivated SARS-CoV-2 particles and patient-derived SARS-CoV-2 RNA, compared different virus concentration approaches, validated the selected concentration approaches and virus quantification by reverse transcription quantitative PCR and reverse transcription digital PCR, including normalization of the virus titers using different controls and fecal indicators. We have then applied the obtained evaluation data to establish a pilot monitoring for SARS-CoV-2 concentrations in wastewater from selected Slovenian wastewater treatment plants. Additionally, we employed HTS, followed by bioinformatics analysis, to detect and quantify the presence of key mutations belonging to SARS-CoV-2 variants of concern. The results enabled us to track the increasing and decreasing concentration of the virus in wastewater and to detect the dynamics of the variants displacements in the population, such as at the appearance of the highly transmissible alpha variant. Both, SARS-CoV-2 concentration and variants monitoring in wastewater influent, were in agreement with observations from clinical data over the same period. The national wastewater monitoring, including different Slovenian wastewater treatment plants, school and retirement home, is currently helping us to follow the SARS-CoV-2 epidemic in Slovenia and support the governmental scientific advisory body. During this pandemic wastewater-based epidemiology has been intensively employed worldwide, becoming an efficient tool, which can help us to better respond to future outbreaks of SARS-CoV-2 or emergence of possible new pathogens. The employment of such approach is also supported by recent EU commission recommendation calling for the establishment of the national wastewater monitoring systems in EU member states.

BIOLOGICAL PROPERTIES OF POTENTIAL *ESCHERICHIA COLI* PROBIOTIC STRAINS LEGM-18 AND ZP

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The use of probiotic nonpathogenic bacterial strains with antibacterial activity against pathogens for the prevention and treatment of intestinal infections on farms is supported by the scientific community. The aim of this work was to study the biological properties and evaluate the probiotic potential of two potential probiotic *Escherichia coli* strains: i) LEGM-18. isolated from a healthy person (1993, Russia) and ii) ZP, a genetically modified strain possessing a conjugative plasmid encoding the colicin E7 synthesis gene and harboring the colicin E7 immunity gene in the chromosome (2015, Slovenia). Two potential probiotic strains, *E. coli* ZP (N4i pOX38a Cmr, Gmr) and *E. coli* LEGM-18, and the control strain *E. coli* M-17 were used in the study. All strains were tested for hydrophobicity (Salt Aggregation Test; Bacterial Adhesion to Hydrocarbons Test), adhesion, antagonism, resistance to bile and pepsin, growth characteristics on different media (Broth Luria-Bertani, M9-glucose, M9-glucose + Casamino Acids), survival after lyophilization and toxicity to rats. It was shown that cell surface hydrophobicity of *E. coli* LEGM-18 and ZP strains was significantly lower than the cell surface hydrophobicity of M-17. The adhesion ability of LEGM-18 and M-17 was moderate, while the ZP strain exhibited low adhesion ability. The antagonistic effect of *E. coli* LEGM-18 against *Proteus vulgaris, Shigella flexneri, E. coli* O157 and *E. coli* 643 was higher than the antagonistic effect of *E. coli* ZP. All strains were resistant to pepsin and bile, safe for rats, easily grown on basic nutrient media, and remained viable during storage for one year. These data indicate that *E. coli* LEGM-18 and *E. coli* ZP are promising strains for development of a new complex probiotic veterinary drug, possessing the advantage of an extended spectrum antagonistic activity, and the ability to affect bacteriocin-resistant enteropathogenic *Escherichia* strains.

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GRAPEVINE LEAF ENVIRONMENTAL DNA SEQUENCING PROVIDES INSIGHTS INTO TEMPORAL SUCCESSION OF PLANT PATHOGENIC FUNGI UNDER ORGANIC AND CONVENTIONAL MANAGEMENT

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Plant growth and health are highly dependent on plant-associated microbes and in recent years there has been an increase in microbiome research of agricultural crops, including grapevine. Despite recent advances, we still lack a systematic overview of the diversity of grapevine-associated fungi and how the grapevine microbiome is influenced by cultivation methods. Beside conventional agriculture, organic farming has been increasing recently to decrease the impact on the environment. In this study, we present the diversity and composition of plant pathogenic fungal communities occurring in the leaves of grapevines under an organic and conventional management. We hypothesized that the type of plant protection used in conventional and organic vineyards would have a great impact on the microbial, and especially the fungal, community structure associated with grapevine plants. To compare fungal communities of the leaves of conventionally and organically managed grapevines, we sampled leaves of the cultivar Bianka at the Eszterházy vineyard situated in Eger, Hungary, during the months of June, July, August and September before harvest (2020). After DNA extraction and Illumina NextSeq sequencing, the quality-filtered and rarefied dataset contained 9,993 fungal Amplicon Sequence Variants (ASVs) in total. Among these, 3,475 ASVs representing 22,486,375 reads were assigned to functional groups, of which 911 ASVs were considered plant pathogen. At genus level, *Phaemoniella* (associated with Esca disease in grapevine) showed the highest number of ASVs, followed by *Alternaria* (causal agent of *Alternaria* blight), *Epicoccum* (often associated with the mycobiome of grapevines), and *Diplodia* (associated with grapevine trunk disease) from the 88 different genera found, with respect to ASV richness. Differences in fungal richness were apparent among months, but not between organic and conventional management types. Similarly, community structure was primarily influenced by sampling month, as shown by NMDS and PERMANOVA analyses, explaining 38% of compositional variation among samples. We identified several indicator ASVs for different months. Beside the strong temporal turnover of leaf-associated fungi, the organic vs. conventional management did not seem to affect leaf fungal communities in a significant manner. It is unclear how much of this community turnover is driven by periodic fungicide applications and how much by seasonality. The lack of significant differences among cultivation types suggest that leaf-associated fungi undergo a seasonal succession.

SARS-COV-2 INVESTIGATION IN WILDLIFE AND ENVIRONMENT

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Almost two years of coexistence with COVID-19 are behind us and it is very much clear that disease is likely to become endemic. To make it manageable, the knowledge of all aspects of the disease and its causative agent is of paramount importance. Human-to-animal contact, as people encroach on animal habitats has shown in the past to cause spillover events of numerous pathogens from animals to humans, and vice versa. When it comes to SARS-CoV-2, it has been proven that humans sporadically can transmit the virus to other animals or even cause an epidemic wave within some species such as outbreak of infection in farm minks. Due to the number of infected people, possible animal susceptibility, high viral recombination rates of coronaviruses and recent reports of environmental, especially wastewater contamination, reverse spillovers of SARS-CoV-2 can be expected. Therefore, the aim of this study was to investigate a possible transmission of SARS-CoV-2 to free ranging and captive wildlife as well as the environment after the first infection wave and during the second one. We tested the environmental samples which were represented by wastewaters (n = 21; nine wastewater treatment plants from seven Croatian counties; from 1st to 3rd treatment stage), surface waters (n = 30; two nature parks and one city lake) and bivalves from the Adriatic Sea (n = 77). Regarding the wildlife species, we tested samples of wild boar (n = 153), red fox (n = 204) and jackal (n = 65), for the presence of SARS CoV-2 RNA and anti-SARS CoV-2 antibodies. We also tested yellow-legged gulls (n = 111) since they feed on garbage pins nearby highly populated cities. To fulfil the epidemiological data, we tested samples of Zoo animals (n = 32) that were consecutively in contact with SARS CoV-2 positive humans. Finally, samples from one bat colony (n = 38) located relatively close to the human settlement were tested as well. The presence of SARS-CoV-2 was examined by applying real-time RT-PCR directed towards different genome targets; the presence of anti-SARS CoV-2 antibodies were examined by two commercially available serological assays (ELISA and VNT). Eight out of nine wastewater plants were positive in influent wastewaters on at least one genome target. Most effluent wastewaters from 2nd and 3rd treatment stage plants were SARS-CoV-2 negative (81.8%), as well as all surface waters and bivalves. However, no wildlife samples were either serologically or virologically positive, regardless of the locality and distance from human settlements. Samples of wild animals from the Zagreb Zoo, which are in daily contact with people, including those proven to be positive for COVID-19, were also negative. Nevertheless, 58% of the bats tested ELISA positive, and neutralising antibodies were detected in 29% of bats. Guano samples (26%) tested positive with SARS-CoV-2 E-gene-Sarbeco real-time RT-PCR protocol. The detected coronavirus sequences were classified within both alphaCoVs and betaCoVs. The discovered betaCoV sequence was phylogenetically clustered with other SARS-CoV-2-like viruses, but not with the original pandemic virus. In conclusion, no evidence of spillover of SARS CoV-2 was found in tested wildlife species regardless of their habitat, however, the SARS CoV-2 pandemic strain derived from wastewater and the SARS CoV-2-like strain detected in bats indicate that a possibility of virus transmission is present, and monitoring must be carried out.

GENOMIC SURVEILLANCE AND MONITORING OF CIRCULATING AND NEWLY EMERGING VARIANTS OF SARS-COV-2 IN HUNGARY

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SARS-CoV-2 as a single stranded RNA virus is constantly changing due to naturally occurring mutations throughout the whole 30 kb viral genome. While a certain amount of genetic variation is expected to occur during infection and active replication, it is of utmost importance to monitor circulating viruses for key and emerging mutations that may affect the nature of the virus. Many mutations have no effect on the ability to spread or cause more severe symptoms, however the emergence of certain variants with mutations that are more beneficial for the virus can lead to increased infectivity. The estimated mutation rate in SARS-CoV-2 is between 1.19 and 3.3×10^{-3}/site/year. Based on specific genetic markers that are associated with changes in receptor binding, reduced neutralization by virus specific antibodies, reduced efficacy of vaccination and treatments, potential diagnostic impact, or increased infectivity or disease severity, SARS-CoV-2 variants are classified as Variants of Interest (VOI), Variant of Concern (VOC) and Variants of High Consequence (VOHC). VOCs including the alpha (B.1.1.7), beta (B.1.351), gamma (P.1) and delta (B.1.617.2) variants and other key mutations, such as E484K receptor binding spike protein mutation are closely monitored by the Hungarian National Public Health Cen-

ter and collaborating partners. Variant surveillance consists of targeted and randomized sample selection and the use of different molecular tools including real-time RT-PCR, digital droplet RT-PCR and next generation sequencing methods. Wastewater surveillance indicated that B.1.1.7 variant first emerged in Budapest in early January 2020 followed by the first diagnosed human cases and rapidly became dominant in the entire country. By mid-February (week 6) 70% of the diagnosed cases were caused by the alpha variant. The B1.351 (South African) variant was first reported in February and the P.1 (Brazil) variant was imported in the beginning of June 2021. Emergence of the most recent B1.617.2 delta variant has been reported since May and now is the dominant variant causing infections. Monitoring and genomic surveillance of the circulating and newly emerging variants and mutations is a particularly important epidemiological task to trace the path of the infection and to help decision makers to apply certain countermeasures such as best vaccination strategies and containment approaches.

INVESTIGATION OF THE SAME *NIM* GENE-INSERTION SEQUENCE CONFIGURATIONS ON THE EXPRESSION OF THE *NIM* GENES AND METRONIDAZOLE RESISTANCE OF *BACTEROIDES FRAGILIS* STRAINS

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For metronidazole rsistance of *Bacteroides* spp. the best-characterized resistance mechanism is that which is mediated by *nim* genes. However, there are some issues that are not supporting this view, e.g. some strains carry a nim gene but are not metronidazole rsistant and sometimes the nim expression do not correlate with metronidazole resistance. Previously we reported some *nimB* and *nimE*-postive *B. fragilis* strains that had gene specific insertion sequence (IS) elements that are thought to regulate the expression of these genes. However despite the same genetic constitution, these strains differred in the levels of metronidazole resistance. Our aim was to investigate this odd relationship. We recorded metronidazole MICs by Etests, isolated total RNA and by gene-specific primers we conducted qRT-PCR. Our results showed that while metronidazole resistance scatters through a wide range for these strains, the expression of the *nim* genes were somewhat constant. From these data we could confirm that the same regulation mechanism (same IS elements) drive the same expression levels of the *nim* genes but to explain the role of the nim genes in metronidazole resistance we should propose the role of epistatic factors or some rate limiting steps also.

EXAMINATION OF THE ANTIBIOTIC RESISTANCE MECHANISMS OF A MULTIDRUG-RESISTANT *PHOCAEICOLA* (*BACTEROIDES*) *VULGATUS* ISOLATE AND THE ROLE OF A NOVEL B-LACATAMASE GENE ON IMIPENEM RESISTANCE IN *BACTEROIDES* ISOLATES

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An imipenem-resistant *P. vulgatus* strain (*P. vulgatus* 2070) has been isolated in Hungary earlier and now we know that additionally to the cfiA-mediated carbapenem resistance of *B. fragilis* there is another carbapenemase gene (*crxA*) for some *B. xylanisolvens* strains too. We sought to determine the carbapenem resistance mechanism of *P. vulgatus* 2070 and also examine its prevalence among other *P. vulgatus* strains too. First we determined the genomic sequence of *P. vulgatus* 2070 strain and using bioinformatic tools identified antibiotic resistance genes and genomic islands. Based on this information we screened a collection of normal microbiota *Bacteroides* (*Phocaeicola*) strains (n = 184) for the resistance genes found in *P. vulgatus* 2070. *P. vulgatus* 2070 was multidrug-resistant (displayed resistance to ampicillin, piperacillin/tazobactam, cefoxitin, imipenem, erythromycin, moxifloxacin and tetracycline) and harboured the identifiable *cfxA, blaHGD1* and *tetQ* antibiotic resistance genes. The *cfxA* and the *tetQ* genes resided on mobilizable and conjugative transposons in the determined genomic sequence, respectively, but *blaHGD1*, a Class A2 β-lactamase gene, was coded in an operon also harbouring a GNAT-type addiction toxin gene. *P. vulgatus* 2070 produced the following β-lactamase activities: >1,000 u/mg with nitrocefin and 3.2 u/mg with imipenem. *BlaHGD1* could be found in all the *P. vulgatus* strains (n = 35) in our collection in addition to a *Parabacteroides distasonis* isolate which were mostly susceptible to imipenem. In conclusion we can say that the imipenem resistance of *P. vulgatus* 2070 may be caused by carbapenemase production. The roles of the *cfxA, blaHGD1* β-lacatamase and the GNAT toxin genes in determination of this resistance is being carried out in our laboratory by gene targeting and qRT-PCR experiments.

SARS-COV-19 VARIANT MONITORING IN SLOVENIA AND WORLDWIDE

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Like any other, the SARS-CoV-2 virus also rapidly changes over time. Most mutations do not affect phenotype of the virus, while some lead to the emergence of new variants with altered properties. These can be associated with virus infectivity, disease severity, vaccine efficacy, or may impact diagnostic tools. Monitoring the spread of variants and the emergence of new mutations is therefore crucial for the appropriate organization of public health as well as the design of preventive social measures. The severity of pandemic caused by SARS-CoV-2 virus demanded a prompt formation of a global network for efficient and transparent variant monitoring, which is based on close cooperation between international public health organizations and national health systems worldwide. As a result, we now operate with over 3 million publicly available curated genome sequences and the number keeps growing. These continue to improve our knowledge on how mutations effect virus phenotype, while the established variant naming system facilitates worldwide communication and enables unified data analysis. Slovenia as a part of the network established national strategy for SARS-CoV-2 variant monitoring. Strategy is a collaboration of multiple institutions and includes whole genome sequencing, monitoring of wastewaters, in vitro virus infectivity testing as well as regular communication of findings with government agencies and public.

DYNAMICS OF SEDIMENT MICROBIAL COMMUNITIES DURING A SEAGRASS MEADOW DECLINE

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Seagrass meadow sediments harbor diverse and abundant microbial communities and are considered to be hot spots for microbial activity. During the last few decades, a severe decline of different seagrass species has been reported. Although the taxonomy of microorganisms in seagrass meadow sediments has been described, little is known about their response to a meadow decline. Illumina MiSeq sequencing of the V4 region of the 16S rRNA gene was performed on sediment samples originating from a declining *Cymodocea nodosa* meadow in order to assess the effect of a meadow decline on microbial sediment communities. Sampling was carried out in surface sediments at monthly intervals. For comparison, sediment communities from an adjacent non-vegetated area were also characterized. Richness estimators and diversity indices displayed a decreasing trend with sediment depth, while community analysis at the level of OTUs showed differentiation primarily by sediment depth and secondly by the presence or absence of seagrass vegetation. Even though the *Cymodocea nodosa* meadow reached a point where almost no leaves were present no clear temporal community variation was observed. Taxonomic analysis revealed a clear dominance of bacterial over archaeal reads with most archaeal sequences classified as Nanoarchaeota, Thermoplasmatota, Crenarchaeota and Asgardarchaeota. The bacterial community was mainly composed of Desulfobacterota, Proteobacteria, Bacteroidota and Chloroflexi. Taken together, our results indicate that changing environmental conditions caused by the decline of the *Cymodocea nodosa* meadow did not have a strong effect on the sediment microbial community composition.

THE FINAL 30 PERCENT: OPTIMIZING THE *ASPERGILLUS NIGER* CITRIC ACID FERMENTATION UP TO THE THEORETICAL MAXIMUM

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For over a century, the filamentous Ascomycete fungus *Aspergillus niger* a ubiquitous soil-borne saprophyte, has been playing a pivotal role as a platform organism in industrial biotechnology. It was then discovered that any *A. niger* strain tested when grown in liquid sugar medium is a superior producer of citric acid (2-hydroxy-propane-1,2,3-tricarboxylic acid), a compound widely used as a flavour agent in food and beverages as well as a pH-regulator, antioxidant and preservative in the pharma and cosmetics industries. The fungal production of citric acid had led to the development of a fermentation technology that accounted for over 95% of the global production. Due to the 60,000 tons / year capacity citrate manufacturing plant being under construction in the Szolnok Industrial Park, Hungary will soon become a major citrate producer/exporter. Citric acid overflow requires a unique combination of unusual nutrient conditions such as excessive levels of car-

bon source, H^+, and dissolved oxygen, and suboptimal concentrations of phosphate as well as certain trace elements, which synergistically influence the yield. Molar yields of citric acid can reach up to 98%, resulting in the practically quantitative conversion of the carbon substrate into organic acid. The biochemical mechanism behind this overflow has attracted scientific interest for several decades, but still only pieces of the puzzle are understood. One reason for this is that labs often apply conditions under which citric acid yields are not comparable with those obtained in the industrial process – reaching molar yields anywhere between 10% and 70% in the literature – and results therefore do not reveal the events which take place under industrial conditions. Here we discuss the operational adjustments undertaken to optimize the *A. niger* citric acid fermentation at lab-scale (6 L) to achieve molar yields close to 100%, thereby mimicking true producing settings.

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INVESTIGATING THE PREVALENCE OF A FUNGICIDE RESISTANCE MARKER AND THE GENETIC STRUCTURE OF A GRAPEVINE POWDERY MILDEW POPULATION IN MÁD (TOKAJ)

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In Hungary, and worldwide, grape is a particularly important crop. One of its most common and significant diseases is the grapevine powdery mildew (GPM) caused by the fungus *Erysiphe necator*. The disease results in significant yield losses, the control is costly, and infected grapes degrade the quality of wine. GPM is routinely controlled by sterol demethylation inhibitor (DMI) fungicides, which are inhibiting the CYP51 enzyme, a crucial biomolecule in the biosynthesis of the fungal cell membrane. Long- term and intensive use of DMIs has resulted in widespread resistance to these fungicides in GPM populations. A common marker of DMI resistance is the A495T point mutation in the *cyp51* gene, which leads to an amino acid change in the target protein, preventing DMI fungicides from fulfilling their function. To our current knowledge, the GPM population in Europe can be divided into two genetically distinct groups: genotypes A and B. The two groups may differ in seasonality, way of reproduction, fungicide resistance, and caused symptoms. To distinguish these groups, several single nucleotide polymorphisms (SNPs) have been described in the β-tubulin (*tub2*) and the translation elongation factor 1-alpha (*ef1-α*) gene sequences. We sampled a GPM population for multiple years in order to (i) investigate the prevalence of the A495T resistance marker, (ii) ascertain the proportion of genotypes A and B, (iii) characterize a local population based on multilocus genotyping, (iv) observe the possible seasonality of these groups and (v) compare the data from areas treated with different plant protection practices. The sampling took place between 2017 and 2019 in a Tokaj vineyard (Mád, Szent Tamás) where two distinct pest management practices have been applied. DNA extractions were performed from single chasmothecia and mycelium. The aforementioned SNPs were PCR-amplified and sequenced or identified by Real-Time PCR in 283 samples. We identified the presence of the A495T marker in the collected samples, although with low prevalence. Genotype B was dominant in the area regardless of the season, and the resistance marker was also more frequent in samples belonging to this group. Genotype A was observed in low quantities and despite the fact that according to previous literature the group only reproduces clonally, we identified the genotype from DNA extractions made from chasmothecia. Using multilocus genotyping, the samples were separated into several distinct groups. Applying this method for monitoring GPM populations and collecting a deeper knowledge about these new groups may allow the development of more effective GPM management practices in the future.

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CHANGE IN MICROBIOME OF BLACK LOCUST AND POPLAR RHIZOSPHERE UNDER MYCORRHIZA AND INORGANIC FERTILIZER APPLICATION

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The effect of chemical fertilization on soil bacterial and fungal communities is a growing concern due to the changes on microorganisms in soil ecosystems. Black locust (*Robinia pseudoacacia* L.) due to its fast and intensive growth, it is the most important tree species cultivated in Hungary. It contributes greatly to soil quality by changing the biodiversity of soil, improving soil chemical properties and fertility, restoring degraded soils and sequestering the organic carbon in soil.

Populus x *euramericana* L. represents the majority of poplar plantations in European countries. In their natural habitats as well as in plantations poplars are colonized by ectomycorrhizal fungi, this interaction is important for tree nutrition and can profoundly modulate plant responses to unfavourable environmental conditions. Mycorrhizal fungal inoculation has the potential to be a useful biotechnological tool that benefits plant development and health, increases plant defense mechanisms and alleviate different stress effects. Black locust and poplar plantation experiments were conducted in Monorierdő, Pest County, Hungary. The study aims to compare the mycorrhizal inoculation and fertilizer applications on bacterial and fungal communities in rhizosphere soil of intensively cultivated plantations using the Illumina Miseq sequencing platform. Our results considered that mycorrhizal inoculation and chemical fertilization have less impact on rhizosphere bacterial community, while the diversity and composition of soil fungal community are more sensitive and affected by chemical fertilizer than mycorrhizal inoculation.

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BACTERIAL DIVERSITY OF THE HIGH-ALTITUDE PERMAFROST REGION LOCATED NEAR THE OJOS DEL SALADO (CENTRAL ANDES, CHILE)

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The Dry Andes of Chile provides a unique site for the research of extremophilic microorganisms. Due to the harsh climate (i.e. extreme aridity, low temperature and strong ultraviolet radiation), the Atacama Desert is considered as a Mars-like habitat. Here, above the snow line, climatic conditions are favorable for permafrost formation. The permafrost, whether we are talking about the high altitude or the arctic permafrost gets increasing attention today due to its importance in the global warming. The ice gives us an insight into the current or past climatic state, furthermore, the accelerated permafrost thawing has great implications for climate change. For example, permafrost degradation can lead to ecological and economic disasters, because it contains an enormous amount of water, organic carbon and potentially pathogenic microbes, which were buried under frozen soil. Therefore, microbial analysis of permafrost has received much attention in the past decades. The aim of our research was to explore the composition of bacterial communities in the permafrost region on the Ojos del Salado volcano. Soil samples from various depths (0, 10, 35 and 60 cm) were taken at different altitudes (4,200, 5,260 and 5,830 m a.s.l.), then they were subjected to 16S rRNA gene amplicon sequencing in order to reveal the bacterial community composition. The most abundant bacterial phyla were the Proteobacteria, Actinobacteria and Bacteroidetes through all our samples, however, their ratio changed with the altitudes, and sometimes with the depth, as well, with Proteobacteria being dominant in the higher altitudes and greater depths, and Actinobacteria dominating the lower altitudes – Bacteroidetes was the most abundant in some of the samples taken from 10 to 35 centimeters depth. Our results for the most abundant phyla were similar to those for Arctic and other high-mountain permafrost soils, despite the distinct methods applied.

INFLUENCE OF FERTILIZERS AND CROPS ON SOIL BACTERIAL DIVERSITY IN LONG-TERM MAIZE AND WHEAT CROPPING SYSTEMS

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New large-scale agricultural technologies and agrochemicals introduced in the 1950's led to great increase in production of food grains, e.g. maize and wheat. Agricultural intensification, however, is widely considered to have negative effects on soil productivity and ecosystem, including soil degradation, re-shaping the soil microbial communities and thus a loss of productivity over time. The aim of our project was to investigate the effects of fertilizers on the composition of soil bacterial communities of crops in a long-term experiment in Martonvásár (Hungary). We collected samples from soils under maize (*Zea mays*) and wheat (*Triticum aestivum*) monocultures, and wheat - maize dicultures, five times in 2018. Each plot was supplied either with inorganic NPK fertilizer or NPK together with farmyard manure (FYM), apart from control plots. 16S rRNA gene amplicon sequencing was applied to determine the composition of bacterial communities. We observed RB41, *Bryobacter, Sphingomonas, Flavobacterium, Gemmatimonas*, SC-I-84, *Candidatus* Udaeobacter, and unclassified genera of Vicinamibacteraceae, Acidobacteriales and Chitinophagaceae in high relative abundance. According to statistical analysis, both crop rotation and fertilization have a significant effect effect ($p<0.05$) on the OTU composition of the bacterial communities. Comparing the plots under different fertilization regimes, communities of maize and wheat monocultures

fertilized with NPK and FYM did not differ significantly from their control plots but from NPK-treated monocultures. Any kind of fertilizer added to the diculture changed the community composition significantly. When the bacterial assemblages of crop rotation types under the same fertilization regime were compared, wheat and maize monocultures were distinctive from each other in case of the control and the NPK+FYM treatment, while dicultures could not be clearly distinguished from monocultures. The application of NPK resulted in significantly different community structures regardless of the crop rotation system. The supplements of inorganic fertilizer with manure mitigated the effects of NPK, because the bacterial communities were more similar to the control plots than to the NPK-fertilized soils. Furthermore, the bacterial community of diculture diverged from monocultures as the result of using only inorganic NPK fertilizer.

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ALTERNARIA SPECIES AND THEIR SECONDARY METABOLITES IN GRAPEVINE (*VITIS VINIFERA*) SHOOTS

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Grapevine harbours complex endophytic fungal communities affecting the metabolism, growing, productivity and stress tolerance of the host plant. According to our previous results and information available from other studies focusing on the grapevine mycobiome, a significant number of fungal species belonging to the genus *Alternaria* are represented among the endophytic fungi colonizing the different grapevine varieties. The genus is a biologically, morphologically and ecologically diverse group. In the recent decades, *Alternaria* species have gained increasing scientific attention primarily in connection with their challenging taxonomy and ability to produce a high variety of secondary metabolites, however data on well-identified species in Hungarian vineyards and their secondary metabolites are limited. In the present study we addressed the molecular identification of endophytic *Alternaria* species associated with the above-ground tissues of grapevine. We also aimed to determine the metabolite profile of the *Alternaria* isolates. Based on the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA, we identified the *Alternaria* species belonging *Alternaria* sect. *Alternaria* in a dominant number among the fungal isolates from asymptomatic leaves and clusters. As the ITS region is not adequate for the species delimitation in *Alternaria* sect. *Alternaria*, five additional genomic loci (RPB2, ALTA1, endoPG, OPA10-2 and KOG1058) were included in our study allowing for a better species resolution. The metabolite profiling was carried out using high performance liquid chromatography (HPLC)-tandem mass spectrometry (MS/MS). We can conclude that the endophytic fungal isolates gained from healthy shoots of grapevine belong to two distinct lineages considered as *A. alternata* and *A. arborescens* species complex, and numerous compounds characteristic to the genus have been found in the different *Alternaria* lineages.

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THE LEAF-ASSOCIATED MYCOBIOME IN THE LIGHT OF GRAPEVINE (*VITIS VINIFERA*) GENOTYPE

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Grapevine (*V. vinifera*), one of the most important cultivated plants worldwide, is naturally inhabited by a wide range of fungi, modulating health, growth and productivity of the host plant. This diverse fungal community may even have an impact on the organoleptic properties of wine and other fermented beverages, so it is essential to have a profound understanding of its diversity and composition. The above-ground tissues of grapevine represent a dynamic and harsh habitat for microbial colonizers due to the exposure to environmental factors such as fluctuating temperature, solar radiation, water limitation etc. It is an important question, what drivers shape the microbial composition of the grapevine phyllosphere, thus contributing to plant health and the quality of the resulting wines. In our work, we investigated the potential differences of the mycobiome associated with asymptomatic leaves of three different cultivars, *V. vinifera* cv. *Furmint*,

cv. *Kadarka* and cv. *Syrah*, grown in the same vineyard under identical environmental and management conditions in the Eger wine region. We focused on the question whether the grapevine genotype has an effect on the fungal communities inhabiting the inner and external tissues of healthy leaves using high-throughput DNA sequencing. We compared richness and relative abundance of various functional groups of fungi, such as saprotrophs, leaf-associated fungi, plant pathogens and mycoparasites and assessed their compositional differences among the three cultivars. While cultivars did not have a significant effect on the richness and relative abundance of fungal functional groups, we did find strong compositional differences among cultivars. Indicators for both cv. *Furmint* and cv. *Syrah* were e.g. the general saprotroph *Aureobasidium* and plant pathogen *Ustilago*, for cv. *Kadarka* and cv. *Syrah* the plant pathogen *Erysiphe*.

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EFFECTS OF AGRICULTURAL PRACTICES ON THE AMMONIA EMISSION FROM SOIL AND ON THE CATABOLIC PROCESSES OF MICROBIAL COMMUNITIES

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Urea-based nitrogen fertilizers are widely used in the agriculture. However, the rapid conversion of urea to ammonia can lead to a significant amount of nitrogen emission from the soil through volatilization, thus greatly reducing the nitrogen use efficiency of the soil and creating higher environmental load. We examined the effect of three agricultural practices in a small-plot field experiment in Őrbottyán, with maize as the crop plant. Two doses of nitrogen (the present N_{max} and a higher dose) and a commercially available urease inhibitor were applied either on the soil surface or worked into the soil. Nitrogen loss was measured as ammonia emission from each plot. The effect of the treatments on the bacterial communities was examined by catabolic fingerprinting with the Microresp method. We found that both the urease inhibitor and the mechanical treatment were able to effectively reduce ammonia emissions. The nitrogen dose, the use of inhibitor and the mechanical treatment all had significant effects on the soil catabolic processes two weeks after their application, however these effects were reduced in one additional month. In conclusion, we found that a nitrogen dose higher than the currently allowed maximum can be safely applied when proper agricultural techniques are used.

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ACTIVATION OF LATENT HHV-6 VIRUS INFECTION DURING LONG-DURATION SPACE FLIGHT - IN THE LIGHT OF "INTERFERON" SPACE EXPERIMENTS

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Stressors of space flight, exposure of astronauts to non-terrestrial hazards have negative effects on immune system and adaptation facilitating latent herpes virus reactivation during and after spaceflight. In our earlier space experiments "Interferon" (Salyut-6) we got evidence of dysregulated immune system as proof of concept. The main purpose of the research is to study the presence and detect activation of human herpesvirus 6 A and B (HHV-6 A, B) as latent infection and the antiviral effect of an immunomodulant wheat germ extract (IWGE) during the special conditions of space flight. HHV-6 B could induce central nervous system alterations not yet described in details and is able to activate latent HIV in the body. Human lymphoid MOLT3 cells were infected with HHV-6B Z29 (Ferrara) strain in various concentrations. Infected and uninfected cells were treated with a licensed immunomodulant compound (Avemar pulvis) in concentrations of 10-250 µg/mL. Cell viability, cytotoxicity, expression of HHV-6B and inhibition of virus expression were quantitatively determined with cytopathogenic effect, immunofluorescent assay and real time PCR. Low IWGE concentrations (up to 100 µg/mL) inhibited replication of HHV-6B in MOLT3 cells by more than 80%, medium level (100-150 µg/mL) selectively destroyed virus infected cells, and concentrations above of 200 µg/mL were cytotoxic, resulted in death of all MOLT3 cells. IWGE have an inhibitory effect on the replication of HHV-6B a HIV transactivating heterologous and opportunistic virus and also inhibits ribonucleotide reductase (RR) enzyme. HHV-6B virus coded for one of the subunits of RR, and IWGE may have the capacity based on it to inhibit the activity of HHV-6B expression. All the methods used during our experiments are suitable to monitor and quantitatively determine virus expression in a dose dependent manner. Our research project seems suitable to monitor and determine activation of latent human herpesvirus-6B infection and manage it by an immunomodulant compound during special conditions of space flight.

WEST NILE AND USUTU VIRUS SEROPREVALENCE IN HUNGARY: A NATIONWIDE SEROSURVEY AMONG BLOOD DONORS IN 2019

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In Hungary, West Nile virus (WNV) has been responsible for a remarkable number of human cases in the last two decades, while the first human Usutu virus (USUV) infection was confirmed only in 2018. A comprehensive serosurvey was conducted among blood donors to assess the WNV and USUV seroprevalence in 2019, one year after the European WNV epidemic. Altogether, 3,005 plasmas were collected and screened for WNV and USUV specific Immunoglobulin G (IgG) antibodies by Enzyme–Linked Immunosorbent Assay (ELISA). All reactive samples were further tested for tick-borne encephalitis virus IgG antibodies by ELISA. Indirect immunofluorescence test and microneutralization assay were used as confirmatory methods. Overall, the WNV seroprevalence was 4.32% (95% confidence interval: 3.61−5.13), and in five blood donors USUV seropositivity was confirmed. The highest seroprevalence was measured in Central, Eastern and Southern Hungary, while the Western part of the country proved to be less affected. The highest WNV seroprevalence was measured in Jász-Nagykun-Szolnok County. There was a statistically strength association between the WNV seroprevalence of 2019 and the cumulative incidence in the period of 2004 and 2019 calculated for every NUTS 3 region. The latest WNV serological screening was performed in 2016 and the prevalence of anti-WNV IgG proved to be 2.19% (95% confidence interval: 1.64–2.90). One year after the 2018 European WNV epidemic, a significant increase in seroprevalence could be observed in the Hungarian population and evidence for USUV seropositivity was also obtained. Spatial pattern of seroprevalence can support to determine the high-risk areas, raising awareness of the need for preventive measures.

INVESTIGATING THE PREVALENCE OF MULTIRESISTANT ENTEROBACTERALES IN BLACK-HEADED GULLS (*CHROICOCEPHALUS RIDIBUNDUS*) AND A COMPARISON WITH CONTEMPORARY HUMAN ISOLATES

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During winter, large number of black-headed gulls (*Chroicocephalus ridibundus*) flocks at the docks of Budapest. These birds are in proximity to humans and water bodies and frequently use anthropogenic food sources such as landfills. To investigate the prevalence of third generation cephalosporin resistant Enterobacterales in gulls and compare ESBL-producing *Escherichia coli* isolates to contemporary human-derived strains. In the winter of 2018-2019, 123 fecal samples were obtained from gulls captured for ringing purposes at the docks of Budapest. Isolates were recovered on eosin-methylene blue media supplemented with 2 mg/L cefotaxime and were identified by MALDI-TOF-MS. Susceptibility testing was perfomed using standard disc diffusion method for ertapenem, ciprofloxacin, trimethoprim-sulphametoxazole, amikacin, gentamicin and tobramycin. ESBL-production was determined by double-disc synergy test, carbapenemase production by modified carbapenem inactivation method and MASTDISCS Combi Carba test. We also collected 134 contemporary ESBL-producing *E. coli* from inpatients of the Semmelweis University. ESBL-producing isolates were screened for the resistance genes *blaSHV, blaTEM, blaCTX-M-1,2,8,9* groups. *E. coli* phylogroups and members of sequence type (ST) 131 clonal lineage were identified by multiplex PCRs. Enterobacterales resistant to cefotaxime were carried by 59% (73/123) of the sampled gulls; total of 123 isolates were recovered (82 ESBL-producers, 31 AmpC-producers and 10 carbapenem resistant isolates). The AmpC-producing isolates were not further investigated in this study. Of the ESBL-producers, 69 were *E. coli*, 10 *Klebsiella pneumoniae* and three *Citrobacter* spp. Among the ESBL-producing *E. coli* isolates, the dominant genes in gull and human isolates were *blaCTX-M-1* group (68% in both sets) and *blaCTX-M-9* group (22% and 27%, respectively). Gull isolates showed lower co-resistance than human clinical isolates; 48% and 80% to ciprofloxacin, 48% and 59% to trimethoprim-sulphametoxazole, 24% and 47% to amikacin and tobramycin, 16% and 44% to gentamicin, respectively. Most gull isolates (70%) belonged to commensal phylogroups (A, B1, C, or E) while phylogroup B2 was predominant among human isolates (74%). Of the gull isolates, 26% (18/68) belonged to phylogroup B2 and 15 of them were pandemic ST131; seven of the C2 and eight of the C1-M27 clades. Of human isolates, 63% (86/136) was ST131, one, one, two, 31 and 51 were members of A, B, C1, C1-M27 and C2 clades, respectively. Of carbapenem resistant isolates, metallo-beta-lactamase production was found in four *E. coli*, two *K. pneumoniae* and one *Enterobacter* spp., one *E. coli* and one *K. pneumoniae* produced OXA-48; one porin deficient *Enterobacter* spp. were found. Gulls may play a role in the dissemination of these agents resistant strains because of their vagrant behavior highlighting the importance of One Health in case of antibiotic resistance.

CANDIDA ALBICANS ALLEL-SPECIFIC GENE PROMOTER ANALYSIS WITH CHIP-SEQ DATA

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Candida albicans is a diploid opportunistic pathogenic yeast. Its main characteristic is that it has no real sexual reproduction. Due to the lack of normal meiotic division, its chromosomes have changed differently over time. Previous work has demonstrated allele-specific gene expression in *Candida albicans*. We assume that one possible explanation for this could be a difference in the promoter or terminator region of the gene. We postulate that differences in these regions, by affecting the binding of transcription factors, may cause allele-specific transcripts. To determine the physical location of the promoter and terminator regions, we used the standard approach of locating the promoter region up to 1,000 base pairs upstream of the gene and the terminator region up to 500 base pairs downstream of the gene, unless the ORF of the next gene is closer. In the case that it is closer, we extended the promoter/terminator regions only to the sequence of the ORF. We first searched for sequence divergence between the two alleles at the promoter, gene and terminator regions using the *Candida albicans* SC5314 A22-s07-m01-r135 genome sequence. In the second step, we used a published dataset containing both RNA-seq and ChIP-seq data from a single set of isolates. These were run on our allele-specific pipeline to obtain the genes that show allele-specific expression (RNA-seq) under the given conditions. From the results of ChIP-seq assays performed under the same conditions, we determined the coverage of the three regions by each gene. By comparing the three results, we infer allele-specific activation of the promoter, ORF, terminator region of the gene.

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TECHNO-ECONOMIC ANALYSIS FOR COMPLEX UTILISATION OF *YARROWIA LIPOLYTICA*

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Among non-conventional yeasts *Yarrowia lipolytica* has a special occupies a very prominent place. It is excellent enzyme-, ergosterol-, citric acid-, oil (Single Cell Oil (SCO)-, Tri-acil-glycerol (TAG-biodiesel, linolic acid), and erythritol producer, having good metal absorption capability (applicable in bioremediation), as well as is a good expression system. However, there are only a few articles dealing with its complex application. Only a few reports on *Yarrowia* based biorefinery present techno-economic analysis and simulation focusing on only 1-1 main product (for example in case of erythritol, and SCO). At the same time the European Cosmetic Ingredients database contains several *Yarrowia* based components, but in scientific reports these can not be found. Therefore, in our recent research we studied in biorefinery concept bioprocesses based on *Y. lipolytica* fermentation and fractionation. A process simulation was created on both experimental and literature survey basis to elaborate and examine such a technology, which recovers most of the value-added products of *Y. lipolytica* cells in the same facility including potential cosmetic ingredients as well.

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INVESTIGATION OF THE INTERACTION OF KERATINOCYTES AND *CANDIDA* SPECIES

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Our skin provides immunological protection against several pathogens. Skin epithelial cells respond to microbial stimuli in various ways, such as through the production of antimicrobial peptides or secretion of cytokines, although phagocytosis of potentially evading microbes was also reported. Relatively little is known about how skin keratinocytes differentiate between the presence of pathogenic and commensal fungi. In this project, we aimed to investigate how human keratinocytes interact with different *Candida* species, as common colonizers of the skin. While *C. albicans* is a common cause of cutaneous candidiasis, *C. parapsilosis* is rarely associated with this disease. For the experiments human skin keratinocyte cell lines (HaCaT, HPV-KER) were applied and challenged with *C. albicans* (SC5314 and WO1) and C. parapsilosis (GA1 and CLIB214) strains. We aimed to determine the extent to which *C. albicans* and *C. parapsilosis* damage human keratinocytes, their attachment to host cells, the keratinocytes' ability to internalize these fungi and to examine cytokine

production in response to stimuli. Our results suggest that *C. albicans* causes significantly more damage to human keratinocytes than *C. parapsilosis* and the HPV-KER cell line was more susceptible to the infection. In both HaCaT and HPV-KER cells, the production of IL-6, IL-8, and CCL5 increased primarily after *C. albicans* infection. Based on the adhesion and phagocytosis studies, there was a low degree of association and internalization in case of *C. parapsilosis* GA1 and CLIB214 compared to *C. albicans* SC5314 and WO1.

LESSONS LEARNED FROM THE SARS-COV-2/COVID-19 PANDEMIC

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In the past almost two years we experienced an unprecedented viral pandemic, the most devastating pandemic since the Spanish Flu more than 100 years ago. Well aware that Coronaviruses have the potential to jump species and cause outbreaks in humans, the scale of this pandemic excelled everything researchers could think of. How was it possible that we could not stop this infection as long as it was geographically restricted? Due to enhanced molecular technologies it took only a short period of time to unravel the pathogen's genome, and, based on this, to quickly develop molecular tools for diagnosis. Nonetheless the virus continued to spread – within China and beyond. Meanwhile tens of thousands of full viral genomes were sequenced using NGS technology. In March 2020, the first mutation in the spike protein (D614G) was noticed but no one was too much worried about it. Meanwhile we thoroughly observe all mutations/virus variants and can see a significant increase in transmissibility of the virus. Although previously thought that recombination events in influenza A viruses are much more dangerous, we learned that the exchange of certain amino acids, especially in the most important spike protein, of SARS-CoV-2 is equally dangerous resulting in higher infectivity of and/or in immune escape variants. How did we try to fight this pandemic? First starting with non-pharmaceutical interventions – essentially the same as 100 years ago – researchers around the globe started to work on vaccines and treatment options at the same time. As we know today, THE antiviral medication was not yet identified, but luckily our clinical colleagues learned to use the right medication at the right time during the course of the disease, and thus we were able to limit the death toll. The development of vaccines was much more successful, and today more than 5.7 billion doses of vaccines were already used. Nonetheless the fear of getting shots with vaccines which are based on rather new technologies are high in the population, and 10-20% of the population refuses vaccination, which makes it difficult to reach herd immunity and consequently to overcome the pandemic. Also, as never before, fake news, mainly distributed through social media, opposed the scientific view on vaccines. We experienced already a number of "infection waves" in Europe – currently we are inmidst wave #4. The strategies how to handle these "infection waves" differed significantly. In my talk I will look into these different strategies. And last but not least I will try to provide an outlook about the future of SARS-CoV-2/COVID-19.

THE TALE OF THE TWO-HEADED BEAST: METATRANSCRIPTOMIC ANALYSES REVEAL DIFFERENCES IN GENE EXPRESSION PROFILES OF *BOTRYTIS CINEREA* DURING NOBLE AND GRAY ROT DEVELOPMENT IN GRAPEVINE

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Botrytis cinerea is well-known for causing gray rot (GR) of grapevine (*Vitis vinifera*). Under certain environmental conditions, however, *B. cinerea* can cause noble rot (NR) of berries, the basis for producing the sweet aszú wines. Little is known about the fungal and plant metabolic genes involved in NR and how they differ from GR. We analyzed metatranscriptomic data generated from healthy (H), NR and GR grape berries collected from the Tokaj wine region. Functional gene expression profiles of *V. vinifera* and *B. cinerea* were compared among rot types, sampling months, and physical characteristics of berries using multivariate analyses of variance, differential gene expressions, gene ontology and functional pathway enrichment. Abundance, richness and composition of functional gene transcripts of *V. vinifera* and *B. cinerea* differed among berry types and sampling months. Furthermore, *B. cinerea* showed higher metabolic activity during NR than in GR, particularly concerning genes involved in pathways contributing to the characteristic aroma profile and changes in berry skin structure associated with NR. We found that harvest time influenced functional gene profiles in NR and GR berries and that many differently expressed genes corresponded to processes linked to sensory differences between NR and GR berries, with implications for wine makers.

OXIDATIVE STRESS ELICITED GENE EXPRESSION CHANGES IN *FUSARIUM VERTICILLIOIDES* MUTANT STRAINS

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Oxidative stress is one of the most severe types of environmental stress, which plant pathogenic filamentous fungi are exposed to. There is a growing body of evidence on that secondary metabolism is associated with oxidative stress in filamentous fungi. Previously, two mutant strains (ΔFvatfA, ΔFvmnSOD) of *Fusarium verticillioides* were constructed in our Department. In this maize pathogenic fungus, the transcription factor FvAtfA is involved in the control of pigment (carotenoid, bikaverin) and mycotoxin (fumonisins B1 and B2) production and also in the orchestration of oxidative stress defense. The antioxidative enzyme FvMnSOD is responsible for the elimination of superoxide radicals formed in the cell. In previous studies, FvMnSOD has not been coupled to secondary metabolite production but the ΔFvmnSOD mutant strain has shown hypersensitivity to menadione, a superoxide generating agent. We optimized the experimental conditions to gain an access to transcriptome changes taking place in submerged, oxidative stress (menadione sodium bisulphite, MSB) exposed cultures of FGSC 7600 (control), ΔFvatfA and ΔFvmnSOD strains. RNAseq data were generated and aligned to the NRRL 20956 reference genome. Expression values for all genes were calculated, and expression patterns for the fumonisin, bikaverin and carotenoid biosynthetic gene clusters were analyzed further. Interestingly, significant downregulation of the carotenoid gene cluster was observed in the absence of the FvAtfA transcription factor with exception of *carB, carRA*, whose expression was slightly improved by oxidative stress. Similarly, six genes in the bikaverin gene cluster were also downregulated in the ΔFvatfA strain, and this downregulation was further enhanced under MSB treatment. It was also observed that oxidative stress alone was a positive regulator of some genes among the twelve tested genes of the fumonisin gene cluster (e.g. *fum10*), but overall, oxidative stress caused a larger down-regulation in the ΔFvatfA deletion strain. In contrast, the lack of FvMnSOD affected secondary metabolite biosynthesis to a lesser extent in comparison to the loss of FvAtfA. However, the expression of three genes in the bikaverin gene cluster (*bik1, bik5, bik6*) was up-regulated by MSB meanwhile some fumonisin biosynthetic genes (*fum13, fum17*) down-regulated under oxidative stress. It is noteworthy that the carotenoid gene cluster did not respond to the deletion of FvmnSOD at all.

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ISOLATION AND CHARACTERIZATION OF A NOVEL CARBAMAZEPINE DEGRADING BACTERIUM AFFILIATING TO THE GENUS *NOCARDIOIDES*

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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 demonstrated that at environmentally relevant concentrations these compounds can exert ecotoxic effect on aquatic organisms. We recently committed to the selective enrichment, identification and isolation of pharmaceuticals-, especially DIC, IBU and CBZ degrading bacteria. Here we report on a novel *Nocardioides* species, isolate CBZ_1T, obtained from a biofilm bacterial community selectively enriched on carbamazepine. A polyphasic approach, including phylogenetic (whole genome sequencing, ANI, dDDH) chemotaxonomic (analysis of polar lipids, cellular fatty acids, respiratory quinones), morphological (TEM), physiological and biochemical studies (API® ZYM, API® 20NE and API® 50CH), was used to accurately determine the taxonomic affiliation of the isolate. Genome resolved metagenome and metatranscriptome studies were performed to determine the activity of strain CBZ_1T in the presence of different pharmaceuticals during enrichments. The proposed new to science bacterial species' biodegradation capacity towards the aforementioned pharmaceutical compounds, without the formation of harmful degradation by-products, was also assessed in microcosm experiments followed by HPLC and bioassays. Phylogenetic, phenotypic and chemotaxonomic data supported the classification of strain CBZ_1T to the genus *Nocardioides* for which the name *Nocardioides carbamazepinivorans* is proposed. Metagenome and metatranscriptome studies showed that during the selective enrichments strain CBZ_1T was active and proliferated well in IBU and CBZ

enrichments, but not in DIC. Strain CBZ_1T was capable of degrading carbamazepine in the presence of glucose without the formation of toxic by-products. Although a multitude of *Nocardioides* isolates have been described as dibenzofuran, p-nitrophenol, chlorophenols, pyridine, atrazine, phenanthrene, carbendazim etc. degrading organisms to the best of our knowledge no *Nocardioides* isolates have been reported so far capable of carbamazepine biodegradation.

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SCREENING FOR DICLOFENAC, IBUPROFEN AND CARBAMAZEPINE DEGRADING BACTERIA SELECTIVELY ENRICHED AND ISOLATED FROM A SUBSURFACE BIOFILM

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Pharmaceutically active compounds (PhACs) are widespread micropollutants in aquatic environments worldwide. The most widely detected, persistent or pseudo-persistent PhACs are diclofenac (DIC), ibuprofen (IBU) and carbamazepine (CBZ). These compounds in environmentally relevant concentrations may exert ecotoxic effect on aquatic organisms such as fish, crustacean and mussels. Wastewater effluents are the main sources of PhACs contamination, surface and groundwaters have the highest concentration of them. Conventional wastewater treatment plants are inefficient in eliminating PhACs. Better alternatives or additional units, preferably biological solutions, are needed for more efficient elimination of PhACs from the wastewater. Amongst the three compounds carbamazepine is the most resistant against biodegradation. In the present study we aimed at selectively enrich, isolate and identify potentially diclofenac, ibuprofen and carbamazepine degrading bacteria. For this purpose, a groundwater biofilm community was used, with high phylogenetic and functional diversity as determined earlier. Selective enrichments for three months were done in mineral salts medium (MSM) supplemented with pharmaceuticals (100 ppm, either DIC, IBU or CBZ). After each month sub cultivations and bacterial isolations took place. A bacterial strain collection containing 47 species level identified isolates was obtained. The bacterial species representative isolates were tested for DIC, IBU or CBZ biodegradation in MSM as follows: (i) first, using the semi-quantitative resazurin screening assay potential pharmaceutical degraders were identified, isolates that showed increased activity in the presence of individual pharmaceutical compounds as sole source of carbon and energy (10 ppm); (ii) second, to get more accurate information about the biodegradation capacity of the selected isolates high pressure liquid chromatography (HPLC) was applied. Parallel to the individual pharmaceutical biodegradation tests, co-metabolic microcosm experiments were also set up; apart from DIC, IBU or CBZ (1.5 ppm) additional carbon sources such as yeast extract (50 ppm) or glucose (500 ppm) were added to the test solutions. Isolates affiliating with the genera *Stenotrophomonas, Rhizobium* and *Brevundimonas* showed the highest DIC, IBU and CBZ biodegradation rates, respectively. These isolates may be used for the development of biotechnological tools for better elimination of PhACs from the wastewater.

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SCREENING ANTAGONISTIC EFFECT OF FLUORESCENT PSEUDOMONADS AGAINST *ASPERGILLUS FLAVUS*

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Fungal pathogens can cause a great number of plant diseases leading to significant crop losses in the field due to postharvest processes. Phytopathogen fungi are capable of producing secondary metabolites poses health hazards to animals and humans. Different practices have been applied to control plant diseases. Rhizosphere bacterial species such as *Bacillus, Streptomyces* and *Pseudomonas* have been proved to be efficient biocontrol agent in managing diseases. Pseudomonads are capable to release soluble and volatile compounds to suppress the growth of phytopathogen fungi, but most research only focus the effects of bacteria on the fungi. In our work we would investigate the metabolomic background of bacterial-fungal interactions. We have selected 60 fluorescent *Pseudomonas* strains and an *Aspergillus flavus* strain from the Szeged Microbiological Collection and we have isolated 20 new *Pseudomonas* strains from corn rhizosphere. Dual culture method was used for preliminary screening of antagonistic behaviour. The method was performed in quintuplicate

for solid-state cultivation. Inoculum of the fungus was placed on the middle of PDA plates and *Pseudomonas* isolates from an overnight culture. Petri dishes were inoculated with fungi and bacteria alone are served as controls. All the plates were incubated up to 8 days. To determine the toxin producing capability of the fungal strain, the toxin content of an agar plug removed from the mycelia from the middle of the plate and was measured by LC-MS method. According to the results of our LC-MS measurements, some strains were shown to inhibit AB1 production (95-99%) and cause significant morphological changes.

GAMMA (Γ)-CORE PEPTIDE DERIVATIVES OF NOVEL TOMATO PLANT DEFENSINS EFFECTIVELY INHIBIT THE GROWTH OF PLANT PATHOGENIC FILAMENTOUS FUNGI

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In the past decades the number of damages caused by plant pathogenic fungi has been constantly rising, and causes billions of euros loss for agriculture. This problem is further exacerbated by the increasing number of phytopathogenic fungi showing high resistance to conventional pesticides. Therefore, it is necessary to develop new plant and crop protection strategies. Defensins and their peptide derivatives with antifungal effect are considered as potential biofungicides. All plant defensins contain an evolutionarily conserved GXC-X[3-9]-C amino acid motif, the so-called γ-core region. Previous studies already demonstrated that the presence of the γ-core region is important for the antifungal effect of plant defensins, and synthetic peptides spanning the γ-core region have high in vitro antifungal activity. Previously we identified four novel defensin-like protein from a tomato plant (*Solanum lycopersicum* L.) genomic database. In the present study we investigated the in vitro antifungal activity of synthetic γ-core peptide derivatives of these defensins against the main filamentous fungal pathogens of tomato plant (*Botrytis cinerea, Cladosporium herbarum, Fusarium oxysporum*) in a broth microdilution susceptibility assay. Based on our results, the hydrophilic and highly positively charged γ-core peptide derivatives are able to effectively inhibit the growth of phytopathogenic filamentous fungi with different efficacy (minimum inhibitory concentration: 12.5 - 400 μg/mL). Concluding the results, we suppose that the antifungal active γ-core peptide derivatives could serve as novel biopesticides in the agriculture after further investigations.

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IDEALISATION THE RANDOMLY AMPLIFIED POLYMORPHIC (RAPD) METHOD FOR COMPARATIVE ANALYSIS OF *SHEWANELLA BALTICA* SPECIES

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The *Shewanella* genus is one of the most abundant γ-proteobacteria in the marine and fresh water environment. Its metabolic versatility and ability to utilize a variety of extracellular electron acceptors is a key feature in its role in the turnover of organic matter, denitrification and also bioremediation. One of its less studied represenatative is *Shewanella baltica* that is mostly isolated from spoiled freshwater and marine foods and can be responsible for spoilage of these meats. From this point of view it has an importance in food safety. On the other hand a well documented characteristic of some *S. baltica* strains is its heavy metal tolerance and electroactive activity that feature could make them proper candidates in special environmental monitoring systems, based on the microbial fuell cell technology. Till know there is a limited number of data available about the clonal relationships of different isolates. Due to its easy execution the Randomly Amplified Polymorphic (RAPD) method is a preferred method to investigate genetic similarities and differences among different bacterial isolates. Several primers and primer combinations are available for this purposes for different bacterium species. In this study we tested the resolutions of different RAPD primers and primer combinations in case of the recently isolated five different *S. baltica* isolates from Hungary. For this purposes we have used the formerly published primers like: ERIC1, ERIC2, A10, F4, R16, N9, AP4, R108, HLWL74. In frame of this presentation the applicability of different primer combinations will be discussed that characterize the five different *S. baltica* strains.

COMPARATIVE AND STATISTICAL ANALYSIS OF 100 STWINTRONS FOUND IN AN *HYPOXYLON* GENOME

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Spliceosomal introns are pervasive in eukaryotic nuclear transcriptomes. Their precise excision from pre-mRNAs requires the action of the U2 spliceosome. Intervening sequences may be complex and include more than one canonical intron unit. We have described a class of nested U2 introns, the stwintrons, which are removed by consecutive splicing reactions. There are different stwintron classes, depending on which of the 3 canonical sequence elements of the external U2 intron is disrupted by the internal U2 intron: [D], 5'-donor-disrupted; [L], lariat branch point (BP) element-disrupted; [A], 3'-acceptor-disrupted. In a [D1,2] stwintron, an internal intron is nested in the 5'-donor element of an external intron between the first and the second nt (5'-$G_1|U_2$). The genesis of new introns remains a mystery. The availability of complete genomes of > 1,000 fungi provides opportunities to study intron gain events throughout a whole phylum as well as amongst closely allied species. Fungal U2 introns are generally small – often < 100 nt. The short but ubiquitous 5'-donor, the BP element, 3'-acceptor sequences are well defined in model genomes. These characteristics enabled the design of a motif search algorithm to predict stwintrons in genome sequences in the absence of expression data. Recently, we found 36 structurally related [D1,2] stwintrons in *Hypoxylon* sp. CO27-5, including 23 highly similar over the complete width [1]. The 23 "sister stwintrons" only occur in CO27-5 and/or EC38. The observed proliferation correlates with elements of high symmetry (long terminal inverted repeats). All insertions occur at new gene positions, all but one stwintron seamlessly integrated in exonic sequences. Crucially, no tandem site duplications are extant, inconsistent with a DNA transposon insertion mechanism. One theorem on propagating introns is that they degenerate much faster than coding sequences, eventually rendering them indistinguishable from regular spliceosomal introns at the primary sequence level [2]. Screening the CO27-5 genome with our motif search algorithm yielded > 100 [D1,2] stwintrons, including the 36 of high sequence similarity. The vast majority of the additional > 70 [D1,2]s are inserted at positions also occupied by [D1,2] stwintrons in the orthologous genes in 17 other Hypoxylaceae species, suggesting that they are older than the sister stwintrons. Here we report on the results of comparative and statistical analyses of these >100 [D1,2] stwintrons, in search of hints that a section of the 70 or so sequence-divergent [D1,2]s may have their origin in ancient internally symmetrical stwintrons.

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[1] Fekete et al (2021) J Fungi 7:710.
[2] Collemare et al ˙(2013) Commun Integr Biol 6:e23147.

PROLIFERATION OF INTERNALLY SYMMETRICAL STWINTRONS AND RELATED CANONICAL INTRONS IN HYPOXYLACEAE SPECIES

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Spliceosomal introns are omnipresent in eukaryotic nuclear transcriptomes. Their excision is catalysed by a specialised organelle, the U2 spliceosome. Intervening sequences may be complex and include more than one canonical intron unit. We have described a class of nested U2 introns, the stwintrons, which are removed by consecutive splicing reactions. There are different stwintron classes, depending on which of the canonical sequence elements of the external U2 intron is disrupted by the internal U2 intron: [D], 5'-donor-disrupted; [L], lariat branch point (BP) element-disrupted; [A], 3'-acceptor-disrupted. In a [D1,2] stwintron, an internal intron is nested within the 5'-donor element [D] of an external intron between the first and the second nt (5'-$G_1|U_2$). The origin of new spliceosomal introns is a mystery. Six different mechanisms of intron acquisition have been proposed [1]. Here we present a unique case of intron proliferation in closely related species of the Hypoxylaceae family [2]. Dozens of [D1,2] stwintrons and canonical introns share specific sequence characteristics that are associated with striking intron proliferation in Hypoxylon sp. CO27-5. We called these related stwintrons and introns of high sequence similarity and internal symmetry, sister (stw)introns. They are predicted to form hairpin secondary structures but the symmetry is such that two intron RNAs may also form a double-stranded molecule. Sister stwintrons proliferate as such in divergent taxa but also can give origin to canonical U2 introns with essentially the same symmetrical features, which would be able to proliferate independently as U2 introns. Sister (stw)introns occur at new gene positions, seamlessly integrated in exonic sequences which are continuous in other Hypoxylaceae. There are no conserved sequence patterns in the bordering exons. Intron phase bias was not observed nor was there a preference for intron generation towards the 5' terminus of pre-mRNAs, suggesting random integration at the genomic level. Crucially,

no tandem site duplications are extant, ruling out the DNA transposon insertion mechanism of propagation. Nevertheless, it remains tempting to speculate that the blatant symmetry of sister (stw)introns is involved in the molecular mechanism of their duplication. We propose two new concepts in which the internal symmetry of the proliferating (stw)intron would enhance duplication to other loci in the genome. In both cases we postulate that intron duplication is a rare by-product of the repair of certain double-stranded DNA breaks (DSBs). The exact location of the new intron insertion would be determined by an impromptu DSB.

Acknowledgements: Supported by the grants EFOP-3.6.1-16-2016-00022) and the NRDI Fund grants NN 128867, K 138489.
[1] Yenerall and Zhou (2012) Biol Direct 7:29.
[2] Fekete et al (2021) J Fungi 7:710.

IDENTIFICATION OF THE POLYSACCHARIDE DEPOLYMERASE OF PHAGE B1, SPECIFIC FOR THE K2 CAPSULAR TYPE OF *KLEBSIELLA PNEUMONIAE*

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Klebsiella pneumoniae is among the leading bacteria that cause nosocomial infections. The capsule of this Gram-negative bacterium is a dominant virulence factor, with a prominent role in defense and biofilm formation. Bacteriophages, which are specific for one bacterial strain and its capsule type, can evoke the lysis of bacterial cells, aided by polysaccharide depolymerase enzymes. In this study, we isolated and characterized a bacteriophage against the nosocomial *K. pneumoniae* 52145 strain with K2 capsular serotype. The phage showed a narrow host range and stable lytic activity. We identified the location of the capsule depolymerase gene of the new phage, which was amplified, cloned, expressed, and purified. The efficacy of the recombinant B1dep depolymerase was tested by spotting on *K. pneumoniae* strains and it was confirmed that the extract lowers the thickness of the bacterium lawn of strains with K2 serotype, as it degrades the protective capsule on bacterial cells. The protein exhibits synergistic effect when it is applied in combination with other phages, which are not capable of evoking lysis without K2 depolymerase activity. As *K. pneumoniae* strains possessing the K2 serotype have epidemiological importance, the B1 phage and its depolymerase are promising candidates for use as possible antimicrobial agents.

CHARACTERISATION OF THE *CITEROMYCES MATRITENSIS*-PRODUCED KILLER TOXIN

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Killer yeasts produce and secrete toxic compounds what are able to inhibit the growth of sensitive cells or even kill them. Killer toxins differ not only among the species but strains belonging to the same species in their mode of action, chemical structure and the coding genetic background. Fungal infections cause world-wide problems in human health care, agriculture and food industry as well. The number of the efficient antifungal drugs is limited and this fact is aggravated by the development of the resistance. Therefore, to find new antifungal compounds is in focus of interest. Natural antifungal compounds have several preferential characteristics e.g. they are less harmful to human health and are environmentally friendly therefore killer toxins produced by yeasts are potential candidates as new antifungal substances. *Citeromyces matritensis*, teleomorph of *Candida globosa*, is an ascomycetous yeast species what can be isolated from different habitat, e.g. plant material and food stuff. The species produces antibacterial compounds effective against *Pseudomonas fluorescens* and *Staphylococcus aureus*. In this study, we present the production of antifungal compound by *C. matritensis* SZMC 26734 strain. The activity spectrum of the toxin was examined in more detail by plate assay against numerous yeast strains including human pathogenic species and food spoilage-causing strains. Species belonging to different genera like *Candida, Cryptococcus, Cutaneotrichosporon, Debaryomyces* proved susceptible to *C. matritensis*. The toxin acts in wide pH range (pH 4 – pH 8), with activity maximum at pH 7. It is thermo-sensitive because it becomes inactive above 30°C. The toxin was produced in either synthetic or nutrient rich medium but the NaCl-content of the medium increased its activity. The mechanism of inhibition is not known but the treatment with the toxin-containing supernatant was lethal to the sensitive cells. Chitinolytic activity of the strain was detected. The *C. matritensis* toxin is probably encoded by chromosomal genes as no DNA plasmids or RNA viruses could be detected. Further study is needed to identify the chemical nature of the toxin and to evaluate its therapeutic potential.

DIFFERENCES IN COMMUNITY-LEVEL CATABOLIC PROFILES (CLCP) CAN REFLECT SOIL CHARACTERISTICS RESULTING FROM VARIOUS LAND USES

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Three different land use types: arable land, meadow and forest soils were compared according to the catabolic response patterns of their microbial community. Characteristic physicochemical parameters were measured and relative substrate induced respiration (rSIR) values were determined by Microresp technique. This method was based on the addition of 23 different organic compounds (carbohydrates, carboxylic acids and amino acids) commonly found in soils and root exudates. Soils from arable land had the highest pH (7.6-7.8) and were rich in nitrate (15-59 mg N/kg) and phosphorous (310-751 mg P_2O_5/kg). In forest and arable soils similar amounts of NO_3^- were detected, while forest showed the lowest pH (6.0-6.4). Both forest and meadow soils contained more total nitrogen (0.4-1.4 m/m %), ammonium-N (8-38 mg/kg) and humus (6.4-27.1 m/m %) than arable land (0.2-0.3 m/m %, 4.5-7.1 mg N/kg and 2.9-3.5 m/m %, respectively). Arable land and meadow could be characterised by a high relative catabolic response for carbohydrates, including monomers of cellulose (glucose) and xylan (xylose). On the other hand, forest soils were significantly different from the other land use types by the increased relative CO_2 production rate for carboxylic acids, especially for 3,4 dihydroxybenzoic acid; an aromatic ring containing substrate which is a lignin degradation intermediate. Furthermore, glutamine and glutamate, the two amino acids having key roles in nitrogen metabolism, induced higher relative respiration in forest soil than in arable land samples. In areas where herbaceous plants dominate (such as arable land and meadow), resident microbiota plausibly has enzyme sets primarily suitable for cellulose and xylan degradation. Contrary in forest, woody plants and their dead material can drive rhizosphere community to secrete higher amounts of enzymes capable of breaking down lignin and its degradation products. As a consequence, specialised enzyme sets of a soil microbial community can respond with higher relative catabolic activity when monomers of cellulose, xylan or lignin are added to them as substrate.

NON-LACTIC ACID BACTERIA FOR BIOLOGICAL CONTROL OF MYCOTOXIN CONTAMINATION IN COMMODITIES

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Mycotoxin contamination in feed and food products is a worldwide problem bearing a life-threatening consequence both on animals and humans. Not surprisingly, *Fusarium* mycotoxins deoxynivalenol, fumonisins, zearalenone, or *Aspergillus / Penicillium* mycotoxin ochratoxin A or *Aspergillus* mycotoxins aflatoxins entering the feed and food chain has been a crucial long-term issue for veterinarians, medicals, agroindustry experts, and researchers working in this field. Although several technologies have been developed, tested, and employed to mitigate the detrimental effects of these mycotoxins, universal methods are still not available to reduce all mycotoxin levels in feed and food in the last decades. Non-lactic acid bacteria (*Lysinibacillus* sp. *Rummeliibacillus suwonensis*, *Bacillus thuringiensis*) isolated from fermented forages were characterized and their mycotoxin resistance and elimination potential were investigated. AFB1 (100 µg/L), DON (1,000 µg/L), ZEA (500 µg/L) did not cause inhibition of the cell growths. Ochratoxin caused significant (73%-54%) growth inhibition. Zearalenone (ZEA, 100 µg/L), aflatoxin B1(AFB1, 24 µg/L), and deoxynivalenol (DON, 200 µg/L) mycotoxins were applied to the cell wall fractions of the bacteria. Cell fractions eliminated ZEA in 100%-16%, AFB1 in 64%-3%, while did not eliminate DON toxin. Cell wall fraction research revealed cell wall protein importance in ZEA elimination. Potential application of the isolates e.g., in fermented forages needs further investigation.

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ARE VOLATILE ISOLATES CONTAINING GLUCOSINOLATE BREAKDOWN PRODUCTS OVERLOOKED IN TESTING THE ACTIVITY AGAINST MYCOTOXIGENIC *PENICILLIUM VERRUCOSUM*?

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Moulds that cause food spoilage are a huge problem in the food industry, especially mycotoxin-producing moulds, which pose a major health risk to food consumers. *Penicillium verrucosum* is one of the major producers of ochratoxin A (OTA) in food. International Agency for Research on Cancer (IARC) classifies OTA as a nephrotoxic, neurotoxic, hepatox-

ic, teratogenic, genotoxic, immunotoxic, embryotoxic, and carcinogenic toxin. Traditionally, mould growth and OTA production in foods have been controlled by the use of various chemical additives, but nowadays there is an increasing demand from consumers to avoid chemical food additives, which has prompted researchers to search for new safe and biodegradable preservatives. Many studies indicate that essential oils (EOs) and volatile isolates (VIs) rich in low molecular weight phenolic and terpenoid compounds are promising alternative antifungal agents, but there haven't been many studies showing the antifungal activity of EOs and VIs rich in glucosinolate breakdown products, such as isothiocyanates, nitriles, epithionitriles, and thiocyanates. For that purpose, the antifungal activity of nasturtium (*Tropaeolum majus* L.) VI was tested. The nasturtium seed powder was subjected to microwave hydrodiffusion and gravity (MHG) for 15 min at a power of 500 W using an ETHOS X device, and further hydrodistillated with Clevenger system for 2.5 hours to obtain a VI. The chemical composition of nasturtium VI was determined by GC-MS/MS, while the minimal inhibitory concentrations (MIC) of nasturtium VI and its pure compounds (benzyl isothiocyanate, 2-phenylacetonitrile) against *P. verrucosum* were determined by microdilution method. GC-MS/MS analysis revealed that nasturtium VI was mainly composed of benzyl isothiocyanate (54.35%), and 2-phenylacetonitrile (37%). Among these compounds, nasturtium VI was the most effective with MIC value of 0.05 mg/mL. Excellent antifungal activity was also found for pure benzyl isothiocyanate with MIC value of 0.10 mg/mL, while pure 2-phenylacetonitrile was 8 fold lower with MIC value of 0.8 mg/mL. For comparison, others found that oregano EO, mainly composed of phenolic compounds thymol (4.5%) and carvacrol (53.4%), had a MIC of 1.09 mg/mL. The most potent of these compounds was thymol with a MIC of 0.12 mg/mL, while carvacrol had a MIC of 0.38 mg/mL. Thus, it can be concluded that nasturtium VI, which is rich in glucosinolate breakdown products (benzyl isothiocyanate, 2-phenylacetonitrile), has cca. 20 times better antifungal activity against *P. verrucosum* than oregano EO. It can be assumed that isothiocyanates are the main compounds that confer excellent antifungal activity to nasturtium VI. This result indicates that VIs rich in glucosinolate breakdown products, which are not typically used in mould control studies, are a promising alternative to phenol-rich essential EOs. Isothiocyanates are lipophilic and therefore can react with membrane-bound enzymes, inhibit fungal growth and cause cell death. In addition, benzyl isothiocyanate is known for its inhibitory effect on mycotoxin production, such as AME, AOH, ALT and TEN. Therefore, it is necessary to determine the effect of nasturtium VI, benzyl isothiocyanate and 2-phenylacetonitrile on mycotoxin production of *P. verrucosum*. It is also important to test synergism between the glucosinolate breakdown products in the further studies.

CARRIAGE OF *STAPHYLOCOCCUS AUREUS* IN WILD HEDGEHOGS (*ERINACEUS EUROPAEUS*) IN HUNGARY AND FIRST DETECTION OF MECC-MRSA IN THE COUNTRY

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In 2011 a new *mecA* gene homologue was described from a bovine isolate in the UK which was named *mecC*. Since its first documentation in livestock, *mecC* gene carrying methicillin resistant *Staphylococcus aureus* (*mecC*-MRSA) was found in wild animals, as well. Especially high prevalence of *mecC*-MRSA was reported among hedgehogs in Sweden (64%) and Denmark (61%). Based on these North-European findings we aimed to survey the Hungarian hedgehog population for *mecC*-MRSA. Altogether 200 hedgehogs were screened for *Staphylococcus aureus* with a culture-based method. The antibiotic susceptibility of the isolates to nine drugs was determined with MIC test strips, their virulence genes and *mec* genes were identified by PCR, and their genetic relatedness was established by PFGE. Whole genome sequencing and spa typing with Sanger sequencing were performed for the single *mecC*-MRSA isolate found. 13 of the 200 animals were carriers of *S. aureus* (6.5%), among which one *mecA* and one *mecC* positive isolate were identified (0.5% each). All isolates were susceptible to non-beta-lactam antibiotics. Toxin genes were not found in them, but the majority possessed genes responsible for adhesion and biofilm production. The *mecC*-MRSA isolate belongs to a new sequence type: ST6736 which is a single-locus variant of ST130, and has a new spa type (t19701). It carries a recently described novel exfoliative toxin (etE). According to our knowledge this is the first report of *mecC*-MRSA from Hungary and the first survey of *Staphylococcus* carriage among wild animals in the country. In our case the *mecC* prevalence was much lower compared to Northern European countries, and more similar to screening results from Austria and Germany. Based on our results the chances of *mecC*-MRSA zoonotic transmission from wild animals currently is very low in Hungary. Nonetheless, awareness of the presence of *mecC*-MRSA is important, as these strains could be overlooked and misidentified in clinical microbiology laboratories where molecular methods such as PCR are not used in the identification of MRSA strains. Therefore *mecC*-MRSA could potentially emerge as a novel human pathogen, especially where a close contact is present between humans and animals.

COPPER IONS MITIGATE MANGANESE(II) ION INHIBITION OF ITACONIC ACID PRODUCTION IN *ASPERGILLUS TERREUS* IN A CARBON SOURCE-DEPENDENT MANNER

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Influence of copper(II) ions on the growth, morphology and itaconic acid formation was investigated in the high-producer strain *Aspergillus terreus* NRRL1960. Cultures were grown on monosaccharides metabolized either mainly via glycolysis (D-glucose, D-fructose) or primarily via the pentose phosphate shunt (D-xylose, L-arabinose), either under manganese(II)-ion paucity (1.5 μg/L) or sufficiency (300 μg/L). The lower Mn(II) concentration is beneficial for itaconic acid yield while the higher concentration is conducive to biomass formation. However, Mn(II) ion inhibition of itaconic acid formation can be moderated by increasing the Cu(II) concentration in the medium. Copper tolerance of *A. terreus* decreases when Mn(II) availability becomes increasingly limiting. Under such conditions, biomass formation on D-glucose or D-fructose could be sustained at concentrations up to 300 mg/L Cu(II), while D-xylose- or L-arabinose supported growth was fully inhibited already at 100 mg/L. Consequently, while Mn(II) inhibition of itaconic acid production could be neutralized by copper ions on D-glucose and D-fructose, this divalent cation antagonism could not be fully exploited to increase production on D-xylose or L-arabinose. Under itaconic acid producing conditions, fungal morphology is typically characterized by small compact pellets (<0.5 mm diameter) with branches of short hyphae consistent of "swollen" cells with increased diameters and reduced length. The biomass remained essentially filamentous when low (3 mg/l or less) concentrations of Cu(II) ions were present in the medium, remarkably, regardless of the Mn(II) concentration. By contrast, pellet diameter increased with increasing extracellular Cu(II) concentrations, whatever the Mn(II) concentration. Our results indicate that copper metabolism and homeostasis is physiologically relevant for overflow metabolism and should be considered when optimising itaconic acid fermentation in *A. terreus* [1].

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ISOLATION OF BACTERIA WITH ELECTROACTIVE POTENTIALS

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In nature different bacteria have evolved strategies to transfer electrons far beyond the cell surface. The electrontransfer potential makes them proper to use in different bioelectrochemical systems such as microbial fuel cells (MFCs). These electron producing systems are in the major focus of interest as they can serve as alternative, renewable energy sources, also in parallel with waste water management and they can also be adequate devices in environmental monitoring systems. In this study we have isolated and selected electroactive bacteria from the environment with two different methods, in two consecutive steps. First, preselection of the isolates was performed on nutrient agar containing Reaction Black 5, an azodye, under anaerobic conditions for three days. Colonies with discoloration halos around them were isolated, grown, conserved and tested further with the WO3 nanorod reduction method. For this latter tests dense strain suspensions were incubated in the presence of WO3 nanorod crystals, synthetised in a special teflon bomb on 180ºC for 16h. The so gained bacterial suspensions were incubated for 20-40 minutes under anaerobic conditions on 30ºC. Blue discolored wells of the 96 well plate suggested that presenting bacteria were able to carry out effective electron transfer and by this able to reduce the WO_3 nanorods. Altogether we could find 54 different bacteria with the capacity for electrontransfer based on the results of the reactive Black 5 method, while 12 from them proved to be effective in the WO3 nanorod reduction assay. Isolates were determined with the help of MALDI-TOF. Candidate bacteria will be further investigated for their applicability in MFC systems.

A NEWLY IDENTIFIED PLAYER IN PITTED KERATOLYSIS

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Pitted keratolysis is a bacterial skin infection, predominantly affecting the pressure-bearing areas of the soles and charaterized with crateriform pits and malodour. Till today our knowledge is limited about the aetiological agents of this superficial skin infection. Only few bacterial species are attributed to the symptoms, like *Kytococcus* (formerly *Micrococ-*

cus) *sedentarius* and *Dermatophilus congolensis*, and some closely not identified isolates from the genuses like *Corynebacterium, Sreptomyces* and *Actinomyces*. Today, treatment of pitted keratolysis is empiric and is based on the application of topical antibiotics, such as erythromycin, tetracycline and clindamycin. Recently, resistant cases have been described and therefore it is reasonable to get more knowledge about the causative agents of this relatively common skin infection. In this study we have analysed the bacterial compositions of the lesions of a recent pitted keratolysis case. Classical microbiological methods were used for bacterium isolation and to exclude the presence fungi. MALDI-TOF and in one case 16sRNA sequence analysis was used for species identification. Enzymatic activities of the isolated species were revealed by testing their protease, gelatinase, lipase and lecithinase activities. Altogether six bacterium species (*Bacillus thuringiensis, Staphylococcus simulans, Staphylococcus haemolyticus, Streptococcus mitis, Steptococcus paransanguinis* and a *Macrococcus* sp.) were identified from the lesions. *B. thuringiensis* was the only species thats' presence could be revealed from all the tested lesions and beside it had a strong keratolytic activity that is a crucial feature in evoking the typical symptoms of pitted keratolysis. These attributes suggested the flagship role of *B. thuringiensis* in evoking the symptoms.

ANTIBACTERIAL EFFECTS OF ESSENTIAL OILS AGAINST *CUTIBACTERIUM ACNES*

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Acne vulgaris is a chronic skin disorder of the pilosebaceous unit. It typically affects areas have high density of sebaceous follicles. It can present as non-inflammatory comedones, inflammatory papules, pustules, nodules and cysts or a mixture of lesions. Twenty percent of the disease can appear severe acne that is likely to lead to scarring. Colonization of the pilosebaceous follicle by *Cutibacterium acnes* (formerly: *Propionibacterium acnes*) is a major factor for the inflammatory reaction and therefore *C. acnes* has been a major target of therapy in inflammatory acne. It is known that several Essential Oils (EO) are antibacterially active against different species, therefore we proposed an investigation of thirty essential oils against this bacterium in order to reveal their potentials as therapeutic agents. First of all, we determined the sizes of the inhibition zones of the EOs on the lawn of the *C. acnes* by using the drop plate method. Compound composition of the most effective EOs were compared with the direct bioautographic method, while EOs with the most marked antibacterial features were incorporated in 3% concentration in to the hydrophilic hydrogel methylcellulose and the hydrophobic unguentum oleosum cream ointments. Results of the drop plate method has revealed that the oregano, thyme, cinnamon, lemongrass, clove and holy basil had the most marked antibacterial effect against *C. acnes*, while oregano, thyme, patchouli, clove and holy basil EOs showed the largest inhibition zones in the bioautographic system. Comparing the two ointment bases, the methylcellulose based hydrogel expounded higher antibacterial effect with EOs in contrast to that of the hydrophobic ointment base. Our work highlights the potential of EO containing creams to combat acne and to offer a naturally alternative therapy instead of using antibiotics, to decrease the global spread of antibiotic resistance.

INVESTIGATION OF DMI-FUNGICIDES RESISTANCE IN GRAPE POWDERY MILDEW (*ERYSIPHE NECATOR*) POPULATIONS IN HUNGARY

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Powdery mildew is one of the most widespread fungal diseases associated to grapevine (*Vitis vinifera*) caused by the obligate biotrophic fungus *Erysiphe necator*. The continuous and intensive use of sterol demethylation inhibitor (DMI) fungicides results in a gradual development of sensitivity by alterations in several genes. The occurrence of resistant *E. necator* populations is generally low and it has been previously shown that pathogens with reduced sensitivity to one certain DMI fungicide could be still sensitive to other DMIs. The main goal of our work was to detect point mutations (SNP) in genes causing DMIs resistance and to describe fungicide sensitivity of Hungarian populations of *E. necator*. In our work we adapted a commonly used method to test whether *E. necator* conidia collected from the experimental vineyard of Eszterházy Károly Catholic University showed resistance to four tested DMI fungicides. Sterile cellophane with 100 µl of each tested fungicide on its surface was laid onto 1.5% water agar, conidia of *E. necator* were spread on the cellophane covered plates. Fungicides were tested at ten different concentrations as triplicates. The tested DMI fungicides were: propiconazole; tebuconazole; myclobutanil and penconazole. Following 1 days of incubation, the ratio of germinating and

non-germinating spores and the length of the germinating spores were determined. The germination rates were compared to the control (conidia spread on 1.5% water agar without fungicides) germination rate. We observed reduced sensitivity to all the tested DMIs except propiconazole when applied on our isolates. With gene sequence analysis we identified a conserved nucleotide variation in the coding region of 14 alfa-demethylase (CYP51), the target of the commonly used sterol demethylase inhibitor (DMI) fungicides. SNP in CYP51 causing tyrosine to phenylalanine substitution amino acid position 136 (Y136F) of the protein is associated with DMI resistance in *E. necator*. In our further studies we would like to expand the list of tested fungicides with other DMIs used in the viticultural practice.

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ORAL PROBIOTICS FOR POTENTIAL HEALTHCARE APPLICATIONS

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Approximately 700-1,000 bacterial strains live together in equilibrium in the human oral cavity. When this sensitive societal equilibrium is getting disturbed, the number of pathogenic microbes may increase and trigger pathogenic processes. Oral diseases, like halitosis, caries, gingivitis and periodontitis, which can frequently lead to dedention as well, affect millions of people all over the Globe. Probiotic strains produce substances, e.g. bacteriocins, to inhibit the growth of pathogens. This can provide a tool that is alternative to synthetic antibiotics, since increasing multi-drog resistance raises serious problems. On the basis of literature and preliminary results, numerous probiotic strains are able to inhibit the growth of pathogenic microorganisms. World Health Organization defines probiotics as "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host". The most extensively studied probiotic strains belong to Lactobacilli, Bifidobacteria, non-pathogenic *Escherichia coli*, Bacilli and yeasts, e.g. *Saccharomyces boulardii*. Many probiotic strains produce antimicrobial substances, e.g. short-chain fatty acids, hydrogen peroxide, nitrogen oxide and bacteriocins. Bacteriocins are ribosomally synthesised antimicrobial peptides, and therefore can be useful alternatives to antibiotics. They can be narrow- or broad-spectrum peptides, which are effective in vivo and in vitro, have low toxicity and can also be produced in situ. As an example, *Streptococcus dentisani* is an anticariogenic probiotic bacterium, which is capable to inhibit the growth of *Aggregatibacter actinomycetemcomitans* and *Streptococcus mutans* and kill *Fusobacterium nucleatum*. *Lactobacillus casei* and *Lactobacillus rhamnosus* inhibit the growth of *S. mutans*, and *L. rhamnosus* while *Lactobacillus plantarum* is effective against *A. actinomycetemcomitans* and *Porphyromonas gingivalis*. Probiotic strains tested in our laboratory, i.e., *L. plantarum, L. rhamnosus, L. casei, L. acidophilus, L. delbrueckii*, *Bifidobacterium thermophilum*, two *Streptococcus dentisani* strains and a commercially available probiotic product – were able to inhibit the growth of several oral pathogenic bacteria. For future applications a family of suitably engineered, stable probiotic consortia should be developed to remedy the various diseases caused by oral pathogenic biofilms [1].

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THE EFFECT OF WHEY PROTEIN AND DENATURED WHEY PROTEIN ON MICROENCAPSULATION OF *LACTOBACILLUS PLANTARUM* 299V BY LYOPHILIZATION

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Probiotics are microorganisms that have health benefits on the host when they are administrated to a sufficient amount. They have numerous beneficial characteristics, e.g., irritable bowel syndrome controlling; endogenous or exogenous pathogens suppression; lactose tolerance improvement; colon cancer risks reduction; body weight regulation; constipation improvement; tooth decay prevention. However, owing to the harsh environments during manufacturing and the digestion (e.g., oxygen, temperature, low pH, high bile salt content). probiotics are difficult to survive through the process of manufacturing, not to mention the lack of their probiotic effect on the host. Microencapsulation is a talented technology that can protect the probiotics by coating them with wall materials to maintain their viability and functionality during the manufacturing and digestion process and they can be applied to preserve their probiotic effect in the gut with an adequate dose level. Whey protein (WP) and denatured whey protein (DWP) are exceptional coating materials with specific physical and chemical properties. WP has excellent emulsification, gelation, and fill-forming properties that can encapsulate the probiotics well. DWP has a high tensile property and low oxygen permeability, which means it can protect probiotics from

harsh gastrointestinal environments. Research related to the effects of singular protein material as a coating material on the viability of probiotics is generally reported. However, the investigation on the comparison between two proteins and their combination, especially the effect of ratio between core-to-wall and wall material ratio rarely been deeply discussed. Hence, the effects of different coating materials, WP and DWP, with different core-to-wall ratio and wall materials ratio on the yield (%), bulk density (kg/m3), encapsulation efficiency (%), and living cell numbers (CFU/g) of microcapsules were investigated. The yield of microcapsules with core-to-wall ratio 1:1varies from 49.35% to 59.76%, and the yield of microcapsules with core-to-wall ratio 1:1.5 varies from 61.12% to 68.02%. The bulk density of the microcapsules with care-to-wall ratio 1:1 and 1:1.5 varies from 260 kg/m^3 to 270 kg/m^3, and from 230 kg/m^3 to 250 kg/m^3, respectively. The encapsulation efficiency of the microcapsules with care-to-wall ratio 1:1 and 1:1.5 varies from 81.49% to 89.38%, and from 61.73% to 85.20%, respectively. The cell numbers of the microcapsules with care-to-wall ratio 1:1 varies from 8.74 log CFU/g to 9.64 log CFU/g, and the yield of microcapsules with core-to-wall ratio 1:1.5 varies from 6.25 log CFU/g to 9.15 log CFU/g. The results showed that the core-to-wall ratio has an extremely significant effect on the yield and bulk density. However, there is no significant effect on the encapsulation efficiency and cell number. Besides, wall materials formulation and ratios have a significant effect on the encapsulation efficiency and cell numbers. The results of our research are very promising and may have some guidance on the production of microcapsules by lyophilization in the food industry.

GENOMIC CHARACTERISATION OF ENTEROHEMORRHAGIC, SHIGA-TOXIN PRODUCING AND ENTEROPATHOGENIC *ESCHERICHIA COLI* ISOLATED FROM BOVINE AND HUMAN SOURCES IN HUNGARY

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Intestinal pathogenic *Escherichia coli* (InPEC) that belong to the enteropathogenic (EPEC), enterohemorrhagic (EHEC) and Shiga toxin-producing (STEC) pathotypes are significant foodborne zoonotic pathogens posing serious health risk. Members of the O157 serogroup are strongly associated with severe disease. The main reservoir of human infections is healthy cattle. We sampled animals across Hungarian cattle farms during 2017-2018 to isolate and extensively characterise EHEC, STEC and EPEC strains. Our goal was to explore their prevalence, genetic variability and mobile genetic elements (MGE), and compare them to isolates originating from human cases of sickness. Of 309 samples tested, 20 were STEC and 18 EPEC strains, accounting for a prevalence of 6.5% and 5.8%, respectively. We also detected a further 5 EPEC strains by screening 184 previously isolated bovine strains for the presence of stx and eae genes. Twelve bovine STEC, (out of them 9 EHEC), 4 bovine EPEC, 3 human EHEC, and 2 human EPEC strains, as well as 5 commensal *E. coli*, which included strains isolated from earlier studies, were used for comparative genomic analysis. The whole genome sequences (WGS) of the selected strains showed multiple types of virulence arrays with several integration patterns for the Stx prophage and the locus of enterocyte effacement (LEE). All of the EPEC strains proved to be atypical (aEPEC), a pathotype of increasing importance. The prevalence of antibiotic resistance was low, as only a single multidrug resistant (MDR) strain was identified. Phylogenetic analysis showed that the STEC strains isolated in our study represent a diverse set of lineages within the pathotype. Our results underline the zoonotic potential of bovine EHEC and EPEC isolates, and point to the crucial role that prophages play in shaping the genomic variability of these pathotypes.

GETTING SALTY: THE EFFECT OF SALINITY AND WATER CHEMICAL TYPES ON BACTERIAL COMMUNITY COMPOSITION BASED ON GLOBAL DATA

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Moderately saline and alkaline environments are expected to become more common globally due to accelerating salinization. On the other hand, human activities also induced environmental changes which threaten the future persistence of aquatic saline-alkaline systems, while their microbial richness is still largely unexplored. To date, still little is known

about the biogeographic distribution of alkaliphilic microbes and their interaction with the surrounding environment. Whether habitats with different salinity and water chemical types (carbonate or chloride anion dominance) have a distinct bacterial composition or how bacterial communities will respond to freshwater-brackish transition is still unclear. To answer these questions, first we compared V3-V4 regions of 16S rRNA gene amplicon datasets obtained from freshwater, brackish and saline aquatic environments worldwide (344 samples from 165 sites) and filtered them rigorously during the bioinformatic analysis to avoid artifacts. In addition to the amplicon dataset, an extensive collection of soda lake metagenomes (150 metagenomes from 40 sites) was created using own data and publicly available datasets. These metagenomes were compared to other saline aquatic habitats and an inventory of the biogeography, diversity and evolutionary origin of keystone alkaline lake microorganisms was created. Our results showed remarkable differences in bacterial community composition of freshwater, soda and saline habitats, along with communities representing transitions between these environments. Interestingly, community structure also depended on the sampled biogeographic region. Above a certain salinity threshold, samples were clearly separated according to the dominant anions (carbonate vs. chloride). In the case of soda lakes, planktonic actinobacteria (acIII-A1, Luna1-A2, uc_Nitriliruptoraceae) and uncultured members of the Rhodobacteraceae family were commonly found. The decrease in relative abundance of certain freshwater groups (LD12, *Flavobacterium, Aquirufa, Limnohabitans, Hydrogenophaga*) indicated transition to brackish waters, while taxa such as *Halomonas* and a few *Synechococcus* lineages were characteristic for chloride-dominated saline waters. Most shared mOTUs were obtained from marine habitats while shared mOTUs were not identified between soda lakes of the Carpathian Basin and other environments. Mapping revealed a wide geographic distribution for certain mOTUs. Isoelectric properties of the predicted proteomes indicated adaptation to saline conditions. Overall, our study indicates that microorganisms of different alkaline aquatic habitats carry special adaptations suggesting important role of eco-evolutionary processes.

CHARACTERIZATION OF NEW MEMBERS OF THE COTH KINASE PROTEIN FAMILY IN *MUCOR CIRCINELLOIDES*

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The CotH protein mediated interaction proved to be crucial for the fungal invasion during mucormycosis. Moreover, IgG antibodies produced against the *Rhizopus* CotH3 protein protected mice with diabetic ketoacidosis (DKA) from mucormycosis and anti-CotH3 antibodies were proposed promising for immunotherapy treatment of human mucormycosis. Thus, our research is mainly focused on the extensive analysis of this kinase family and the clarification of their role in the virulence of *Mucor*. We performed the functional analysis of the CotH proteins, which involved tracking the phenotypic alterations of genetically stable cotH mutants. Eight putative spore-coat genes were disrupted in *M. circinelloides* by an in vitro plasmid-free CRISPR/Cas9 method. Growth ability of the mutants under different conditions (stressors, hydrogen peroxide) were examined. Inner spore structure was investigated by transmission electron microscopy. The phagocytic assay and acidification of macrophages were performed with a macrophage-like cell line J774.16. Possible changes in cell wall structure were monitored using fluorescence microscopy and flow cytometry analysis. Pathogenicity of the mutants was examined in *Drosophila melanogaster, Galleria mellonella* and a murine model of mucormycosis. Cell wall stressors affected differently the *cotH* mutants. Deletion of some of the *cotH* genes resulted in variances in the structure of the inner spore coat, differences in spore size distribution, fungal growth and sporulation. Acidification of phagosomes is not affected by the examinated CotH proteins, and the absence of CotH proteins did not affect the survival of spores after in vitro interaction with macrophages. The *cotH3*, *cotH4* and *cotH5* mutant strains showed reduced virulence.

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ESTABLISHING STRUCTURE-ACTIVITY RELATIONSHIPS (SARS) FOR NEWLY IDENTIFIED FUNGAL PEPTAIBOLS: A COMBINATION OF EXPERIMENTAL AND THEORETICAL TECHNIQUES

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Today more than 375 species from the genus *Trichoderma* have been characterized, and further attention has been drawn to the species owing to their application in biocontrol of plant pathogenic microorganisms and their plant growth-promoting effects. Numerous *Trichoderma* strains play important roles in agriculture and biotechnology due to their secondary metabolites, which include the largest group of peptaibiotics: the peptaibols. Peptaibols are identifiable by their characteristic properties, which include the acetylated N-terminus and an amino-alcohol group like phenylalaninol (Pheol) or isoleucinol (Ileol) at the C-terminus. A key characteristic is the presence of non-proteinogenic amino acids, such as α-aminoisobutyric acid (Aib), hydroxyproline or D-isovaline (Div). They are characterized by a high degree of variability in their sequences due to the synthesis carried out by the non-ribosomal peptide synthetases (NRPSs) with modular structures. The potent antagonistic effects of peptaibols are due to their ability to aggregate and form ion channels in the cell membrane, which ultimately leads to cell death, thereby, providing protection for plants against pathogens. Modern molecular modeling techniques such as accelerated molecular dynamics (aMD) are used to uncover the folding process of peptaibol sequences and provide a deeper insight into their structure, which can be further utilized to establish correlational relationships between conformation and bioactivity of peptaibols. In the present study, purified peptaibol extracts from six *Trichoderma* species belonging to clade Longibrachiatum were tested against nine commonly known plant-pathogenic Gram-negative and Gram-positive bacterial strains and their minimal inhibitory concentration (MIC, mg/mL) values were determined. The peptaibols were found to be primarily effective against Gram-positive bacteria, however, the *T. longibrachiatum* f. *bissettii* strain showed inhibitory effect to the Gram-negative *Rhizobium radiobacter* strain, apart from showing the strongest inhibitory effect to several other Gram-positive bacteria. The peptaibol sequences were modelled using the aMD technique and their structures compared with the MIC-test results to correlate folded peptaibol dynamics affected by their amino-acid content with their expressed bioactivity. The structure-activity relationships established in this manner will lead to effective selection of peptaibiotic intervention required for plant disease management.

SURFACTIN PRODUCTION OF *BACILLUS* STRAINS ISOLATED FROM RHIZOSPHERE OF VARIOUS VEGETABLES

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Surfactins are cyclic lipopeptides consisting of a β-hydroxy fatty acid of various chain length and a peptide ring of seven amino acids linked together by a lactone bridge, forming the cyclic structure of the peptide chain. These compounds are produced mainly by *Bacillus* species and possess numerous biological effects such as antibacterial, antifungal and antiviral activities. For their surfactin production profiling, several *Bacillus* strains isolated from vegetable rhizospheres were identified by GC-MS technique. Then a HPLC-HESI-MS method was developed to simultaneously carry out the quantitative and qualitative characterizations on the extracted ferment broths. More than half of the examined *Bacillus* strains produced surfactin and the MS2 spectra analyses of their sodiated precursor ions revealed a total of 29 surfactin variants and homologues, some of them with extremely high number of peaks with different retention times, suggesting large numbers of variations in the branching of their fatty acid chains. Results supported the conclusions of our former studies stating that the appearance of previously rarely encountered group of surfactins with methyl esterified aspartic acid in their fifth amino acid position may be encountered in considerable numbers and the fatty acid chain lengths to vary between 12 and 18 carbon atoms.

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DETERMINATION OF INDOLE-3-ACETIC ACID BIOSYNTHETIC PATHWAYS IN FUNGAL ENDOPHYTES

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Indole-3-acetic acid (IAA) known as widely produced it's phytohormone in plants and plays a crucial role in plant physiology including cell division and elongation, tissue differentiation, root initiation and phototropic response. Both plants and microorganisms have been reported as IAA producers. Tryptophan is known to serve as a general precursor of IAA synthesis, but it can be also synthesized via a Trp-independent pathway. According to the recent literature, there are around five Trp-dependent IAA biosynthesis pathways that have been demonstrated in microorganisms namely the indole-3-acetamide (IAM), indole-3-pyruvate (IPyA), tryptamine (TAM), indole-3-acetonitrile (IAN), and tryptophan side-chain oxidase (TSO) pathways. In the present study, 62 fungal endophytes were isolated from 8 Mongolian medicinal plants and were identified based on molecular taxonomical tools. Furthermore, IAA production of the isolates were examined by HPLC-MS/MS analysis and the IAA biosynthetic pathways of the IAA producers were determined using also HPLC-MS/MS techniques. As the results, fungal endophytes were able to produce IAA through four different pathways such as IAM, IPyA, TAM and TSO. In fact, it was the IPyA pathway that most frequent within the fungal endophytes. Moreover, numerous isolates have conducted two or more biosynthetic pathways for their IAA biosynthetic pathway and on the contrary, some fungal endophytes have functioned only one pathway to produce IAA.

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INHIBITION OF QUORUM SENSING BY CONVENTIONAL ANTIBIOTICS AND RESISTANCE MODIFIERS

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Multidrug resistant organisms (MDROs) are one of the most severe inevitable issues which hinder the effective treatment of disease and administration of various anti-microbial agents. Quorum sensing (QS) is the regulation of gene expression depending on cell-population density. Bacteria produce and release chemical signal molecules called autoinducers that increase in concentration as a function of cell density. Gram-positive and Gram-negative bacteria use different QS systems to regulate their physiological activities including symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation. Drug repurposing (or drug repositioning) is an approach to identify new applications for approved drugs that are outside the scope of the original medical indication. In our study, the antibacterial, anti-QS and biofilm inhibiting properties of well know antibiotics, efflux pump inhibitors, proton pump inhibitors, and antipsychotic drugs were determined in Gram-positive and Gram-negative bacteria. The antibacterial activity of the compounds was assessed by broth microdilution method. The anti-biofilm activity of compounds was determined on Gram-negative, biofilm-producing *Salmonella* Typhimurium 14028s, *E. coli* ATCC 25922 strains and on Gram-positive *Staphylococcus aureus* ATCC 25923 and methicillin resistant *S. aureus* 272123 strains using crystal violet (CV) staining. The inhibition of quorum sensing (QS) was determined using the sensor strain *Chromobacterium violaceum* 026 and the AHL producer strain *Enterobacter cloacae* 31298 by agar diffusion method. It can be concluded that five compounds including gentamicin, ciprofloxacin, thioridazine (TZ), phenyl-arginine-β-naphthylamide (PAβN), and chlorpromazine (CPZ) demonstrated highly efficient QS inhibitory effect. Gentamicin had a biofilm inhibiting activity on *E. coli* and *S.* Typhimurium, ciprofloxacin inhibited the biofilm formation by *S.* Typhimurium. The antipsychotic drug thioridazine inhibited the biofilm produced by the reference *S. aureus* strain. Furthermore, PAβN demonstrated anti-biofilm activity on Gram-negatives such as *E. coli* and *S.* Typhimurium. Furthermore, CPZ could inhibit the biofilm formation of *S.* Typhimurium and *S. aureus*. In the future these compounds might be used as anti-biofilm agents, however various aspects of the mode of action of these compounds are not clearly understood yet. Agents with anti-virulence properties may represent new potential antibacterial therapeutics in the future. QS-based modulation of bacterial virulence could be an attractive strategy because the pharmacokinetic parameters and tolerability of most of these compounds have already been investigated.

SELENOESTERS AS EFFLUX PUMP INHIBITORS IN BACTERIA AND CANCER CELLS

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The presence of resistant pathogens and tumour cells leads to a decrease in the efficacy of many antibacterial and antitumor agents, which causes a serious problem in selecting the appropriate therapy. One of the most important of the many resistance mechanisms is the overproduction of efflux pumps. Through this mechanism, bacteria and tumour cells pump the drugs out of the cells, so that the drug does not reach the concentration required to elicit its effect. In previous experiments, selenoesters have been shown to be effective derivatives and have excellent biological activity. Based on these results, new selenoesters were synthesized and the antibacterial and antitumour effects of these compounds were studied in vitro. Thus, fifteen selenoesters (eight ketoneselenoesters and seven cyanoselenoesters) were investigated in this study. The antibacterial activity of selenoesters was determined on Gram-positive sensitive and resistant *Staphylococcus aureus* strains, and on Gram-negative *Salmonella enterica* subsp. *enterica* serovar Typhimurium strains. The eruption of mature biofilm and the anti-biofilm activity were tested on biofilms produced by *Pseudomonas aeruginosa* (CCM 3955) and *S. aureus* (ATCC 25923). For the evaluation of the anti-efflux pump activity, *S*. Typhimurium and *S. aureus* strains were used. Human colon adenocarcinoma (sensitive Colo205 and resistant Colo320) and human embryonal lung fibroblast MRC-5 cell lines were used in cytotoxicity assays. The apoptosis inducing effect of the selenoesters and the ABCB1 pump inhibitory effect were further investigated using the Colo320 cell line. After determining the antibacterial effects, the ketoneselenoesters showed to be more effective than the cyanoselenoesters. All of our compounds had anti-biofilm activity, the efflux systems were successfully inhibited in *S. aureus* MRSA strain. All cyanoselenoesters were found to be selective, having activity only against cancer cells. All ketoneselenoesters inhibited effectively the ABCB1 pump, furthermore some of the ketone selenoesters had ATPase inhibitory activity and one ketoneselenoester was also able to induce early apoptosis. These results suggested that ketone- and cyanoselenoesters could be effective compounds reducing the resistance mechanism in bacteria and in tumor cells. Selenium-containing compounds could provide alternative and effective scaffolds to overcome efflux-related MDR in bacteria and cancer cells. Nevertheless, the mode of action of the compounds needs additional investigation.

ARBUSCULAR MYCORRHIZAL SYMBIOSES OF TOMATO UNDER HEAT AND DROUGHT STRESS, FOCUSING ON VARIOUS PHOSPHATE TRANSPORTERS

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To mitigate the stresses caused by climate change is a big challenge of agriculture. Moreover the depleting phosphorus reserves and growing populations also put a rush on professionals to find a solution. The utilisation of arbuscular mycorrhizal fungi (AMF) could be a solution to these, as plants with this symbiotic partner show better growth, higher yield, and can withstand biotic and abiotic stresses more than their not colonised counterparts. Our goal was to monitor arbuscular mycorrhizal tomato roots in response to simple and combined heat and drought stresses, with a focus on different phosphate transporters. After the germination tomato plants were grown in a climatic chamber for seven weeks in pots with drilled out bottom on porous foam in a plastic container filled with water following the modified method of Snow and Tingey [1]. After the growth period mycorrhizal (*Funneliformis mosseae*) and non-mycorrhizal plants were subjected to single drought, single heat and combined drought and heat stresses. Drought stress was applied for two weeks with the reduction of the water level, heat stress was applied for one week in a gradual manner with temperatures peaking at 42°C, while combined stress contained both treatments. Our results revealed a definite response to *Funneliformis mosseae* under heat, drought, and combined (heat+drought) stresses. Mycorrhizal plants showed better growth, altered defense enzyme activities and gene expressions (phosphate transporters belonging to PHT1 family) under the applied stresses compared to control. We concluded that the higher vigor and stress effect avoidance of mycorrhizal plants was a result of enhanced phosphorus uptake through the direct and the mycorrhizal pathways.

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[1] Snow and Tingey (1985) Plant Physiol 77:602.

CATABOLIC ACTIVITY PROFILES OF SOIL MICROBIOTA IN A LONG-TERM CROP ROTATION EXPERIMENT BY APPLYING MICRORESP METHOD

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To maintain agricultural soil quality, crop rotation and organic amendments are recommended. The so called long-term agricultural experiments provide a good opportunity to examine the possible changes in chemical and biological properties of soils. The aim of this work was to compare three crop rotation treatments in combination with three fertilization treatments, to reveal the long-term (60 years) changes of soil quality. In the present study, long-term experimental fields on loess soils near Martonvásár were examined: 1) maize, 2) winter wheat, 3) winter wheat – maize diculture combined with a) control, b) NPK and c) NPK + manure treatments (4-yearly) were studied. The arable soil is a long-term maize-winter wheat diculture experiment changing crops after 2 years. Soil samples were taken from the top layers 5-times during the vegetation period from April to October in 2018 and 2019. The applied catabolic activity profiling of the soil microbial communities is a simple and high throughput method which can be combined with genetic diversity approach. The MicroResp method using colorimetric microplate-based detection of evolved CO_2 was applied using 23 different organic substrates (sugars, amino acids and carboxylic acids). There were significant differences according to soil fertilization treatments and seasons but not with plant cultures. In addition, significant interactions forced to evaluate the result separately in each season. To evaluate the responsibility of substrates for the soil groups' separation, SIMPER test was used. Generally, only one or a few, somewhat different substrates were responsible for the sample distinctions. To explore the influence of soil physical or chemical properties on the catabolic activities, redundancy analysis was applied, showing the importance of soil pH and organic carbon content as the main influencing factors.

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THE EXAMINATION OF THE INTERACTION BETWEEN *CANDIDA* SPECIES AND ORAL PATHOGENIC BACTERIA ON THE LEVEL OF EXTRACELLULAR VESICLES

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The human oral cavity is colonized by more than 700 microorganisms, including bacteria, viruses, fungi, protozoa and archaea, collectively called as oral microbiota. As a result of various environmental effects, such as smoking, alcohol consumption, or infections, the microbial composition may change, which can result dysbiosis. Dysbiosis can lead to the development of various diseases, such as oral candidiasis. Oral candidiasis most commonly caused by the overgrowth of *Candida albicans*, which can reduce the bacterial diversity. We are investigating the interaction between *Candida* and oral pathogenic bacteria which can not only occur directly, but also indirectly on the level of extracellular vesicles (EV). For our experiments we used *Candida albicans* SC5314, *Candida parapsilosis* CLIB214 strains, *Staphylococcus aureus, Enterococcus faecalis* and *Pseudomonas aeruginosa*. We succesfully optimized the fungal and bacterial EV isolation protocol from liquid media. The characterisation of the fungal EVs by transmission electron microscopy and NanoSight showed round shaped particles with diameters between 50 and 150 nm. Bacterial EVs were characterized by NanoSight, it also showed round shaped particles with diameters between 50-250 nm. We examined the effects of EVs released by *Candida albicans* and *Candida parapsilosis* on the growth and biofilm formation efficiency of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus faecalis*. Extracellular vesicles from *C. albicans* and *C. parapsilosis* had different effects on the growth of *Staphylococcus aureus* and *Enterococcus faecalis*. Fungal EVs also affected the biofilm formation efficiency of bacterial species, *C. parapsilosis* EVs significantly increased the biofilm formation efficiency of *Staphylococcus aureus*. Next, we examined the effect of EVs released by *S. aureus, E. faecalis* and *P. aeruginosa* on the growth capacity and biofilm formation efficiency of *C. albicans* by coincubation experiments. The bacterial EV treatment significantly reduced the CFU of *Candida albicans* cells. Using fluorescence microscopy we found that the fungal cells and bacterial EVs colocalize after 4 hour of incubation, and the fungal cells form hyphae with various efficiency, while the bacterial cells and fungal extracellular vesicles do not colocalize. Based on these results, it can be assumed that there is an interaction between fungal and bacterial cells on the level of extracellular vesicles.

THE EFFECTS OF *CANDIDA ALBICANS* ON THE PROGRESSION OF ORAL SQUAMOUS CELL CARCINOMA USING IN VIVO MICE MODEL

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Tumors of the oral cavity cause serious medical challenges worldwide. According to the WHO, there were 350,000 new registered cases in 2018, and approximately 170,000 deaths could be attributed to lip and oral cavity associated malformations. 90% of the oral cavity tumors are oral squamous cell carcinoma (OSCC). According to many research, OSCC can develope oral candidiasis due to the unique immune-suppressive environment and the applied tumortherapy. The most frequent causative agent of oral candidiasis is *Candida albicans*. During our research we examined the effect of *Candida albicans* on the progression of OSCC. Based on our in vitro results, we developed an in vivo xenograft model, in which we injected HSC-2 human tumor cells into the tounge of immunsuppressed mice (OSCC xenograft), and after that we caused oral candidiasis (OC-OSCC xenograft). Histological staining of epithelial-mesenchymal transition markers (E-cadherin, vimentin, p63) was carried out, and then transcriptomical examination was prepared to explore the molecular mechanisms. Based on our results it is clear, that we succesfully developed an in vivo xenograft mice model to examine the effect of oral candidiasis on the progression of OSCC. In the samples of the OC-OSCC xenograft group a higher expression level of p63 and vimentin was detected, while the E-cadherin expression was decreased compered to the samples from the OSCC xenograft group. Based on the histophathological examination a severe inflammation, thrombosis and epithelial-mesenchymal transition was caused by *C. albicans* and was detected in the samples of the OC-OSCC xenograft group. During transcriptomical examination we succesfully identified marker genes, which are, according to the literature data, have a proven role in the OSCC invasion, and furthermore the expressions of these genes were significantly higher as a result of oral candidiasis. Our in vivo results suggest that oral candidiasis increase the progression of OSCC.

INVESTIGATION OF OENOLOGICAL PROPERTIES OF NON-*SACCHAROMYCES* YEASTS

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Yeasts have been used to make food, e.g., baking bread or making alcoholic beverages, for thousands of years. *Saccharomyces cerevisiae* and *Saccharomyces bayanus* are frequently used species in wine and beer production. Previously, incidence of the non-*Saccharomyces* species during fermentation was considered as undesirable. In contrast, scientists and winemakers now believe that the new and non-traditional yeast species can also be useful for the fermentation industry. These species can contribute to the aroma complexity of the drinks or stability of the colour compounds. In addition, craft beers are gaining ground nowadays. Craft beer producing companies want to produce unique alcoholic beverages with new and special flavours. They frequently use new raw materials, methods and non-traditional yeasts, which are indispensable for producing beverages with different flavours and alcoholic contents. In our work, we investigated the physiological properties of different members of the genus *Schizosaccahomyces* (*S. pombe, S. pombe* var. *malidevorans*, *S. japonicus, S. octosporus, S. cryophilus*), which are essential for the fermentation of fruit juices. Our first task was to find a medium and conditions that were equally suitable for optimal reproduction of the different strains. Their assimilation and fermentation ability, alcohol and sulphur tolerance were tested. We also investigated their sugar, temperature and pH requirements. Our data indicate that the different *Schizosaccharomyces* species have unique characteristics and can be suitable for juice fermentation.

INVESTIGATION OF THE PARTS OF ZINC HOMEOSTASIS IN THE HUMAN FUNGAL PATHOGEN *CANDIDA PARAPSILOSIS*

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Reports show that *Candida parapsilosis* is often the second or third most commonly isolated *Candida* species from blood cultures and is associated with outbreaks of infection in neonatal intensive care units. Both species have several virulence factors by which they can adapt to the host's enviroment and, therefore, can cause an infection. These factors include

adhesins, as well as the ability to form biofilms, secrete hydrolytic enzymes, such as acidic proteinases and lipases. These *Candida* species also have the ability to obtain growth-limiting heavy metal ions from their environment, such as zinc. This capability is key since host niche represents a zinc-limited environment that is one way to inhibit microbial growth. Hence these pathogens need to possess a zinc transport system that allows them to access bound zinc ions from the host environment upon infection. In contrast with this a high zinc ion concentration can also be a way of microbial elimination as it occurs in the phagosomes of *Mycobacterium tuberculosis* infected macrophages. In the case of *C. albicans*, the way of zinc acquisition is intensively studied, but we lack any information on the components playing role in the zinc homeostasis of *C. parapsilosis*. Thus we aimed to in silico predict potential zinc transporters in *C. parapsilosis*, create homozygous knock out mutants and expose them to various types of stressors and zinc limiting conditions however, we visualized the intracellular zinc ion level and its localisation inseide the yeast cells. By flow cytometric and confocal microscopy methods we analyzed the kinetics of uptake of the zinc transporter mutants by macrophages, their killing efficiency and investigated the zinc ion level in the phagolysosome during in vitro infection.

PRODUCTION OF BIOACTIVE PHENOLICS FROM HAWTHORN FRUIT MATERIAL SAMPLED IN TURKEY

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Bioactive phenolics can possess health-protective effects including antioxidant, antimicrobial, anti-inflammatory and anti-cancer activities. Many of them can be used in the food-industry as an additive in functional foods as well as a preservative in packaged foods. In fact, such compounds can be obtained in high amount from antioxidative plant materials though variety of extraction methods. Those methodologies that performed by enzyme-assisted techniques are the most ecofriendly approaches for this purpose. Studies have proven that the hawthorn fruit substances are rich in many nutrients including phenolics, however, the extraction is generally operated with a harsh solvent-assisted technologies. In this study, we used a carbohydrase-assisted extraction approach to obtain free phenolic compounds from a hawthorn fruit (*Crataegus orientalis* Pall. ex M.Bieb. subsp. *orientalis*) substance collected in the area Takkalı Mountain, Konya, Turkey. To prepare cellulolytic extract for the experiments, wheat bran-based solid-state fermentation with the zygomycete *Rhizomucor miehei* was conducted. The obtained enzyme extract and a commercial *Aspergillus niger* pectinase was used to treat the hawthorn fruit residue previously subjected to oven-drying. Then, the total phenolic content and antioxidant activity of the extracts were determined and compared. Results showed enhanced phenolic content and antioxidant activity in samples treated with the *R. miehei* cellulase cocktail compared to the enzyme-free control. Concentrations of many phenolic compounds, e.g., dihydroxybenzoic acid, protocatechuic acid, caffeic acid, vanillic acid, vanillin, p-coumaric acid and quercetin increased after enzyme treatments according to analytical HPLC. Additionally, antimicrobial activity of the phenolic extracts was also studied against foodborne pathogenic and spoilage bacteria. Both the planktonic and the biofilm cells of some bacteria tested were inhibited by the extracts in vitro. Taken together, our results indicated that the enzyme treatment operated by *R. miehei* cellulases increased the extractable phenolic yield and antioxidant activity of the hawthorn residue.

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EFFECT OF MINERAL TREATMENTS ON SOME ENZYMATIC ACTIVITIES AND CO_2 PRODUCTION OF ACIDIC SANDY SOILS

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In the Institute of Agricultural Chemistry and Soil Science of Debrecen University (Hungary) in a model experiment (2017; 2019), the effect of four minerals (alginate, perlite, zeolite, dolomite) are examined on some enzymes activities and CO_2-production of acidic humus sandy soils. Soils originated from Nagykáta (pH_{H_2O} = 5.2; the minerals examined are the alginate, perlite, zeolite) and Debrecen – Pallag (pH_{H_2O} = 6.4; the mineral examined is the dolomite). The applied doses from alginate, perlite, zeolite were 20t/ha, from dolomite 3t/ha. In the amelioration experiment all treatments en-

riched NPK fertilizers taking into account the nutritional requirements of test plants (millet, *Panicum miliaceum* L.; „Yellow-podded - Maxidor" bean, *Phaseolus vulgaris nanus* L.). On the bases of results, the water management parameters were influenced positively by alginate, perlite, and dolomite treatments. The water content changed between average in controls 11. 45–18.70%, it is a normal in the season, but the alginate, perlite, and dolomite caused an increased result from 23% to 30%. The urease enzyme activity (195.3-493.6 mg NH_4^+/kg soil / 2h) increased in all treatments significantly, the saccharase activity increased by alginate (6.62 mg glucose / kg soil / 24h), perlite (6.29 mg), and dolomite (5.39 mg) in treatments compared the controls (average 4.12 mg). The dehydrogenase activity changed in smaller value (50.03-51.98 µg INTF / g soil / 2 h), caused positive effects by perlite, and dolomite in soils. The phosphatase activity also changed positively (144.9-160.4 mg P_2O_5 / kg / 2h) except by dolomite amendment. The CO_2 - production didn't change significantly in the experiments (73.6-83.9; 143.5-151.2 mg / kg / 14 days). There was some correlation between the examined soil variable. In alginate, perlite and dolomite experiment positive medium-strong correlation proved between moisture content and urease (r = .938; .810; .943), as well as saccharase activity (r = .783; .820; .642). The changing of soil moisture content influence positive value the soil respiration (CO_2-production) (r = .919 alginite; .889 perlite; .823 zeolite). In alginate and perlite experiment positive strong correlation was between changing of saccharase activity, and CO_2-production (r = .514; .686). Between the enzyme activities we proved correlation varying size and direction in experiments. Due to human activity and intensive land use, acidic sandy agricultural areas have an increasing tendency. Plant cultivation is not safe on these areas without melioration activities and reasonable nutrient supply. All these activities need to be harmonize with the principles of environmental protection. Based our results minerals applied in agriculture can be possible alternatives to the sustainable land use.

MICROAEROBIC DEGRADATION OF XYLENE: AN ENRICHMENT APPROACH COUPLED WITH GENOME-RESOLVED METAGENOMICS

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Among monoaromatic hydrocarbons xylenes are considered one of the most common environmental contaminants. Their occurrence is mainly associated with oil and petroleum hydrocarbon spills, and discharges from petroleum industries. Since many microorganisms can use xylenes as source of carbon and energy under aerobic conditions, biodegradation of the contaminated environments is usually an obvious solution. However, in subsurface environments the availability of oxygen is always restricted. Under oxygen-limited conditions, the degradation rate of para- and ortho-xylenes is low, which makes these compounds persistent in subsurface environments. It is well known, that markedly different bacteria degrade aromatic hydrocarbons under microaerobic and fully aerobic conditions. The key enzymes playing crucial role in the microaerobic degradation of aromatic hydrocarbons are the I.2.C-type extradiol dioxygenases, which are mainly harboured by members of the order Burkholderiales. Nevertheless, still little is known about bacteria capable of degrading xylenes under microaerobic conditions. To reveal the diversity of these bacteria and the *C23O* genes encoding extradiol dioxygenases which play role in this process, aerobic and microaerobic xylene-degrading enrichment cultures were set up and investigated with a multi-omics approach. The Illumina 16S rDNA amplicon sequencing based analysis of the enriched bacterial communities revealed key differences between the composition of the aerobic and microaerobic xylene-degrading enrichment cultures. Members of the genus *Sphingobium* were only abundant in the aerobic enrichments, while members of the genera *Rhodoferax* and *Azovibrio* were characteristic in the microaerobic enrichments. Members of the genus *Pseudomonas* were the most dominant in both type of enrichments, but significantly higher dominancy of these bacteria could be observed in the aerobic cultures. However, identification of bacterial isolates revealed that different lineages of the genus *Pseudomonas* were dominant in the microaerobic enrichments than in the aerobic cultures. In the microaerobic cultures a yet undescribed member of the genus *Pseudomonas* was considerably dominant among the isolates. By applying genome-resolved metagenomics we were able to assemble 12 bacterial genomes from one of the microaerobic enrichment cultures, including *Rhodoferax* sp. and *Azovibrio* sp. as well. This analysis revealed that members of the genus *Rhodoferax* could play prominent role in the degradation of aromatic hydrocarbons under microaerobic conditions.

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ANTIBIOTIC RESISTANCE IN THE FOOD CHAIN: EXTENDED SPECTRUM B-LACTAMASE PRODUCING BACTERIA FROM A DOMESTIC PIG HOLDING AND FROM CONTEMPORARY HUMAN ISOLATES

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Production of extended-spectrum beta-lactamases (ESBLs) is a major problem in human and veterinary medicine. ESBL producers appear in human and animal microbiomes and can serve as sources of infection in veterinary and human healthcare, in accordance with the One Health principle. Moreover, food animals may contribute to spreading of resistant bacteria or their resistance genes to humans through the food chain. This study follows up the carriage of ESBL producers in pigs throughout their rearing as well as in contemporary human samples. The litters of three sows (forty piglets) were followed up. Fecal samples were collected at the age of 1, 35, 65, 90 and 120 days. Sows were sampled after farrowing. Eighty-seven human fecal isolates were collected at University of Debrecen. The samples were inoculated onto eosin-methylene blue agar plates supplemented with 2 mg/L cefotaxime. Isolates recovered were identified using MALDI-TOF MS and their antibiotic susceptibility tested using the EUCAST disk diffusion method. ESBL production was tested using the double-disk synergy test. ESBL gene families (*blaCTX-M* and its subgroups, *blaTEM, blaSHV*) and phylogroups were detected by polymerase-chain reactions. Two of the three sows were positive, three of the piglets belonging to the negative sow was positive as well at day 1; 5 more of them yielded positive samples later. Ten and two piglets of the two carrier sows were positive at day 1, then 3 and 7 piglets, respectively, proved to be positive in the piggery. A total of 163 samples was tested, the prevalence of ESBL producing bacteria was 29.4% (48/163); 41 *Escherichia coli* (82.0%), 5 *Citrobacter freundii* (10.0%); one *Enterobacter cloacae* and one *Morganella morganii* (2.0%) were found. Co-resistance rates were relatively low; 31.3% (15/48) was resistant to co-trimoxazole, 16.7% to gentamicin (8/48), 2.1% (1/48) to amikacin and tobramycin. All isolates were susceptible for ciprofloxacin, ertapenem, imipenem and meropenem. In case of human isolates, co-resistance rates were higher, 44.2% (46/87) was resistant to co-trimoxazole, 52.9% (55/87) to gentamicin, 54.8% (57/87) to tobramycin and amikacin, 47.1% (49/87) to ciprofloxacin. One isolate (1.1%) was resistant to ertapenem. The most porcine isolates carried *blaCTX-M-1* group (62.5%; 30/48), *blaCTX-M-2, -8, -9* groups were not detected, prevalence of *blaTEM* and *blaSHV* was 2.1% (1/48), and of 10.4% (5/48). In case of human samples, the prevalence of *blaCTX-M-1* was 68.6% (72/105). Most of the pigs became ESBL carriers sooner or later, regardless of the status of their sow, ESBL producers seem to be present from farrow to finish in the monitored pigs. This creates a possibility for transmission to individuals through the food chain.

AEROBIOLOGICAL INVESTIGATION OF FUNGAL AND BACTERIAL POLLUTION OF SALT CHAMBERS IN HUNGARIAN KINDERGARTENS

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Salt chambers (SCs) are popular settings for prevention or complementary therapy for pulmonary diseases in European countries. In Hungary, many kindergartens operate SCs. Despite this, there are no standards for their operation, including hygienic requirements, e.g., air quality. SCs may provide a special habitat for microbes adapted to live in high saline environments. The aims of this study were to investigate the microbial air quality in SCs and test whether they harbour halotolerant fungi. To measure the concentration of microscopic fungi and bacteria, 100-100 L air was sampled in 21 SCs with an Andersen-type air sampler (MAS-100 Eco). Outdoor samples were also collected as references. Air samples were incubated on blood agar at 37°C for three days to detect bacteria. Fungi were detected on malt extract agar with 10% chloramphenicol incubated at 25°C for five days. Concentrations (C) were expressed in colony forming units (CFU/m^3). Threshold levels were defined as total C of indoor bacteria > total C of outdoor bacteria+200 CFU and indoor C of a given fungal morphotype > outdoor C of a given fungal morphotype +50 CFU. Sporulating filamentous fungi were identified at genus level, with a Carl Zeiss Jenaval light microscope at 300× magnification. Isolates of selected filamentous fungi exceeding threshold levels were identified by the ITS (ITS1-5.8S- ITS4) regions of the rDNA repeats. The airborne concentration of bacteria varied between 710 and 27,000 CFU/m^3, and was higher in all samples collected indoors than outdoors. Their concentrations were 68.5 times higher indoors than outdoors as an average and exceeded threshold in all SCs. The airborne concentration of fungi varied between 0 and 610 CFU/m^3, and was higher in all samples collected indoors than outdoors. In the indoor and outdoor samples 17 and 26 fungal taxa were detected, respectively. Most common genera in the indoor air were *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp. (*A.* sect. Fumigati, Nigri, Versicolores, Circum-

dati) and *Rhodotorula* spp; while the outdoor composition was slightly different, as it was dominated by *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp. (*A*. sect. Fumigati and Nigri), *Phoma* sp., *Cephalotrichum* spp. and *Geotrichum* spp. In 6 of 21 salt rooms, the concentration of indoor molds was above the threshold level. In 38.89% of the salt rooms, yeast levels were more than the double of the outdoor value. It can be concluded that general standards are needed to ensure adequate microbial air quality in SCs.

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HUMAN AND BOVINE CORONAVIRUSES FOUND IN SLOVENIA IN THE PERIOD FROM 2010 TO 2016

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Coronaviruses (CoV) are widely distributed pathogens of human and animals and can cause mild or severe respiratory and gastrointestinal disease. Antigenic and genetic similarity of some CoVs within the *Betacoronavirus* genus is evident. Therefore, for the first time in Slovenia, we investigated the genetic diversity of partial 390-nucleotides of RNA-dependent-RNA polymerase gene (RdRp) for 66 human (HCoV) and 24 bovine CoV (BCoV) positive samples, collected between 2010 and 2016 from human patients and cattle with respiratory disease. The characterised CoV strains belong to four different clusters, in three separate human clusters HCoV-HKU1 (n = 34), HCoV-OC43 (n = 31) and HCoV 229E (n = 1) and bovine grouping only as BCoVs (n = 24). BCoVs from cattle and HCoV-OC43 were genetically the most closely related and share 96.4–97.1% nucleotide and 96.9–98.5% amino acid identity. The genetic comparison of HCoV and BCoV from patients with clinical signs of respiratory disease did not provide evidence for zoonotic transmission of BCoV from bovine patients to humans or vice versa in Slovenia. This study is the first genetic comparison of these three HCoVs circulating in Slovenian human and BCoV in cattle populations and their phylogenetic relationship with CoVs available in GenBank database.

PREVALENCE AND MOLECULAR CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATES FROM CHILDREN'S PLAYGROUNDS IN HUNGARY

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Staphylococcus aureus is one of the most important human pathogenic bacteria. Besides direct person to person contact, indirect transmission via environmental exposure also occurs. Presence of *S. aureus* on children's playgrounds and on toys was described in international studies, however, no such data was available on the prevalence and characteristics of *S. aureus* on Hungarian playgrounds and outdoor gym equipment. In this study, altogether 531 environmental samples were collected from 15 Hungarian cities: 355 samples from playgrounds and 177 samples outdoor gyms. After inoculation on blood agar plates, suspicious colonies (beta-hemolysis, yellowish pigment) were picked up and *S. aureus* isolates have been identified by biochemical tests and nucA PCR. Antibiotic susceptibility of the isolates was tested to 9 antibiotics. Presence of virulence factor genes was detected by PCR. Clonal diversity of the isolates was tested by PFGE and MLST. *S. aureus* isolates were found in 10 samples from 3 playgrounds (2,81%). None of the samples from outdoor gym equipment were positive for *S. aureus*. Two of the isolates were resistant to penicillin and erythromycin and four isolates were resistant to ciprofloxacin. None of the isolates were MRSA. Presence of *spa* (10), *fnbA* (10), *fnbB* (5), *icaA* (8), *cna* (7), *sea* (2), *hla* (10), *hlb* (2) and *hlg* (6) virulence genes were detected. The isolates belonged to seven different pulsotypes by PFGE. With MLST, we have detected isolates belonging to ST8, ST22, CC182, CC398 and CC45. We have found a new sequence type, ST6609, part of CC45. In conclusion, *S. aureus* isolates are present on Hungarian playgrounds, in lower prevalence compared to international data. These strains carry several virulence factors and some of them are resistant to commonly used antibiotics. The isolates were clonally diverse, and some of the identified clones were detected previously during clinical and carriage studies. The isolates can potentially cause skin and soft tissue infections in children.

MECHANISM OF ACTION AND POTENTIAL TARGETS OF *NEOSARTORYA* (*ASPERGILLUS*) *FISCHERI* ANTIFUNGAL PROTEIN 2

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In the consequence of emerging number of antifungal drug-resistant fungal strains the incidences of human mycoses dramatically increased worldwide in the past years. The facts that only a few classes of antifungal drugs are available, and that they can cause serious side-effects and damage the host's organs permanently hamper patient management. Therefore, there is a substantial demand for development of fundamental new, effective and safely applicable antifungal strategies to prevent and treat fungal infections. The features of NFAP2; a small molecular weight, cysteine-rich, cationic antifungal protein secreted by the filamentous ascomycete, *Neosartorya* (*Aspergillus*) *fischeri* NRRL 181 correspond well to these challenges. NFAP2 effectively inhibits the growth of several human pathogenic *Candida* spp. (including azole-resistant strains) in vitro and in vivo, eradicates biofilms of these species, it does not show toxic effect on mammalian cells, and it is able to interact synergistically with several licensed antifungal drugs. Key steps in its possible future application as antifungals drug are understanding the mechanisms of action, and revealing the molecular targets in fungal cells. We demonstrated that NFAP2 is a membrane-interacting protein showing specificity to bound to ergosterol constituted fungal cell membrane. In short time NFAP2 is able to disrupt the cell membrane integrity by a pore-forming mechanism, which results in cell content loss and cell death, finally. Molecular dynamics studies and functional mapping of NFAP2 suggested that the mid-N-terminal, highly flexible and positively charged loop region of the molecule is responsible for bounding to fungal cell membrane; and an oligomerization process for the pore-forming ability. Protein-lipid overlay assay coupled with western blot procedure indicated that NFAP2 has a high binding affinity to phosphatidic acid (PA) and phosphatidylinositol 5-phosphate (PI5P). Considering that PA, a signalling lipid, and that it is known to recruit cytosolic proteins to membranes; it is possible that interaction of NFAP2 with PA required for the entry of this protein into fungal cells and for its intracellular toxicity. Phosphatidylinositol signalling plays an essential role in many biological processes; e.g. filamentous growth of yeasts and providing protection against azole antifungals. These interactions with PA and PI5P suggest potential intracellular protein targets of NFAP2. Applying far-western blotting assay, we already identified some candidates. Taking into consideration the above discussed results, it is very likely that NFAP2 exerts its antifungal mechanism of action via two different ways: 1) Plasma membrane disruption, and 2) binding to an intracellular protein target after internalization.

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MOLECULAR STUDIES TO REVEAL THE MICROBIAL COMMUNITIES OF OLIGOTROPHIC ENVIRONMENTS

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The present study aims to reveal the prokaryotic community structure of three springs located in the regions of Transylvania: Piricske, Somlyó Nagy-borvíz and Taploca The number of the bacteria after DAPI staining was determined by epifluorescent microscopy, then to reveal the microbial community structure high-throughput amplicon-sequencing experiment was applied. The microscopic cell counts showed $4.78*10^4$ cells/mL in Piricske, $1.66*10^5$ cells/mL in Somlyó Nagy-borvíz, and $7.23*10^4$ cells/mL in Taploca. The values of the Shanon diversity index showed 5.04 and 0.77 in the Piricske water sample, 2.44 and 1.91 for Somlyó Nagy-borvíz water sample and 6.47 and 4.41 for Taploca, for bacteria and archaea, respectively. Taploca water samples were characterized with the highest diversity in both archaea and bacteria followed by Piricske and Somlyó Nagy-borvíz for archaea, and Somlyó Nagy-borvíz and Piricske for bacteria. All the samples obtained from the region of Transylvania were characterized by the presence of the phyla Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Nitrospirae, Patescibacteria and Proteobacteria for bacteria and Nanoarchaeaeota, Euryarchaeota and Diapherotrites for archaea, however, some taxa were characterizing each sample such like the high presence of Caldiserica and Spirochaetes phyla in Somlyó water sample, Kiritimatiellaeota, Elusimicrobia and

Omnitrophicaeota in Piricske water sample, and Planctomycetes and Gemmatimonadetes in Taploca water sample. The phylum Altiarchaeota was completely absent in Taploca, instead, the latter was dominated by Thaumarchaeota. Many of the detected taxa are known to thrive under nutrient-depleted conditions. They are able to survive due to their high degree of specialization (e.g. interactions between various microbial populations). The revealed taxa can have a central role in the different in geochemical cycles, e.g. Nitrospirae (present in all samples) in nitrogen cycle or the detected Methanobacteria are present in high number in Somlyó water sample, or Nitrososphaeria in Taploca and members of the order of Desulfobacterales in case of Piricske water sample.

COMPARISON OF SPECIES-SPECIFIC MULTIPLEX PCR AND API 20 STREP FOR THE IDENTIFICATION OF VETERINARY CLINICAL *ENTEROCOCCUS* ISOLATES

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Bacteria of the genus *Enterococcus* are intrinsically resistant to several commonly used antimicrobial drugs. In addition, enterococci have a great ability to acquire new resistance mechanisms, which can lead to multidrug-resistant isolates. The species of the greatest clinical importance are *Enterococcus faecium* and *Enterococcus faecalis*. Because of the naturally occurring differences in the susceptibility of these species, rapid and accurate identification among enterococci isolates is very important for choosing appropriate antimicrobial therapy. Since the identification by routine standard methods, including commercially standardized system such as API (bioMérieux, France), is time consuming and not always reliable, nowadays identification by molecular methods is recommended. The purpose of this investigation was to compare the performance of multiplex PCR and API 20 STREP in determining *Enterococcus faecium* and *Enterococcus faecalis* species among enterococcal isolates from animals. In this study, the enterococci isolates from clinical samples of cats and dogs in Croatia were investigated. A total of 40 isolates were tested for specific $ddl_{E.\ faecalis}$ and $ddl_{E.\ faecium}$ genes by multiplex PCR assay. API 20 STREP (bioMérieux, France) was performed for the same isolates. *Enterococcus faecalis* ATCC 29212 and *Enterococcus faecium* ATCC 6057 were included as reference strains. ith the multiplex PCR assay, 32 isolates were identified as *Enterococcus faecalis*, and 8 isolates as *Enterococcus faecium*. API 20 STREP identified 28 isolates as *Enterococcus faecalis*, 11 isolates as *Enterococcus faecium* and one isolate as *Enterococcus avium*. Overall, among 40 isolates, 35 (87 %) were equally identified as *Enterococcus faecalis* or *Enterococcus faecium* with both API 20 STREP and multiplex PCR. For five isolates (13 %), API 20 STREP did not result in correct identification. Four isolates were identified as *Enterococcus faecium* by API 20 STREP, but the PCR identified them as *Enterococcus faecalis*. Isolate identified as *Enterococcus avium* was recognized as *Enterococcus faecium* with the multiplex PCR assay. Considering the differences in innate susceptibility between two investigated enterococcal species, correct identification is crucial for choosing appropriate antimicrobial therapy. The use of species-specific PCR, as a method for the identification, is strongly recommended, especially in complicated infections caused by multidrug-resistant enterococci.

IN VITRO ASSAY FOR IDENTIFYING CARBOHYDRATES WITH PREBIOTIC NATURE ON A PROBIOTIC STRAIN OF *LACTOBACILLUS PLANTARUM*

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The gut microbiota plays a major role in the human physiology and it is being increasingly recognised that a balanced and healthy gut biota has great importance in the development and maintenance of overall health of the host. In human gut microbiota dysbiosis, one of the tools to modulate and reinstate the healthy microbiome composition is a nutritional intervention with clinically proven probiotic strains strengthened by prebiotic compounds. Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth of a limited number of bacterial species. Nowadays, identifying and developing novel prebiotics have been gained an emerging interest. In this study, we present an in vitro assay developed for screening and identifying compounds with prebiotic nature. For this purpose, we evaluated the growth promoting effect of plant- and fungi-based carbohydrates on a probiotic strain of *L. plantarum*. In advance, an isolation strategy was worked out to extract the polysaccharide fraction of different plant seeds and fluids. Oligosaccharides were also generated followed by chromatographic fractionation and structural characterisation. Three different carbohydrate candidates were identified as potentially effective prebiotics.

EFFECT OF A CLIMATE MANIPULATION ON SOIL MICROBIAL COMMUNITIES IN A SANDY GRASSLAND

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A multiannual extreme drought and rain manipulations (ExDRain-project) have been running to study the effect of local climate change in a multidisciplinary way in a sandy grassland near Fülöpháza (Hungary). Microbial communities have important roles in ecosystem functioning and the understanding of their change due to the climate change is an important task. Therefore, our aim was to reveal the effect of local climate change on soil microbial diversity and analyse relationship among them and other measured parameters. The experimental site was sampled extensively exactly before the manipulations and thereafter in first, third and fifth year resulting in 156 composite soil samples from 48 experimental pots. Community DNA was extracted from every composite soil sample with Qiagen DNeasy® PowerMax® Soil Kit. Prokaryotic 16S rRNA gene was amplified by the Earth Microbiome primer pair, whereas fungal nuclear rRNA ITS2 region was amplified by the ITS3_KYO2 - ITS4 primer pair. The products were analysed by Next Generation Sequencing (NGS) on the Illumina platform. The raw sequences were processed and analysed using the pipeline of the SEED2 software. Soil parameters (e.g., soil organic carbon, total nitrogen, mineral nitrogen forms, particle size) were also measured. Statistical analyses were carried out with the R software. The 16S rRNA gene amplicon sequencing resulted in a total of 80,000 – 217,000 raw reads and 35,000 – 98,000 high-quality prokaryotic reads / sample. Whereas sequencing of fungal nuclear rRNA ITS2 region resulted in a total of 8,000 – 400,000 raw reads and 5,000 – 263,000 high-quality fungal reads / sample. Details of microbial diversity and relationship among the different measured parameters will be presented.

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ENDOPHYTIC PROKARYOTES OF DARK SEPTATE ENDOPHYTIC FUNGI REVEALED BY NGS METABARCODING

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Mosse described long time ago a bacterial like structure from an arbuscular mycorrhiza forming fungi. By now many endohyphal or hyphae associated bacteria have been identified. Dark Septate Endophytes (DSE) are worldwide distributed root endophytic fungi without exactly known role in the ecosystems. Previously many DSE were isolated from the sandy grassland near Fülöpháza and preliminary results suggested that these fungi have bacterial symbionts. Hence our aim in the present project was to describe this bacterial diversity with the help of Next Generation Sequencing (NGS). Genomic DNA from 15 DSE strains was isolated by the NucleoSpin Plant II DNA Isolation Kit. Thereafter prokaryotic 16S rRNA gene was amplified by the Earth Microbiome primer pair. The products were sequenced on the Illumina platform by Michigan State University, RTSF Genomics Core. The raw sequences were processed and analysed using the pipeline of the SEED2 software. The 16S rRNA gene amplicon sequencing resulted in a total of 65,000 – 205,000 raw reads and 140 – 6,600 high-quality prokaryotic reads / sample. The large loss of sequences during analysis is partially due to massive amplification of mitochondrial DNA. The first results show that most of the sequences belong to the Phyla Proteobacteria, Firmicutes, Actinobacteria, Bacteroidota, Bdellovibrionota and Acidobacteriota. But Archaeal sequences belonging to Crenarcheota were also found. Further details of the phylogenetic composition of the prokaryotic symbionts will also be presented.

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GENOME-BASED FUNCTIONAL CATEGORIZATION OF ECTOMYCORRHIZAL FUNGI

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Ectomycorrhizal (EcM) fungi are widespread species establishing symbiotic relationships with most boreal, temperate and some tropical trees that constitute approximately 60% of tree stems on the Earth. Therefore, their essential role in

carbon and nutrient dynamics in terrestrial ecosystems is inevitable. The EcM lifestyle convergently emerged at least 80 times through the evolution and can be found in three fungal phyla (Mucoromycota, Ascomycota, Basidiomycota). Despite their ecological importance and widespread distribution, our knowledge of functional diversification among EcM species is scarce. Moreover, several EcM species forage from the same source (photosynthetic assimilates) while coexisting on one tree-individual, implying the need for functional differences among species. Here, we hypothesize that the co-existence of several EcM species within one habitat could be achieved by evolving a high functional diversification that can be observed in fungal genomes. To test our hypothesis, we attempted to delineate functional groups of EcM species based on gene copy numbers of 187 publicly available EcM genomes. We excluded genomes with low quality to decrease the number of false negative hits for orthologous genes (BUSCO

CHARACTERIZATION OF *RTA*1 GENES AND THEIR ROLE IN THE AZOLE RESISTANCE MECHANISM OF *MUCOR CIRCINELLOIDES*

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Mucor circinelloides belongs to order Mucorales, an opportunistic human pathogenic filamentous fungus which can cause mucormycosis, an aggressive fungal infection. This rare and mostly lethal disease is seen manifested in immunocompromised patients with solid organ transplantation, chronic renal failure, neutropenia, poorly controlled diabetes, and nowadays in COVID-19 patients. Surgical debridement for both analysis and treatment and ever-increasing azole resistance of these fungi make the diagnosis and treatment of this dreaded disease far more challenging. The high mortality rate linked with this mycosis pertains to the antifungal drug resistance mechanism of the fungi. Therefore, identification of the molecular mechanisms which confers this drug resistance is of prime importance as this will lead to better diagnostics and will help in the development of new treatment strategies. *Rta1* gene (resistance to 7-aminocholesterol), has a role in fungal resistance to the ergosterol biosynthesis inhibitor 7-aminocholesterol. It confers high and specific resistance to 7-aminocholesterol and many other fungicidal chemicals and plays important role in stress management strategies of the fungi. Genome of *M. circinelloides* encodes four *rta1* genes (*rta1a, rta1b, rta1c* and *rta1d*). The main goal of this study is to characterize the functions of these genes. To functionally characterize the exact roles of these genes in the azole resistance mechanism of *M. circinelloides*, knock-out mutants were created using CRISPR-Cas9 system. Morphology, growth- and germination ability of the created mutants were analysed. The susceptibilities of these mutants to different azoles and amphotericin B were investigated as well. Deletion of *rta1a* and *rta1b* genes resulted in increased sensitivity to ketoconazole. The relative transcript level of the *rta1* genes was measured after azole treatment and at different temperature using quantitative real-time PCR. *rta1c* showed the highest transcript level at different temperature, while the transcript level of *rta1d* significantly increased after azole treatment.

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EFFECT OF FIVE ESSENTIAL OILS ON THE AFLATOXIN PRODUCTION OF *ASPERGILLUS PARASITICUS* GROWN ON WHEAT SUBSTRATE

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Foods and feeds are often contaminated by mycotoxin-producing fungi causing serious problem for food safety and food security. Earlier studies demonstrated that several essential oils (EOs) have antifungal effect and they could also modify the secondary metabolite production of certain fungi. In this study the effects of five essential oils (EOs; cinnamon, clary sage, juniper, lemon, and marjoram) on the aflatoxin production of *Aspergillus parasiticus* cultivated on wheat substrate have been investigated. EOs were used in different concentrations (0,052, 0,105, 0,21, 0,42 mg/cm^3) in the vapor phase and aflatoxin production, as well as the ratio of various aflatoxins (AFB1, AFB2, AFG1, AFG2) were determined. Measurements were carried out on the 5th and 10th days of the incubation period. According to our experiments, cinnamon EO expressed inhibitory effect on the aflatoxin production of *A. parasiticus*. Increasing the concentration of cinnamon EO in the vapour phase resulted in decreased aflatoxin levels in the substrate. Lemon and clary sage EOs could not significantly decrease the aflatoxin production. At the same time, using marjoram EO, stimulation of aflatoxin production by *A. parasiticus* has been observed. We measured the changes in the components of the aflatoxin complex too. On wheat, the toxins

produced in the highest quantities and proportions were AFG1 (63-73%) and AFB1 (19-38%). With increasing incubation time, the ratios of various aflatoxins changed, and by the end of incubation the ratio of AFG1 decreased in both control and treated samples while the ratio of AFB1, AFG2 and AFB2 increased. The least change in the proportions of aflatoxins was observed using lemon and clary sage EOs on vapour.

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INVESTIGATION OF THE INTERACTION BETWEEN *CANDIDA* EXTRACELLULAR VESICLES AND ORAL SQUAMOUS CELL CARCINOMA CELL LINES

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Oral cancer is the 16th common cancer worldwide, 90% of those diseases is oral sqamous cell carcinoma. In relation to the presence of the tumor the normal oral microbiota can change and it might be lead to other diseases like oral candidiasis. A previous study of our laboratory has shown that the colonisation and diversity of yeast cells was significantly higher in the patient's mouth diagnosed with oral squamous cell carcinoma (OSCC) compared to the healthy individuals. Furthermore, in the tumorous mouth the colonisation of yeast cells was higher on the surface of the tumor compared to the healthy surface. The interaction between *Candida* and tumor cells can not only occur directly through different receptors but also indirectly on the level of extracellular vesicles (EV), which is recently poorly understood. Our aim is to examine the interaction between *Candida albicans* and oral squamous cell carcinoma (OSCC) cell lines on the level of extracellular vesicles. In order to investigate the interaction between oral squamous cell carcinoma (OSCC) and *Candida* we used two OSCC cell lines and *Candida albicans* SC5314 and *Candida parapsilosis* CLIB214 strain. We successfully optimized the fungal extracellular vesicle (EV) isolation protocol from solid and liquid media for both species. Transmission electron microscopy and NanoSight analysis of the EV samples from *C. albicans* and *C. parapsilosis* represented round shaped EVs with 100 to 500 nm size distribution. We also developed an extracellular vesicle isolation protocol to isolate EVs released by hyphae form of *C. albicans*. To investigate the effect of fungal EVs on OSCC cells we examined the uptake of the fungal EVs by the OSCC cell lines with Imaging Flow Cytometry. We examined the effects of the fungal EV treatment on the metabolic activity and proliferation of the tumor cells with CCK-8, and BrdU assay. In the interest of to investigate the effects of the fungal EVs to the OSCC cells viability we use LDH assay. CytoSMART Lux2 cell culture video monitoring device was used for the examination of the effects of fungal EVs on the wound-healing capacity of OSCC cells. As a result of the proliferation, metabolic activity and viability experiments we found that fungal EV treatment did not cause any changes on the mentioned cell functions. Imaging Flow Cytometry analysis showed, both tumor cell line able to take up the *C. albicans* EVs with high efficiency at 37°C but at 4°C we could not detect uptake events. According to the wound-healing capacity of the HSC-2 cells we could not detect any changes, but we could observe morphological changes of the cells and single, detached migrating cells as a result of the fungal EVs presence. From these results, we can conclude that there may be an interaction between OSCC cells and Candida.

CLINICAL CHARACTERISTICS AND OUTCOMES OF COVID-19 IN PATIENTS HOSPITALIZED AT THE UNIVERSITY HOSPITAL FOR INFECTIOUS DISEASES „DR. FRAN MIHALJEVIĆ" IN ZAGREB, CROATIA, DURING THE FIRST WAVE OF THE EPIDEMIC

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Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December 2019 in China, and spread across the world by the beginning of 2020, causing the World Health Organization to declare a pandemic in March 2020. The first documented case in Croatia was recorded on February 25th, 2020 with over 373,330 people infected so far. The aim of this study was to describe the clinical characteristics and outcomes of COVID-19 in patients hospitalized at the University Hospital for Infectious Diseases „Dr. Fran Mihaljevic" in Zagreb, Croatia during the first wave of the epidemic. A retrospective study was conducted on the cohort of patients hospitalized at our Hospital with documented COVID-19, confirmed with a positive RT-PCR test for SARS-CoV-2. All

hospitalized patients during the first wave of the epidemic, between February 25 and June 18, were included in the study. The demographic, epidemiologic and clinical data was obtained from the patients' medical charts. During the study period, a total of 175 patients with COVID-19 were hospitalized. All patients were over 18 years of age, with a median age of 59 (range 22–99) years of whom 110 (62.8%) were male. A total of 110 (62.9%) patients had one or more comorbidities. Regarding disease severity, 24 (13.7%) had a mild form, 33 (18.9%) moderate form, 54 (30.9%) severe form and 63 (36%) critical form of the disease. Oxygen therapy was used in 104 (59.4%) patients, admission to ICU in 33 (18.9%) patients, 39 (22.3%) patients required mechanical ventilation and 11 (6.3%) renal replacement therapy. We found a significant correlation between patients' fatal outcomes and vital parameters at admission, such as higher respiratory frequency ($p = 0.026$), Modified Early Warning Score ($p = <0.001$), as well as lower oxygen saturation ($p = 0.001$). Furthermore, we identified a significant correlation between patients' fatal outcomes and several laboratory findings at patients' admission, such as higher leukocyte counts ($p = 0.004$) and percentage of lymphocytes in the peripheral blood ($p = 0.040$), as well as higher levels of serum ALT ($p = 0.001$), creatinine ($p = 0.027$), CK ($p = 0.006$), troponin ($p = 0.003$), LDH ($p = <0.001$) and CRP ($p = <0.001$) were correlated with poor outcomes. Chest X-ray imaging showed interstitial pneumonia in 122 (69.7%) patients, pulmonary consolidation in 8 (4.6%), pleural effusion in 13 (7.4%) and no pulmonary changes in 35 (20%) patients. Regarding treatment, 59 (22.3%) patients received hydroxychloroquine, 11 (6.3%) glucocorticoids, 12 (6.9%) lopinavir/ritonavir and 8 (4.6%) azithromycin. Overall, 21 (12%) of the hospitalized patients died. COVID-19 has become a major respiratory threat during 2020 with a case fatality ratio of 12% among hospitalized patients with confirmed SARS-CoV-2 infection. Poor outcome is associated with several clinical and laboratory parameters. Further investigation is needed to clarify the pathogenesis, outcomes and treatment options for COVID-19 in the future.

PURIFICATION AND CHARACTERIZATION OF B-GALACTOSIDASE ENZYMES FROM *LICHTHEIMIA RAMOSA* AND *RHIZOMUCOR PUSILLUS*

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The β-galactosidase (lactase, EC 3.2.1.23) is an important enzyme in the food industry due to its hydrolytic activity used for lactose conversion. The enzyme is applied to produce lactose-free products that are beneficial for people having lactose intolerant condition. In addition, it can be used for ethanol and sweet syrup production from whey lactose. The β-galactosidase efficiently catalyzes transgalactosylation reactions which can be useful in the synthesis of galacto-oligosaccharide prebiotics. These non-digestible sugars can promote the growth of both the beneficial gut microorganisms and the probiotic additives. Fungi are excellent sources of β-galactosidases and some enzymes have been purified and characterized from these microorganisms. However, there are only a few biochemical data on enzymes purified from zygomycetes fungi. In our previous work, several β-galactosidase active strains were identified in the zygomycetes genera *Mucor, Rhizomucor, Rhizopus, Mortierella, Umbelopsis* and *Lichtheimia*. The *Rhizomucor pusillus* and *Lichtheimia ramosa* presented good β-galactosidase production ability and high enzyme activity in a solid-state condition containing wheat bran as fermentation material. In this study, we aimed for an in-depth analysis of β-galactosidases of *R. pusillus* and *L. ramosa* fungi. Namely, we planned to scale-up the solid-state production, and purify and characterize the enzymes produced. Ammonium sulfate precipitation followed by gel filtration and ion-exchange chromatography techniques were applied for the β-galactosidase purification. A size-exclusion polishing step on a Sephacryl column was carried out to improve the purity of the enzymes. Molecular weight of the purified enzymes was about 80-90 kDa determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Both enzymes showed optimum activity at 50 °C and pH 6.0 incubation conditions. To our knowledge, this work presents the first characterized β-galactosidase from *Lichtheimia*.

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ENHANCEMENT OF PRODUCTION OF SOLUBLE CARBOHYDRATES DURING BIOLOGICAL PRETREATMENT OF WHEAT BRAN

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Despite the pretreatment of recalcitrant lignocellulosic residues by a complex microbial community is a promising approach to enhance the hydrolysis of carbohydrates, this still faces many challenges such as the selection of species that can co-exist in the same community and possess synergistic effect, and the optimization of the cultivation method to enhance

the lignocellulose degradation process etc. In this study, some bacteria, *Bacillus, Rhodococcus* and *Pseudomonas* strains were evaluated individually as well as constructed in different communities to figure out the characteristics of each ecosystem, providing insights into key determinants of the microbial consortium performance in the biological pretreatment of wheat bran. The results showed that the microbial consortium accounts for the higher conversion to released glucose in comparison with monoculture in submerged pretreatment of wheat bran. A high amount of reducing sugars with less carbohydrate loss was observed when using consortium in solid stage pretreatment with moisture over 70%. Typically, *Pseudomonas putida* co-existed with *Bacillus* community and *Rhodococcus fascian*s showed a synergistic relationship by releasing a higher amount of glucose after 72 hours than its combination with other ligninolytic strains. Additionally, the combination of a bacterial consortium with filamentous fungus *Aspergillus niger* improved the bioprocessing potential. The higher amount of fermentable carbohydrates was released in the solid stage and submerged cultivation claimed the dominant roles of *Aspergillus niger* and bacterial consortium, respectively. Our results indicated that a mixture consortium of fungi and bacteria may have great potential in the increase of efficacy of biological pretreatment.

ENRICHMENT AND ISOLATION OF SULFUR-OXIDIZING BACTERIA FROM A BIOFILTER TREATING H_2S-CONTAINING AIR

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Among environmental elements air plays a crucial role in the life of all living creatures at Earth. Various human activities produce vapours and gases that, in addition to their harmful effects, even cause unpleasant odours. Odours affect a person's well-being, which can lead to even more serious damage to health. To remove unpleasant odours, chemical, physical, and biological processes also exist. Biological methods take advantage of the metabolic activity of microorganisms. In industrial technologies, sulfur-containing compounds can also cause unpleasant odour. These compounds include hydrogen sulfide (H_2S), which not only has an unpleasant odour but is also toxic to organisms. The aim of the present study was to enrich and isolate sulfur-oxidizing bacterial strains from water sample of a biofilter, treating H_2S-containing air at a food industrial site. The water sample was taken from a biofilter, which functioned as a biological deodorant. Microbial community of the water sample was revelaed by 16S rDNA Illumina amplicon sequencing. Enrichment culturing was also applied to gain chemolithothrophic sulfur-oxidizing bacterial strains. The microbial community of the water sample was dominated by members of the genus *Acetobacter* (>40%), followed by *Streptomyces* (36%), *Lactobacillus* (8%), *Acidithiobacillus* (5%), *Paenibacillus* (2%), *Thiomonas* (1%) and *Pararhodospirillum* (1%). The bed of the biofilter was pine bark, on which no biofilm was visible and we were unable to isolate bacterial DNA from its surface. From the enrichment cultures a single bacterial strain was isolated, which was member of the genus *Thiomonas*, and was most closely related to *T. perometabolis*, and *T. intermedia*, which are frequently used to treat H_2S-containing air.

GENOME-CENTRIC INVESTIGATION OF ANAEROBIC DIGESTION USING SUSTAINABLE SECOND AND THIRD GENERATION SUBSTRATES

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Biogas production through co-digestion of second and third generation substrates is an environmentally sustainable approach. Green willow biomass, chicken manure waste and microalgae biomass substrates were combined in the anaerobic digestion experiments. Biochemical methane potential test showed that biogas yields of co-digestions were significantly higher compared to the yield when energy willow was the sole substrate. To scale up the experiment continuous stirred-tank reactors (CSRTs) are employed, digestion parameters are monitored. Furthermore, genome-centric metagenomics approach was employed to gain functional insight into the complex anaerobic decomposing process. This revealed the importance of Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes phyla as major bacterial participants, while Methanomicrobia and Methanobacteria represented the archaeal constituents of the communities. The bacterial phyla were shown to perform the carbohydrate hydrolysis. Among the representatives of long-chain carbohydrate hydrolysing microbes Bin_61: Clostridia is newly identified metagenome assembled genome (MAG) and Bin_13: DTU010 sp900018335 is common and abundant in all CSTRs. Methanogenesis was linked to the slow-growing members of the

community, where hydrogenotrophic methanogen species *Methanoculleus* (Bin_10) and *Methanobacterium* (Bin_4) predominate. A sensitive balance between H_2 producers and consumers was shown to be critical for stable biomethane production and efficient waste biodegradation.

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SARS-COV-2 REPLICATION-TRANSCRIPTION COMPLEX: IDENTIFYING PROMISING TARGETS FOR ANTIVIRAL THERAPY

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Coronaviruses (family Coronaviridae, order Nidovirales) are important pathogens. In humans, coronaviruses primarily cause upper and lower respiratory tract disease which, in infections caused by newly emerging coronaviruses (SARS-CoV, MERS-CoV, SARS-CoV-2), may progress to pneumonia, acute respiratory distress syndrome (ARDS), and (multi) organ failure with fatal outcome. Coronaviruses employ a multi-subunit protein complex, which is generally referred to as the replication-transcription complex (RTC), to replicate and transcribe their exceptionally large positive-stranded RNA genomes of approximately 30 kb. The coronavirus RTC also includes enzyme functions that counter the commonly high error frequency of viral RNA polymerases. The individual protein components of the RTC are called nonstructural proteins (nsp) 1 to 16 and are produced from two large polyproteins by proteolytic processing by two types of virus-encoded proteases. Over the past few years, significant progress has been made regarding the structural and functional characterization of coronavirus RTC-associated enzymes and their cofactors. RTC functions include more or less universal activities of plus-stranded RNA viruses, like an RNA polymerase (*nsp12*) and helicase (*nsp13*), but also a number of rare or even unique domains such as endo- and exoribonuclease, RNA methyltransferases, RNA polyadenylate transferase and protein-specific nucleotidyltransferase activities. The talk will summarize some of this recent work, focusing on newly discovered enzymatic functions of the coronavirus RTC, the structure and assembly of the coronavirus RTC and the potential use of coronavirus replicase gene-encoded functions as targets for antiviral drugs.

REGULATORY CROSSTALK BETWEEN CORE GENOME AND HORIZONTALLY ACQUIRED GENES INFLUENCES BIOFILM EXPRESSION IN *STAPHYLOCOCCUS AUREUS*

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Biofilm formation is a common theme in the bacterial world conferring protection against unfavorable conditions, including the action of antibiotics. Here we report on an unprecedented regulatory interplay between plasmid-borne and core genome genes involved in biofilm control in the significant human and animal pathogen *Staphylococcus aureus*. Thus, we recorded downregulation of biofilm formation in an *S. aureus* clinical strain upon acquisition of a (multiresistance) plasmid which carried a biofilm gene cluster in addition to a pre-existing biofilm locus on the *S. aureus* chromosome. Unexpectedly, we found the plasmid-borne and core genome biofilm loci (although being different at nucleotide and amino acid sequence levels) to undergo a regulatory crosstalk, with the two respective transcription repressors associated with the loci functionally replacing each other and eventually hindering biofilm formation. We interpret the suppressed biofilm production as a mechanism to reduce fitness costs that might come upon plasmid-mediated acquisition of a second biofilm gene cluster copy. However, as biofilm formation also represents a beneficiary trait, we discuss (from an evolutionary point of view) how the newly acquired DNA traits might be integrated into the preexisting regulatory networks of *S. aureus* to allow expression of this important virulence factor. Together, the data shed light on the functional consequences of gene transfer events and their putative implications for the adaptation and evolution of bacterial pathogens.