Magyar Mikrobiológiai Társaság
és a MMT Alapítványa
www.mmt.org.hu

A Magyar Mikrobiológiai Társaság 2022. évi Nagygyűlése

és a

XV. Fermentációs Kollokvium

ABSZTRAKTFÜZET

Aranyhomok Szálló, Kecskemét

2022. október 12-14.

IDENTIFICATION AND ANALYSIS OF DOZENS OF UNIQUELY OCCURRING [D1,2] STWINTRONS IN THE GENOME OF THE WOOD SOFT-ROT FUNGUS *XYLARIA LONGIPES*

VIKTÓRIA ÁG-RÁCZ, NORBERT ÁG, FRUZSINA PÉNZES, LEVENTE KARAFFA, MICHEL FLIPPHI, ERZSÉBET FEKETE

Department of Biochemical Engineering, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

Spliceosomal introns are pervasive in eukaryotic nuclear transcriptomes. Their precise excision from pre-mRNAs requires the action of the U2 spliceosome. Intervening sequences may include more than one canonical intron unit. We have described a class of nested U2 introns, the stwintrons, which are removed by consecutive standard splicing reactions in an "inside-out" order. 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'-G1|U2). Fungal U2 introns are generally small – often <150 nt. The short but ubiquitous 5'-donor- (6 nt), the BP element- (6 nt), and 3'-acceptor sequences (3 nt) are well defined in model genomes. These characteristics enabled the design of a motif search model to predict stwintrons in whole genome sequences, involving degenerated motifs for U2 intron 5'-donors, BP sequences and 3'-acceptors. For [D1,2]'s, these include two hybrid motifs of 7- and 8 nt, respectively, that consist of nt of the external- as well as of the internal intron. Furthermore, four distance ranges between the five sequence motifs at the 5'- and 3'-splice sites, and the BP elements were defined. Individual motifs and distance ranges were modified in parallel to increase the stwintron complement detected. Our stwintron motif searches are sufficiently effective to identify 91 genuine [D1,2] stwintrons in the *Xylaria longipes* genome (GenBank Accession no. NQIL00000000) (Sordariomycetes; Xylariales; Xylariaceae; *Xylaria*). Splicing was checked by screening local RNA sequence reads (extant SRAs) and a comparative approach was used to track (stw)intron position conservation patterns within the host genes. We compare the results of statistical analyses of this batch of *X. longipes* stwintrons, their integration sites and the bounding exonic sequences with our earlier analyses of the compatible group of 81 uniquely occurring [D1,2] stwintrons found in *Hypoxylon* sp. CO27-5, a member of another family of Xylariales, the Hypoxylaceae. There were no evident sequence-similar stwintrons detected in *X. longipes*, although some [D1,2]'s were found at unique gene positions compared with the orthologous genes in the related species *X. polymorpha*, *X. cubensis* and *X. flabelliformis*.

Acknowledgements: Supported by the Hungarian National Research, Development & Innovation Fund thrue grant K 138489 and Doctoral Student Scholarship Program, grant RH/527-3/2021 to V.Á-R. of the Co-operative Doctoral Program.

BIOEFFECTOR POTENTIAL OF *BACILLUS* STRAINS ISOLATED FROM RECOMPOSTING SPENT MUSHROOM COMPOST

HENRIETTA ALLAGA[1], DÓRA HORKICS[1], ÁDÁM BORDÉ[1], TERÉZIA KOVÁCS[2], CSABA CSUTORÁS[3], JUDIT BAJZÁT[3], NÓRA BAKOS-BARCZI[3], AMANDA SÁNDORNÉ SZŐKE[3], LILLA LUCA LUKÁCS[3], ANDRÁS MISZ[3], CSABA VÁGVÖLGYI[1], LÁSZLÓ KREDICS[1]

[1]Department of Microbiology, Faculty of Science and Informatics, University of Szeged; [2]Biological Research Centre, Szeged; [3]Új Champignons Ltd., Budapest, Hungary

The quality of the casing layer used in champignon production is influenced by its microbiological composition. Bacteria present in spent *Agaricus* compost include *Bacillus*, *Alcaligenes*, *Pseudomonas*, and *Microbacterium* species. Certain representatives of the genera *Bacillus* and *Pseudomonas* are considered as beneficial. Our aim is to use spent mushroom compost for developing alternative casing layers. Microorganisms are isolated from spent mushroom compost samples taken during its natural recomposting process. A total of 15 *Bacillus* strains were isolated and identified from the samples. The resulting *B. licheniformis* (4), *B. velezensis* (4), *B. subtilis* (4), *B. cereus* (2) and *B. paralicheniformis* (1) strains were tested for their pH-, temperature-, and water activity-dependence, extracellular enzyme activities, and indole acetic acid production. In vitro confrontation assays revealed that a *B. licheniformis* and 3 *B. velezensis* strains may have good antagonistic potential against both mushroom-pathogenic moulds (*Lecanicillium*, *Trichoderma*, *Hypomyces* spp., etc.) and fungal plant pathogens (*Gaeumannomyces*, *Fusarium* spp., etc.). A *B. velezensis* strain was selected for the treatment of spent mushroom compost as well as for tomato plant growth experiments. The dry matter content of tomato plants grown in *Bacillus*-treated recompost material and untreated recompost material ranged from 8.94 to 10.0 %, while plants without *Bacillus*-treatment as well as control plants grown in potting garden soil ranged from 5.41 to 14.56 %. The total chlorophyll content of plants grown in media prepared from treated and untreated recompost varied from 2027.32 to 1730.53 µg/g for treated, and from 1388.44 to 590.01 µg/g for untreated compost. We also determined the photosynthetic parameters of the plants such as Fv/Fm and YII. Based on

the measured photosynthetic paratmeters and chlorophyll content there were no differences between the treatments. Ascorbate content was different in each treatment and was higher in potting soil.

Acknowledgements: Supported by Hungarian Ministry for Innovation and Technology (2020-1.1.2-PIACI-KFI-2020-00111) and by grant ÚNKP-21-4 (New National Excellence Program) to H.A.

IDENTIFICATION OF "PIGGY-BACK IMPORT" MECHANISM DURING THE PEROXISOMAL TRANSLOCATION OF A VITAMIN B3-DEGRADING ENZYME

JUDIT ÁMON, GABRIELLA VARGA, CSABA VÁGVÖLGYI, ZSUZSANNA HAMARI

Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

According to our recent results, the catabolic pathway of nicotinic acid (also known as vitamin B3) in eukaryotes differs from catabolic routes discovered in prokaryotes. The pathway in *Aspergillus nidulans* includes seven enzymes (Hxn/S/X/V/T/W/M and HxnN), which consequtively converts nicotinic acid to 6-hydroxynicotinic acid, 2,5-dihydroxypyridine, 2,3,6-trihydroxypyridine, 5,6-dihydroxypiperidine-2-one, 3-hydroxypiperidine-2,6-dione, alpha-hydroxyglutaramate and alpha-hydroxyglutarate, respectively. We showed that conversion of 6-hydroxynicotinic acid to 2,5-dihydroxypyridine by the 6-hydroxynicotinic acid monooxygenase HxnX is taken place in the peroxisomes. HxnX is translocated to the peroxisomes by having a canonical peroxisomal targeting signal 1 (PTS-1) on its carboxy-terminus. The other enzymes of the pathway do not possess any localization signals, henceforth we hypothesized that the rest of the pathway reactions occur in the cytoplasm. Remarkably, the Gfp-HxnW fusion protein showed peroxisomal localization. We hypothesised that the translocation of HxnW depends on HxnX. To test our hypothesis we checked the Gfp-HxnW localization in *hxnX*Δ background and we found that the peroxisomal localization is lost. To further support our hypothesis, we carried out a bimolecular fluorescence complementation (BiFC) analyses by which we confirmed that physical interaction is established between HxnW and HxnX. Here we show the BiFC results. By these localization studies we prove that HxnW enters the peroxisomes by piggy-backing of HxnX. We also propose that the piggy-backing co-translocation mechanism is not only a tool for HxnW to enter the peroxisomes but also a simple way to regulate the stoichiometric balance of HxnX and HxnW in the peroxisomes in order to keep the efficacy of the catabolic process optimal.

Acknowledgements: Supported by the Hungarian Government and the European Union within the frames of the Széchenyi 2020 Programme through grant GINOP-2.3.2-15-2016-00035 and by the NRDI Office (NKFI-K16 119516). The infrastructural background was established with the support of GINOP-2.3.3-15-2016-00006 grant (Széchenyi 2020 Programme).

SYNTHETIC THREE-SPECIES ALLOTETRAPLOID *SACCHAROMYCES* WINE YEAST HYBRIDS WITH COMPLETE EUPLOID PARENTAL SUBGENOMES

ZSUZSANNA ANTUNOVICS[1], ADRIENN SZABÓ[1], LINA HEISTINGER[2], DIETHARD MATTANOVICH[2], MÁTYÁS SIPICZKI[1]

[1]Department of Genetics and Applied Microbiology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary; [2]Department of Biotechnology, Institute of Microbiology and Microbial Biotechnology, University of Natural Resources and Life Sciences Vienna (BOKU), Vienna, Austria

The genus *Saccharomyces* comprises eight „natural species": *S. arboricola*, *S. cerevisiae*, *S. eubayanus*, *S. jurei*, *S. kudriavzevii*, *S. mikatae*, *S. paradoxus*, *S. uvarum* [1] and many strains of chimeric genomes that are called „interspecies hybrids". Two groups of the chimeric, mostly brewing strains the so-called „hybrid species" *S. bayanus* and *S. pastorianus* [2]. Our research group also isolated some natural chimera strains from Tokaj winery region. These strains were sterile, did not form spores. Allodiploid sterility is mainly due to the failure of the chromosomes of the subgenomes to pair in meiosis I [3] which results in the abruption of the meiotic process ("first sterility barrier"). Genome duplication also occurs in yeast interspecies hybrids [4, 5] and the resulting allotetraploid hybrids also produce viable allodiploid gametes. Their viability is frequently misinterpreted as the breach of the sterility barrier by genome duplication [6]. The two sterility barriers (the double sterility barrier) ensure the reproductive (biological) isolation of the *Saccharomyces* species. Due to the second sterility barrier, which has no counterpart in plants, the interspecies *Saccharomyces* hybrids remain sterile even upon whole-genome duplication. Our recent study shows a non-GMO strategy to gain two-species 10-1653 *S. uvarum*- 10-1653 *S. kudriawzevii* („*kudvarum*") ura3 hybrids are crossed with *S. cerevisiae* leu2 resulting „*cekudvarum*" tri-species prototrophic hybrids. FACS analysis determined 2C and 4C amounts of DNA in the „*kudvarum*" and „*cekudvarum*" hybrids, respectively. Since the *S. kudriavzevii* and *S. uvarum* strains were stable heterothallic haploids and the *S. cerevisiae* was diploid, we inferred from the FACS results that the „*kudvarum*" hybrids had allodiploid genomes and the „*cekudvarum*" hybrids had allotetraploid genomes. In the electrophoretic karyotypes, the hybrids had equivalents of all chromosomal bands of the parents. Furthermore to identify each chromosome individually, we tested the hybrids for the presence of orthologues of a group of selected genes as chromosome-specific molecular markers that covered all chromosomes of all parental strains. The RFLP analysis of these markers identified complete sets of *S. kudriavzevii* and *S. uvarum* chromosomes in the two-species „*kudvarum*" hybrids, and the three-species „*cekudvarum*"

hybrids also had all *S. cerevisiae* chromosomes. The hybrids usually had parental mitotypes or, less frequently, recombinant mitotypes [5]. In this study the two-species „*kudvarum*" hybrids received their mtDNA from *S. kudriavzevii*. This was then replaced with the mtDNA of *S. cerevisiae* in the three-species „*cekudvarum*" hybrids. In both cases the mitochondrial genome was inherited uniparentally.

[1] Alsammar and Delneri (2020) FEMS Yeast Res 20: foaa013.
[2] Libkind et al (2011) Proc Natl Acad Sci USA 108: 14539.
[3] Ryu et al (1998) Curr Genet 33: 345.
[4] Karanyicz et al (2017) Appl Microbiol Biotechnol 101: 4617
[5] Szabó et al (2020) Front Microbiol 11: 838.
[6] Naseeb et al (2021) Proc Natl Acad Sci USA 118: e2101242118.

STUDY ON THE bZIP-TYPE TRANSCRIPTION FACTORS NapA AND RsmA IN THE REGULATION OF OXIDATIVE STRESS DEFENSE, MITOCHONDRIAL VOLUMETRIC RATIO AND STERIGMATOCYSTIN PRODUCTION OF *ASPERGILLUS NIDULAS*

BERNADETT BÁKÁNY[1, 2, 3], RÉKA ANTAL[1], PÉTER SZENTESI[3], LÁSZLÓ CSERNOCH[3], WEN BING-YIN[4, 5], TIBOR NAGY[6], ÉVA LEITER[1, 7], TAMÁS EMRI[1, 7], NANCY P. KELLER[5, 8], ISTVÁN PÓCSI[1, 7]

[1]Department of Molecular Biotechnology and Microbiology, Faculty of Science and Technology; [2]Doctoral School of Molecular Medicine; [3]Institute of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; [4]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; [5]Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, USA; [6]Department of Applied Chemistry; [7]ELRN-UD Fungal Stress Biology Research Group, Faculty of Science and Technology, University of Debrecen; Debrecen, Hungary; [8]Department of Bacteriology, University of Wisconsin, Madison, USA

Basic leucine zipper (bZIP) transcription factors play a crucial role in environmental stress response of eukaryotes. In this work, we studied the effect of gene manipulations, including both deletion and overexpression of two selected bZIP transcription factors, NapA and RsmA in the superoxide and catalase production, sterigmatocystin formation, mitochondrial morphology and hyphal diameter of *Aspergillus nidulans*. We found that NapA was crucial in the neutralization of oxidative stress by negatively regulating intracellular reactive species production and positively modulating catalase activities, meanwhile RsmA affected catalase activities slightly negatively. Concerning sterigmatocystin production, the highest concentration was determined in the *ΔrsmAΔnapA* double deletion mutant but increased sterigmatocystin level was also found in the OErsmAOEnapA strain. Our results indicate that NapA coordinated sterigmatocystin production via regulating oxidative species level while RsmA modulated toxin production independently of the redox regulation of the cells. Our further studies using laser scanning microscopy confirmed that the mitochondrial volumetric ratio was negatively correlated with *napA* gene expression. The gene expression of *rsmA* was positively influenced the relative superoxide ratio in the second hyphal segment. In addition, the expression of *rsmA* had negative impact on catalase production and mitochondrial volumetric ratio according to statistical analyses. Neither genetic manipulation of *napA* or *rsmA* genes nor 0.2 mM tBOOH treatment affected hyphal diameter. Taking into account the complex regulatory network of NapA and RsmA on the oxidative stress response, mitochondrial volumetric ratio and secondary metabolite production of *A. nidulans* as well as their observed influence on each other's expressions we can assume that NapA and RsmA may interact with each other either genetically or even physically.

INVESTIGATION OF THE STRUCTURE-ACTIVITY RELATIONSHIPS OF PEPTAIBOLS FROM TWO *TRICHODERMA ROSSICUM* STRAINS

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

Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Until now, more than 375 species from the genus Trichoderma have been described. Due to the potential application of several representatives of the genus in biocontrol of phytopathogenic microorganisms, and the plant growth-promoting effects of *Trichoderma*, further attention has been drawn to the newly described species. Numerous *Trichoderma* strains have a significant role in biotechnology and agriculture in consequence of the production of secondary metabolites, to which the largest group of peptaibiotics, the peptaibols also belong. They are synthesized by non-ribosomal peptide synthetases (NRPSs) which have modular structures, thereby the peptaibols are characterized by a high degree of amino acid variability in their sequences. In our study, purified peptaibol extracts from two strains of *Trichoderma rossicum* (TUCIM 3235 and TUCIM 889) were tested against commonly known eleven Gram-negative and Gram-positive bacterial strains. Their minimal inhibitory concentration (MIC, mg ml^{-1}) values were determined. The two strains produce sequences with similar amino acid composition but different lengths. For a deeper insight into their structures, modern molecular modeling techniques such as accelerated molecular dynamics (aMD) were used to uncover their folding processes. The aMD simulations can improve the knowledge of correlational relationships between conformation and bioactivity. For structure-activity relationships (SARs), the peptaibol

sequences were modelled using the aMD simulation technique and compared with the MIC-test results to correlate folded peptaibol dynamics affected by their amino-acid content and sequence length to their expressed bioactivity. The established relationships between structural characteristics and bioactivity will lead to effective selection of peptaibiotic intervention potentially applied for plant disease management.

POSACONAZOLE IN VITRO AND IN VIVO EFFICACY AGAINST FOUR *CANDIDA AURIS* CLADES

DÁVID BALÁZSI[1], LAJOS FORGÁCS[1], ANDREW M. BORMAN[2], RENÁTÓ KOVÁCS[1], FRUZSINA KOVÁCS[1], NOÉMI BALLA[1], FRUZSINA NAGY[1], ZOLTÁN TÓTH[1], BALÁZS BENCE[1], LÁSZLÓ MAJOROS[1]

[1]Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; [2]National Mycology Reference Laboratory, Bristol, UK

Candida auris is a multidrug-resistant fungus. In most of the cases the echinocandins are the first line drugs in case of invasive *C. auris* infections. However, triazoles also prove to be effective. We compared daily 6 mg/kg of posaconazole efficacy in a neutropenic murine bloodstream infection model against 10 isolates representing four *C. auris* clades (South Asian n = 2; East Asian n = 2; South African n = 2; South American n = 4; two of which were of environmental origin). As a result of the time-kill assay, it can be stated that posaconazole is a strong antifungal static agent against *Candida auris*. Five days of posaconazole treatment significantly increased the survival rates in mice infected with all of the isolates. The most effective was against the East-Asian clade and the least effective was against the isolates derived from Israel (South American clade). Posaconazole treatment regardless of clades decreased the fungal burden in mice by four orders of magnitude in the kidney and the heart ($P<0.001$) and one order of magnitude in the brain ($P<0.001$). The histopathological examination in posaconazole-treated mice confirmed our results revealing small aggregates of yeast cells in the kidneys and hearts, and in the cerebra and cerebelli. Our data showed that regardless of lethality and fungal tissue burden experiments posaconazole is highly effective in a neutropenic murine model against the four major *C. auris* clades.

BACTERIOPHAGE-MEDIATED BIOCONTROL AGAINST *XANTHOMONAS ARBORICOLA* PV. *JUGLANDIS*

DOMINIKA BALI[1], CSENGE GÁL[2], SARSHAD KODERI VALAPPIL[3], SZILVIA PAPP[2], GÁBOR RÁKHELY[3], TAMÁS KOVÁCS[1, 2]

[1]Biopesticide Ltd.; [2]Enviroinvest Corp., Pécs; [3]Department of Biotechnology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

The genus *Xanthomonas* includes a wide range of plant pathogens varying in host specificity and host interaction. Our research focuses on the walnut blight caused by *Xanthomonas arboricola* pv. *juglandis* (Xaj), one of the most frequent infective diseases of walnut, resulting in serious economic losses. Nowadays, the effectiveness of methods of protection against Xanthomonads infections does not exceed 70%. Currently, copper spray, dithiocarbonate, is most often used to stop the disease. A new European Union law prohibits the routine use of antibiotics in agriculture from 2005, so antibiotics cannot be a temporary solution either. Bacteriophage-based biocontrol can be a promising solution to stop and/or prevent *Xanthomonas* bacteriosis. Importantly, bacteriophages can be used in bioproduction. Ten novel bacteriophages were isolated from soil samples taken under infected walnut trees. We have collected 35 samples from Hungary, and 5 samples from Transylvania. Six isolated polyvalent bacteriophages were chosen for further characterization including their morphological, physiological and genomic analyses. Plaque morphology showed clear plaques referring to a possible strict lytic lifecycle. Transmission electron microscope tests were also performed for morphological characterization. A possible strict lytic lifecycle was also confirmed by genome sequencing, where complete genomes of the novel bacteriophages were determined. We performed multimode reader examinations, so we present also efficacy study results tested on ten Xaj strains. All tested bacteriophages were effective against the tested host bacterium, which could be strengthened when bacteriophages were used in a cocktail. As a result of the host specificity tests, we could conclude that the examined bacteriophages belonged to the broad host specificity group. A significant reduction in symptoms was observed on different *Juglans regia* varieties following phage application compared to the control in a field trial using a bacteriophage cocktail containing six bacteriophages. Based on our results, bacteriophage-based biopesticides can provide an effective tool for the biocontrol of *X. arboricola* pv. *juglandis*.

THE EFFECT OF CASPOFUNGIN IN COMBINATION WITH POSACONAZOLE AGAINST ECHINOCANDIN SUSCEPTIBLE AND RESISTANT *CANDIDA AURIS* ISOLATES

NOÉMI BALLA[1, 2], FRUZSINA KOVÁCS[1, 2], ZOLTÁN TÓTH[1], ÁGNES JAKAB[1], LÁSZLÓ MAJOROS[1], RENÁTÓ KOVÁCS[1]

[1]Department of Medical Microbiology, Faculty of Medicine; [2]Doctoral School of Pharmaceutical Sciences, Faculty of Pharmacy, University of Debrecen, Debrecen, Hungary

Candida auris is an emerging potentially multi-resistant pathogen causing invasive infections and nosocomial outbreaks worldwide. It has been published more than 90% and 30% of the isolates are resistant to fluconazole and amphotericin B, respectively; furthermore, the resistance to echinocandins is emerging in the isolates in several countries. For example, an alarming 37% resistance rate to caspofungin was reported in a multicenter analysis derived from India. In this study, the in vitro interaction of caspofungin was determined in combination with posaconazole against echinocandin susceptible (FKS wild type) and resistant (FKS mutant) *C. auris* planktonic and sessile isolates. In case of planktonic cells turbidimetry-based chequerboard microdilution was used for susceptibility testing. For one-day-old biofilms, antifungal susceptibility was evaluated using the XTT colorimetric assay-based chequerboard microdilution method. Drug-drug interactions were assessed utilizing the fractional inhibitory concentration indices (FICIs) and fluorescent microscopy. Median planktonic minimum inhibitory concentrations (pMIC) of *C. auris* isolates to caspofungin ranged 0.5–1 mg L^{-1} and 2– >2 mg L^{-1} for echinocandin susceptible and resistant strains, respectively. In case of posaconazole the median pMICs were between 0.06 and >0.25 mg L^{-1}. In case of one-day old biofilms, MIC values (sMIC) were >32 mg L^{-1} regardless of echinocandin susceptible and resistant phenotype. The sMICs for caspofungin and posaconazole in combination showed 8-128-fold and 4–256-fold decreases, respectively. In addition, caspofungin in combination with posaconazole showed synergistic interaction in case of all sessile isolates tested (median FICI range 0.033–0.375), which was further confirmed the synergy volume ranges calculated by Bliss independence model. The results obtained by statistical interaction analyses correlated well with the fluorescent LIVE/DEAD viability assay. LIVE/DEAD viability staining revealed that caspofungin-exposed wild type and FKS-mutant *C. auris* biofilms exhibited increased cell death in the presence of posaconazole compared to untreated biofilms, caspofungin-exposed sessile populations or posaconazole treated biofilms. The present study is the first analysis evaluating the interaction of an echinocandin and posaconazole against *C. auris* biofilms. We have shown that posaconazole is capable of causing a synergy in combination with caspofungin against *C. auris* especially against biofilms. Our results support the simultaneous use of posaconazole and caspofungin for the treatment of *C. auris* related infections.

Acknowledgements: R.K. supported by the Janos Bolyai Research Scholarship of the Hungarian Academy of Sciences. This research was supported by the Hungarian National Research, Development and Innovation Office (NKFIH FK138462).

ERGOSTEROL BIOSYNTHESIS AND AZOLE RESISTANCE IN *MUCOR LUSITANICUS*

KITTI BAUER[1], CSILLA SZEBENYI[1, 2], BENCE RAFAEL[1], SÁNDOR KISS[1], BERNADETT VÁGÓ[1], MÓNIKA VARGA[1], CSABA VÁGVÖLGYI[1], TAMÁS PAPP[1, 2], GÁBOR NAGY[1, 2]

[1]Department of Microbiology, Faculty of Science and Informatics; [2]ELKH-SZTE Pathomechanism of Fungal infections Research Group, University of Szeged, Szeged, Hungary

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. Mucoromycota species are generally resistant to the majority of the routinely used antifungal drugs, such as azoles. The main target of azoles is the lanosterol demethylase (Cyp51/Erg11), 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/Erg11, other enzymes (Erg6, Erg3, Erg11) 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 azole resistance of *M. lusitanicus*. Relative transcript level of 10 ergosterol biosynthesis specific genes (e.g. *erg2*, *erg3*, *erg6*, *erg7*, *erg24*, and *erg25*) was analyzed using quantitative real-time PCR, after posaconazole treatment. The relative transcription level of certain genes significantly increased after posaconazole treatment. Genome of *M. lusitanicus* encodes three sterol C-24 methyltransferase genes (*erg6a*, *erg6b* and *erg6c*), which catalyzes the conversion of zymosterol to fecosterol and it plays a role in the alternative ergosterol biosynthesis pathway in yeast. Furthermore, 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, sterol 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. An in vivo survival test was performed in *Galleria mellonella* and the virulence of MS12-*Δerg6a* strain significantly decreased. No changes were observed in MS12-Δ*erg6b* and MS12-Δ*erg6c* compared to the control strain under the previously tested conditions. The sterol content in the mutant strains was analysed by LC-MS. Ergosterol, zymosterol and eburicol levels were decreased in MS12-*Δerg6a* strain. Our results suggested that Erg6 play important role in the main ergosterol biosynthesis pathway and in the alternative pathway as well. However, in MS12-*Δerg6a* strain the 7-dehydrodesmosterol content significantly increased. We assumed that the lack of erg6a activates a second alternative biosynthetic pathway from zimosterol to 7-dehydrodesmosterol.

INVESTIGATION OF THE GENETIC DIVERSITY OF *BOTRYTIS CINEREA* POPULATION IN TOKAJ HEGYALJA USING RAPD METHOD

ÁGNES BELÁK, MÓNIKA KOVÁCS, ANDREA TACZMAN-BRÜCKNER, ANDREA POMÁZI

Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary

Botrytis cinerea is a widespread plant pathogenic fungus with a very broad host spectrum. In case of grapes, it is known not only as a plant pathogen that causes serious damage, but also as a filamentous fungus that plays a significant role in making botrytised wine, but noble rot only occurs under certain climatic conditions. Several studies emphasize the significant variability within *B. cinerea* populations, therefore our aim was to investigate to what extent the random amplified polymorphic DNA (RAPD) method can be used to detect the genetic diversity of the Tokaj Hegyalja population, and whether among the amplified markers are ones that are characteristic to *B. cinerea* species. In the study, the M13 and fifteen RAPD primers were tested. The *B. cinerea* strains were selected in such a way that they included those from intact grape berries, szamorodni clusters, and aszú berries as well. In addition to *B. cinerea*, the RAPD reaction was also performed with filamentous and yeast strains belonging to other genera. After optimizing the reactions, we were able to produce patterns for 12 of the 16 tested primers, which were well reproducible during the repetitions. The degree of amplification was different depending on the strain, however, the patterns characteristic of each strain could be recognized in the repetitions. The study showed that different genetic variants are present within the population of *B. cinerea* in Tokaj Hegyalja. In case of several primers we observed that some isolates from szamorodni cluster give a different pattern than the other strains. The examination of reproducibility was also important from the point of view of being able to select those RAPD markers that appear consistently during the reactions and may be suitable for diagnostic purposes. In the case of eight of the 16 primers we were able to identify an amplicon or amplicons that were related to *B. cinerea* species. These so-called SCAR (Sequence Characterized Amplified Region) markers can form the basis of the specific detection of *B. cinerea*.

CHARACTERIZATION OF NFAP2 RESISTANCE-EVOLVED *CANDIDA ALBICANS* STRAINS

GÁBOR BENDE[1], NÓRA ZSINDELY[2], LÁSZLÓ BODAI[3], KRISZTIÁN LACZI[1], CSABA PAPP[4], ATTILA FARKAS[5], ZSOLT KRISTÓFFY[1], PETRA KIRÁLDI[1], GÁBOR RÁKHELY[1, 6], LÁSZLÓ GALGÓCZY[1, 7]

[1]Department of Biotechnology; [2]Department of Genetics; [3]Department of Biochemistry and Molecular Biology; [4]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged; [5]Institute of Plant Biology; [6]Institute of Biophysics; [7]Institute of Biochemistry, Biological Research Centre, Eötvös Loránd Research Network, Szeged, Hungary

In recent years the emerging number of (multi)drug-resistant *Candida* isolates has hampered the management of life-threatening candidiases. Therefore, there is an urgent need of fundamentally new and safe antifungal strategies in the anti-*Candida* therapy with minimal risks for resistance development. The *Neosartorya fischeri* antifungal protein 2 (NFAP2) is a promising candidate in this respect. In a microevolution experiment, we already observed that *Candida albicans* is able to adapt (or develop resistance) only to the 1×MIC (minimum inhibitory concentration) of NFAP2 in comparison to the licensed antifungal drug, fluconazole (FLK), whereas this yeast easily can survive even at 32×MIC of FLK. In the present work we characterized the NFAP2-resistant *C. albicans* isolates in comparison with the FLK-resistant one, and the ancestor wild-type strain (CBS 5982). Whole genome sequencing revealed that the FLK-resistant strain carries single nucleotide variations (SNVs) and an insertion in the clathrin heavy chain, and deletions in PTC2 (type 2C protein phosphatase) genes; while the NFAP2-resistant strains carry SNVs in BNI4 (a scaffold protein that tethers chitin synthase III), a multiple nucleotide variation in Pga58p (putative GPI-anchored protein) and eIF4G (eukaryotic translation initiation factor 4 G), and deletions also in *PTC2* genes. NFAP2-uptake experiments indicated decreased internalization by NFAP2-resistant isolates, and as a consequence reduced cell death. Scanning electron microscopy showed altered cell surface of NFAP2- and FLK-resistant isolates, what was more prominent in the presence of NFAP2. Metabolic adaptation experiments revealed that NFAP2- and FLK-resistance developments have fitness cost. The resistance development diminished the virulence of the NFAP2- and FLK-resistant isolates in a *Galleria mellonella* infection model.

Acknowledgements: L.G. was financed by the Hungarian National Research, Development and Innovation Office - NKFIH, FK 134343 project.

MICROBIOLOGICAL AND MULTITOXIN ANALITICAL EXAMINATION OF SMALL GRAIN CEREALS IN HUNGARY, 2020

ATTILA BERÉNYI[1], CSABA VÁGVÖLGYI[2], BEÁTA TÓTH[1]

[1]Cereal Research Non-Profit Ltd.; [2]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

In the last ten years we can find a definite tendency of climate change in Hungary. Now the weather conditions show strong Mediterranean characteristic in the country. These changes initiate the appearance of new pathogens, among them, toxin-producing fungal species, which previously were characteristic for the southern area of Europe. Therefore, it is important to monitor the presence of these species, as well as their toxin production. The aim of our study was the monitoring of cultivated winter wheat species (mainly from the portfolio of the Cereal Research Non-Profit Ltd.). Twenty different winter wheat samples from three different locations (Szeged, Törökszentmiklós and Iregszemcse) have been investigated. Sequence-based molecular method was used for the identification of the fungal species responsible for infection. A new, efficient multitoxin analytical method was developed for the measurement of mycotoxin contamination. This allows the determination of fourteen toxins (e.g., trichotechenes, aflatoxins and fumonisins) in one step. For this method an Agilent 1260 Infinity II HPLC coupled with an Agilent Ultivo QQQ MS system was used. The average internal fungal infection level was 39.6% for the three locations. In case of Szeged the average infestation level was 56.2%: Alternaria species were responsible for almost 75% of this value, however, *Rhizopus* (11.11%) and *Penicillium* (4.32%) species were also present at considerable level. In Törökszentmiklós the average fungal infection level was 39.3%. The *Alternaria* dominance was characteristic also for this location (65.31%), followed by *Cladosporium* (14.29%) and *Stemphylium* (10.2%). The lowest infection level (23.1%) was detected for Iregszemcse, with the values of 56.14%, 19.3%, and 15.79% for *Alternaria*, *Cladosporium* and *Stemphylium*, respectively. *Epicoccum*, *Fusarium* and *Aspergillus* species were found in traces at all the three locations. The measured toxin contamination levels of the experimental samples have not reached the current EU limit values except one case: the detected aflatoxin B1 level (4 µg kg-1) was higher than the EU risk limit (2 µg kg-1) in Szeged. Trichothecenes, which are mainly produced by *Fusarium* species, were responsible for a significant part of the toxin contamination. A Szeged-Törökszentmiklós-Iregszemcse (east-west) order can be established from the point of view of toxin contamination and fungal infection. There have been cases of low toxin contamination with high level of fungal infection, and vice versa, so for maintaining the food safety the toxin contamination must be investigated.

Acknowledgements: Supported by the Doctoral Student Scholarship Program of the Co-operative Doctoral Program of the Ministry of Innovation and Technology financed from the National Research, Development and Innovation Fund.

TRACE METAL IONS IN FUNGAL ORGANIC ACID FERMENTATIONS

VIVIEN BÍRÓ, ALEXANDRA MÁRTON, KATICA KRAMCSÁK, ANDREA KUN, ERZSÉBET FEKETE, MICHEL FLIPPHI, LEVENTE KARAFFA

Department of Biochemical Engineering, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

Organic acid accumulation is probably the best-known example of primary metabolic overflow. Both bacteria and fungi are capable of producing various organic acids in large amounts under certain conditions, but in terms of productivity – and consequently, of commercial importance – fungal platforms are unparalleled. For high product yield, chemical composition of the growth medium is crucial in providing the necessary conditions, of which the concentrations of four of the first-row transition metal elements, manganese (Mn^{2+}), iron (Fe^{2+}), copper (Cu^{2+}) and zinc (Zn^{2+}) stand out. Three of them – Mn, Fe, Cu – provide the necessary redox and catalytic activity for many important biological process-es. They possess a stable +2 oxidation state and can generate many additional stable states, which allows them to change their oxidation states in biological reactions. Manganese concentrations > 5 µg L^{-1} decrease the final yield of citric acid in A. niger and itaconic acid in *A. terreus*, respectively. Various methods have therefore been patented or published to remove the surplus manganese ions from the growth medium, but a more convenient strategy is to counteract their effect. Both for *A. niger* citric- and *A. terreus* itaconic acid fermentations, low product yield in the presence of high Mn-concentrations can be counteracted by increasing the copper concentration in the culture broth. We recently described that the ratio of copper to manganese – rather than their respective absolute concentration – modulates itaconic acid production yield on D-glucose and D-fructose. In this study we demonstrate that the high-affinity Mn^{2+} transport in *A. niger* is inhibited – in addition to copper – also by Zn^{2+} roughly to the same extent.

ALTERNATIVE OXIDASE PARALOGOUS GENES IN ASPERGILLACEAE SPECIES

VIVIEN BÍRÓ, ALEXANDRA MÁRTON, KATICA KRAMCSÁK, ANDREA KUN, ERZSÉBET FEKETE, MICHEL FLIPPHI, LEVENTE KARAFFA

Department of Biochemical Engineering, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

Ubiquinol:oxygen oxidoreductase [non-electrogenic] (EC 1.10.3.11) is a terminal oxidase in branched mitochondrial electron transport. The enzyme transfers electrons from ubiquinol directly to molecular oxygen, releasing the chemical energy

associated with these electrons as heat instead of using that energy to generate proton motive force. This “alternative oxidase” (AOX) thus bypasses the electrogenic cytochromes downstream in the respiratory chain. It contributes to sustained recycling of reduction equivalents (NADH; NADPH; FADH2) into their oxidized forms, dissipating surplus reducing power whilst decreasing oxidative stress, a byproduct of the action of the electrogenic components of the respiratory chain. AOX is near ubiquitous in the fungal kingdom, including in the early divergent Cryptomycota and Microsporidia phyla of unicellular obligate endoparasites. However, AOX is absent from the so-called genetic “model” species *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. By contrast, the presence of multiple aox genes is widespread within the Aspergillaceae family (Ascomycota; Pezizomycotina; Eurotiomycetes; Eurotiales) and amongst its various species of industrial relevance, like *Aspergillus niger*, *A. oryzae*, *A. terreus* and *Penicillium chrysogenum*. Here, we provide indirect evidence for four independent events that resulted in multiple *aox* genes in *Aspergillus* & *Penicillium* taxa. Paralogous genes usually originate from gene duplication and vertical inheritance.
A second path to acquire paralogs is by horizontal transfer of genetic material, including between widely divergent, long separated microbial clades. We present indications for *aox* gene transfer from an *Aspergillus* host to a taxon of biocontrol *Trichoderma* species (Pezizomycotina; Sordariomycetes; Hypocreales; Hypocreaceae). The absence of paralogous aox genes from some taxa within a clade with multiple *aox* genes can generally be attributed to gene loss of the paralog(s) acquired earlier in the lineage. In the Nidulantes subgenus, species of the series Versicolores have 3 *aox* genes while species in other section Nidulantes series (including *A. nidulans*) have one: the original *aoxA* gene is always conserved. Yet species of the sister section Usti have acquired their second, section-specific aox gene independently from those surviving to date in, e.g., *A. sydowii* & *A. versicolor*. *A. calidoustus* (section Usti) has a third resident *aox* gene of a fourth origin, which seems to match with that of the second aox gene found in *A. niger–A. welwitschiae* (section Nigri), despite the evolutionary distance. In both species, this particular *aox* paralogous gene is divergently orientated from a gene encoding a rare paralog of an alternative NADH:ubiquinone oxidoreductase [non-electrogenic] (EC 1.6.5.9) likewise associated with branched electron transport.

Acknowledgements: Supported the Hungarian National Research, Development & Innovation Fund, grant K 138489.

THE PHYSIOLOGICAL EFFECTS OF THE SUPPLEMENTATION AND EXPRESSION OF *ASPERGILLUS NIDULANS gfdB* IN OTHER *ASPERGILLUS* SPP. ARE SPECIES-SPECIFIC

VERONIKA BODNÁR[1], ANITA KIRÁLY[1], ERZSÉBET OROSZ[1], TAMÁS EMRI[1], ZSOLT KARÁNYI[1], ÉVA LEITER[1], RONALD DE VRIES[2], ISTVÁN PÓCSI[1]

[1]Department of Biotechnology and Microbiology, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary; [2]Fungal Physiology, Westerdijk Fungal Biodiversity Institute & Fungal Molecular Physiology, Utrecht University, Utrecht, the Netherlands

The exceptional diversity, productivity, technological applicability, and great endurance of the *Aspergillus* genus are utilized in numerous industrial microbial biotechnological processes. The extreme solute-tolerant *Aspergillus wentii* is one of the most promising industrially relevant moulds applied for industrial enzyme and biodiesel productions. Based on previous hyperosmotic stress studies, osmophility is widespread among the Aspergilli and the growth stimulatory effect of either 2.0 M sorbitol or 1.0 M NaCl was the most significant for *A. wentii* and *A. glaucus*. In these fungi, osmophility was hypothesized to be connected to the lack of the *gfdB* gene, putatively encoding a NAD+-dependent glycerol-3-phosphate dehydrogenase enzyme in many *Aspergillus* spp., including the filamentous fungus model organism *Aspergillus nidulans* [1]. To get more information on the stress biological background of the observed osmophilic phenotypes, in this study we aimed to characterize *A. nidulans gfdB* supplemented *A. wentii* strains to compare the results with previous studies with the *A. glaucus* wild-type and c’ *gfdB* and the *A. nidulans* wild-type and *ΔgfdB* strains. We also characterized the evolution of stress tolerance in selected *Aspergillus* species, and the outcomes of this study supported the original hypothesis of [2] on the possible involvement of GfdB in the appearance of xerophilic/osmophilic phenotypes in these moulds. On the other hand, the insertion of *A. nidulans gfdB* into the genome of *A. wentii* did not improve the environmental stress tolerance of this species, which was demonstrated previously in both wild-type *A. nidulans* and *A. glaucus* c’ *gfdB* strains [3, 4].
These findings warn us that any modification of the Aspergilli's stress response system may cause complex, unpredictable physiological changes that should be taken into account in any future targeted industrial strain development projects aimed at increasing the general stress tolerance of these moulds.

[1] de Vries et al (2017) Genome Biol 18: 28.
[2] Miskei et al (2009) Fungal Genet Biol 46: Suppl 1: S105.
[3] Király et al (2020) *Fungal Biol 124:* 352.
[4] Király et al (2020) *J Basic Microbiol 60:* 691.

ACQUISITION OF ANTIFUNGAL RESISTANCE INFLUENCES VIRULENCE ATTRIBUTES OF *CANDIDA AURIS* MICROEVOLVED STRAINS IN MOUSE SYSTEMIC FUNGAL INFECTION MODEL

FLÓRA BOHNER[1], CSABA PAPP[1], TAMÁS TAKÁCS[1], ANDRÁS SZEKERES[1], MÓNIKA VARGA[1], CSABA VÁGVÖLGYI[1], RENÁTA TÓTH[1], ATTILA GÁCSER[1, 2, 3]

[1]Department of Microbiology; [2]HCEMM-USZ Fungal Pathogens Research Group; [3]MTA-SZTE Lendület Mycobiome Research Group, Faculty of Science, University of Szeged, Szeged, Hungary

In the recent years, *C. auris* has become one of the most recognized human pathogenic fungal species worldwide. Based on prominent phylogenetic differences between *C. auris* isolates, five clades were differentiated, corresponding to the geographical sites of frequent isolation. These clades are usually associated with distinct antifungal susceptibility patterns and specific clinical manifestations. With the exception of Clade II and V, all the other clades were previously linked with invasive nosocomial fungal infection outbreaks, especially in the ICU setting. Outbreak associated isolates are also often non-susceptible to commonly applied antifungal drugs such as fluconazole. The occurrence of multidrug resistance (generally triazole and polyene resistance) is also quite prevalent (approx. 40%). PDR (pan drug resistant) isolates have also been identified, which is another signature of this species. Several antifungal resistant strains were generated from two (0381, 0387) independent triazole susceptible isolates using the in vitro microevolution method. The effects of the acquired resistance were further examined by comparing the generated strains to their originating clinical isolate. Collected data implies that acquisition of triazole resistance potentially alters sterol content and cell wall composition of *C. auris* strains, that effects the fitness of the fungal cells under host modeling conditions. For instance, 0381 POSevo strain showed a severe growth defect in the presence of cell wall perturbants and membrane detergents, while 0387 derived stains tolerated the presence of caffeine and congo red better than the wild type isolate. Based on intravenous murine infection model, in vitro triazole resistance also altered the virulence of 0381 and 0387 originated strains in an isolate dependent manner. The fungal burden (especially in the brain) slightly increased when BALB/c mice was challenged with 0381 originated strains, while 0387 derived strains showed attenuated virulence in systemic candidiasis model. This indicates that in *C. auris*, stress-related fitness loss linked to the development of antifungal resistance does not necessary correlate with the pathogenic potential of the strains. Resistant strains were also sequenced and compared to their susceptible isolate. 0381 FLUevo and VORevo strains harbored an SNP in *TAC1b* that corresponded with increased CDR1 efflux pump expression. Furthermore, all 0387 originated evolved strain harbored the same loss of function (LOF) mutation in the '*B9J08_002818*' gene. In *C. albicans*, the orthologous gene (*BCY1*) is responsible for coding the regulatory subunit of the PKA kinase, suggesting that it has a key role in the fungal cAMP/PKA pathway. This data suggests that the cAMP/PKA pathway might be involved in the development of antifungal resistance in *C. auris*.

DETAILED ECOPHYSIOLOGICAL CHARACTERIZATION OF *BACILLUS LICHENIFORMIS* STRAINS ISOLATED FROM SWEET POTATO FOR THE DEVELOPMENT OF A FOLIAR TREATMENT FORMULATION

ÁDÁM BORDÉ[1], HENRIETTA ALLAGA[1], RAMTEKE W. PRAMOD[2], TAMÁS MONOSTORI[1], CSABA VÁGVÖLGYI[1]

[1]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary; [2]Department of Biotechnology, Dr. Ambedkar College, Deekshbhoomi, Nagpur, India

Agriculture requires efficient technologies which control the pests of cultivated plants. A green and environmentally friendly approach is biological control which could effectively reduce or mitigate pests and/or their effects. Development an efficient microorganisms-based product requires the careful selection and detailed preliminary characterization of the microorganisms that make up this preparation. During our research targeting the development of a foliar treatment formulation we carried out detailed ecophysiological studies on eight *Bacillus licheniformis* strains isolated from sweet potato, which included the examination of the temperature and pH dependence, the salt tolerance of these strains, as well as the activity of four extracellular enzymes (lipase, protease, chitinase, cellulase). Furthermore, we also measured the production of indoleacetic-acid, siderophore, ammonia and phosphorus solubilization capacity of these strains. Furthermore, we are currently investigating the antagonistic effect of *B. licheniformis* strains against plant phytopathogens in in vitro antagonism tests, with a focus on sweet potato pathogens. In addition, we are also conducting a field test to examine the effect of the three most promising strains (SZMC 27713, SZMC 27714, and SZMC 27715) on yield in sweet potato. Our long-term goal is to develop a foliar treatment formulation with high levels of depsipeptide content, stabilized by chitosan nanoparticles, which will be applicable to a wide range of agricultural and horticultural crops.

Acknowledgements: Supported by the Hungary-Serbia CBC Programme (PLANTSVITA; HUSRB/1602/41/0031).

IN VITRO TRANSCRIPTOME LEVEL INTERACTIONS BETWEEN MYCOPARASITIC *TRICHODERMA ATROVIRIDE* AND HAPLOID *ARMILLARIA OSTOYAE* UNCOVERED BY DUAL RNA-SEQ PROFILING

LIQIONG CHEN[1], SIMANG CHAMPRAMARY[1, 2], NEHA SAHU[3], BORIS INDIC[2], ATTILA SZŰCS[1], GÁBOR NAGY[1], GERGELY MARÓTI[4, 5], BERNADETT PAP[5], CSABA VÁGVÖLGYI[1], LÁSZLÓ NAGY[3, 6], GYÖRGY SIPOS[2], LÁSZLÓ KREDICS[1]

[1]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged; [2]Functional Genomics and Bioinformatics Group, Institute of Forest and Natural Resource Management, Faculty of Forestry, University of Sopron, Sopron; [3]Synthetic and Systems Biology Unit, Biological Research Center, Eötvös Loránd Research Network, Szeged; [4]SeqOmics Biotechnology Ltd, Mórahalom; [5]Institute of Plant Biology, Biological Research Center, Eötvös Loránd Research Network, Szeged; [6]Department of Plant Anatomy, Institute of Biology, ELTE-Eötvös Loránd University, Budapest, Hungary

A destructive forest pathogenic member of the genus *Armillaria*, *A. ostoyae* is known to cause root rot diseases on woody plants worldwide. Efficient control measures limiting the growth and impact of this severe underground pathogen are currently under extensive investigation. Our previous results revealed that a soilborne fungal isolate, *Trichoderma atroviride* strain SZMC 24276, exhibited high antagonistic efficacy, suggesting its potential to be utilized as a potential biocontrol agent. In vitro dual culture assay results indicated that the haploid A. ostoyae strain SZMC 23093 is highly susceptible to the mycelial attack of *T. atroviride* SZMC 24276, offering the opportunity to study the genetic background of the *Trichoderma* antagonistic effect and the Armillaria defense mechanisms. The present study analyzed the transcriptome of *A. ostoyae* SZMC 23093 and *T. atroviride* SZMC 24276 in vitro with dual culture assays, using high-throughput next-generation sequencing technology. The study conducted time-course analysis, functional annotation, and analysis of differentially expressed genes including antagonism-related candidate genes from TA and defense-related ones from *A. ostoyae*. The transcriptome data revealed that the multiple biocontrol mechanisms of *T. atroviride* were activated even before physical contact of the interacting fungi. These included the transcriptional activation of genes involved in the production of antibiotic secondary metabolites and carbohydrate-active enzymes (CAZys). The early transcriptional impact of *T. atroviride* on *A. ostoyae* before mycelial contact is suggested to induce multiple defense reactions such as the production of quinolinic acid, a metabolite with antifungal properties. The CAZy expression profile of *T. atroviride* narrowed down mainly to fungal cell wall degrading enzymes with important roles in mycoparasitism (β-glucanases, chitinases) after physical contact, while further defense strategies are supposed to be deployed by *A. ostoyae*, such as the overexpression of peptidases probably involved in detoxification. However, in spite of the multiple defense mechanisms of *A. ostoyae*, the interaction ended with *Trichoderma* overgrowing and killing *Armillaria*, indicated by the entire lack of *Armillaria* transcripts in the post-mycoparasitic stage of the interaction.

Acknowledgements: Supported by the Hungarian Government and the European Union (GINOP-2.3.2-15-2016-00052).

INVESTIGATING THE DIVERSITY OF ENRICHMENT MICROBIAL COMMUNITIES DEGRADING IBUPROFEN OR DICLOFENAC

ANDREA CSÉPÁNYI[1], ANNA BEDICS[1], MÁRTON PÁPAI[1], ABDULLAH JAWDAT[1], TIBOR BENDEK[1], BALÁZS KRISZT[2], ANDRÁS TÁNCSICS[1]

[1]Department of Molecular Ecology; [2]Department of Environmental Safety, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

In last decades pharmaceuticals have become one of the most frequent micro-contaminants of wastewater treatment plants (WWTPs) and have emerged also in surface waters and mostly every level of aquatic system. The most represented group of newly reported pharmaceuticals are the non-steroidal anti-inflammatory drugs (NSAIDs) e.g. ibuprofen (IBU) and diclofenac (DIC). The high consumption values of these pharmaceuticals together with the limited and uncontrolled elimination rate of these compounds in WWTPs have caused their continous presence in aquatic enviroments, which lead to long-term exposure to wild-living organism and might induce harmful responses. For these reasons it is important to discover new perspectives of enhancing wastewater treatment processes e.g. the biodegradation, which utilize decontaminating properties of bacteria. Our idea was to look for potential degraders in the microbial community of an aromatic hydrocarbon contaminated environment. To achive this goal aerobic enrichment cultures were set up by using groundwater sample of a deeply studied Hungarian aromatic hydrocarbon contaminated site. In the triplicate enrichments IBU or DIC was applied as sole carbon and energy source at a concentration of 100 mg L-1. Diversity of enrichment communities was analized by terminal restriction fragment length polymorphism (T-RFLP) in every month and finally (after 3 months) by Illumina 16S rRNA gene amplicon sequencing. In the IBU-degrading enrichments the two most dominant classes were Alphaproteobacteria (54.9%) and Gammaproteobacteria (38.5%). The most abundant community members belonged to the genera *Pseudomonas* (36.5%), *Hyphomicrobium* (22.9%), *Azospirillum* (13.4%), *Rhizobium* (7.1%) and *Sphingomonas* (2%). In the case of the DIC-degrading enrichment cultures the most dominant class was the Gammaproteobacteria (85.5%) with high abundance of the genus *Pseudomonas* (73.1%), followed by Alphaproteobacteria (13.7%) represented mainly by the genera *Hyphomicrobium* (3.4%), *Nitrobacter* (3.3%) and

Azospirillum (2.8%). Furthermore, after three consecutive subcultivations, eight pure bacterial strains were isolated from the enrichments, which may lead us to find new IBU- and DIC-degrading bacteria, which can be the basis of an effective biodegradation technique in the future.

INVESTIGATION OF LOW TEMPERATURE HEAT TREATMENT AND UV IRRADIATION OF DIFFERENT CULTIVATED MUSHROOMS

CSABA CSUTORÁS[1], ANNA JUHÁSZ[1], NÓRA BAKOS-BARCZI[2], JÓZSEF RÁCZ[2]

[1]Institute of Food Science, Károly Eszterházy Catholic University; [2]Magyar Gomba Kertész Ltd., Demjén, Hungary

Magyar Gomba Kertész Ltd. is one of the most significant mushroom producers in Hungary: not only white button (champignon) mushroom (*Agaricus bisporus*) but also oyster mushroom (*Pleurotus ostreatus*) and some exotic mushrooms (e.g., shiitake, *Lentinula edodes*; *P. eringii*, Eringii sp.) produced. Our modern compost plant produces compost not only for our own mushroom houses, but substantial amount of compost sold in domestic and export markets. As part of our recent R&D activities we develop new methodologies aiming at the production of new functional food products from various mushrooms. Such approach could be the application of low temperature heat treatment of foods (sous-vide technology) as a potential way for the production of high-value raw materials of mushroom-based food products. We investigated the effect of heat treatment and UV irradiation on the protein, vitamin D and ergosterol content of white button and shitake mushrooms. Vitamin D and ergosterol content of mushrooms can be influenced by heat treatment and/or UV irradiation. There is a complex isomerization equilibrium between the different steroid structures. Previtamin D plays an outstanding role in the equilibrium, besides vitamin D other products can be formed like tachisterol, lumisterol and provitamin D. We managed to develop an optimal treatment of mushrooms for highest vitamin D and ergosterol content.

AN ALTERNATIVE MOLD REMEDIATION STRATEGY AGAINST INDOOR MOLDS OF CHURCH OF KÉZDIALBIS

KINGA DÁN[1], LILIÁNA TÓTH[1], GÁBOR BENDE[1], GÁBOR RÁKHELY[1, 2], LÁSZLÓ GALGÓCZY[1, 3]

[1]Department of Biotechnology, Faculty of Science and Informatics, University of Szeged; [2]Institute of Biophysics; [3]Institute of Biochemistry, Biological Research Centre, Eötvös Loránd Research Network, Szeged, Hungary

Deteriorative indoor molds of cultural heritage buildings cause a significant problem in the conservation. The recently applied anti-mold remediation methods can cause further damages, especially to murals, painted statues, and wooden furniture. Therefore, there is a need for new and effective mold control and remediation strategies in the conservation of cultural heritage buildings. The topical application of aqueous solution of the antifungal proteins from *Neosartorya fischeri* (NFAP, NFAP2) can provide an alternative solution for this problem. To support this hypothesis, we collected indoor mold samples from the cultural heritage Calvinistic church of Kézdialbis (Transylvania, Romania). The collected samples were identified by macro- and micromorphology, and molecular markers (ITS4 and RPB2). Based on these, we identified indoor molds belonging to the genera *Aspergillus*, *Cladosporium*, and *Beauveria*. We investigate the in vitro antifungal efficacy of NFAP and NFAP2 against these isolates in a broth microdilution susceptibility test. Both proteins were able to inhibit the growth of the isolated fungi at different minimum inhibitory concentrations.

Acknowledgements: K.D. holds a Székely Forerunner Fellowship from the Forerunner Federation USA. L.G. was financed by the Hungarian National Research, Development and Innovation Office - NKFIH, FK 134343 project.

INVESTIGATION OF THE MICROBIAL TERROIR OF MÁD WINE REGION: HOW SOIL MICROBIOTA MODULATE REGIONAL DISTINCTIVENESS AND THE MUST CONTENT

PÉTER DÁVID[1], ANNA ANITA SZILÁGYI-RÁCZ[1], PÉTER FAUSZT[1], GÁBOR FIDLER[1], ZOLTÁN KÁLLAI[2], ATTILA CSABA DOBOS[3], LÁSZLÓ CSIGE[4], LÁSZLÓ STÜNDL[1], JUDIT REMENYIK[1], MELINDA PAHOLCSEK[1]

[1]Institute of Food Technology, Faculty of Agriculture, Food Science and Environmental Management; [2]Department of Genetics and Applied Microbiology, Faculty of Science and Technology; [3]Centre for Precision Farming R&D Services, Faculty of Agriculture, Food Science and Environmental Management, Debrecen; [4]Mád Wine Academy, University of Debrecen, Mád, Hungary

Soil microbiota plays a critical role in the production of high-quality terroir wines by transmitting microbiota colonizing grape surfaces. Metabolites produced by the billions of these microbes are the main contributors to the minerality and fragrance of wine. The world heritage Tokaj-Hegyalja (Tokaj-foothills) region is famous of its high terroir expression. The topography and

the orientation of the vineyard, geology, the composition of the ground and the climatology basically define the soil microbiom of the vineyard-plot. The soil microbiome is responsive to biotic and abiotic changes. According to our theory, the soil microbiome is a complex biological system whose composition bears the imprints made by the combined effect of all these factors. We explored the terroir of the Mád Wine Region which is a historical wine region located in northeastern Hungary. One prior aim of this study was to investigate the soil microbiota of Tokaj-Hegyalja region in Mád. Culture independent next-generation sequencing was applied to decipher the vitivinicultural terroir of Tokaj-Hegyalja region. We explored in detail the 100% core soil microbiota. Shot-gun sequencing allows us to know what genes are present to infer the functional pathways (supporting biofunctions such as mobilization of absorbable nutrients, plant growth stimulation, production of phytohormones, cellulose-degradation, suitable soil structure, biocontrol, antimicrobial peptide and siderophore production) that are encoded. Furthermore, network analyses were performed to focus more on the connections between the microbial community members. Dissimilarity-based networks were generated to visualize the changes in complex microbial relationships. To uncover the dynamic changes in interactions between microbial species over time (veraison vs. harvest) dissimilarity-based networks were also made. It is still questionable what correlations can be demonstrated between the soil microbiome and the microbes and the flavonoids of the must. Another central objective of this study was to investigate the correlation between must flavonoid components and the soil fungal and bacterial microbiota. According to flavonoid content we managed to rank the must samples is four distinct categories. On the basis of these four must clusters descriptive microbial barcodes were made on the basis of matching soil microbiota. In conclusion this study was made to establish links between topography, climate, soil composition and vineyard microbial community composition, biodiversity, metabolic profiles, and soil resistome load.

LABORATORY DIAGNOSTICS OF MONKEYPOX VIRUS IN HUNGARY

DÁNIEL DÉRI[1, 2], JUDIT HENCZKÓ[1, 3], GYULA ZSIDEI[1], LILI JÁRMI[1], PETRONELLA OLÁH[1], KATALIN TARCSAI[4], ERZSÉBET BARCSAY[4], ZOLTÁN KIS[1], BERNADETT PÁLYI[1]

[1]National Biosafety Laboratory, National Public Health Center; [2]Doctoral School of Biology, Faculty of Science, Eötvös Loránd University; [3]Doctoral School of Pathological Sciences, Semmelweis University; [4]Department of Virology, National Public Health Center, Budapest, Hungary

In May of 2022 several cases of Monkeypox virus (MPXV) infections were reported from multiple European countries, where the virus is otherwise not endemic. Since early May to 12 August, more than 31.000 confirmed cases have been reported worldwide from 89 countries. Primary risk group is formed by men who have sex with men (MSM), since the virus circulates mostly amongst them, but women and children cases have been reported as well. In Hungary the laboratory diagnostics of MPXV is carried out by National Biosafety Laboratory (NBL) of National Public Health Center (NPHC), Budapest. Several types of clinical samples are tested from each patient using a multiplex real-time quantitative polymerase chain reaction (qPCR) with primers detecting the DNA of Orthopoxviruses (OPXV) and specifically MPXV. Besides qPCR, whole genome sequencing (WGS) on Illumina and Nanopore platforms using metagenomics methods, serological tests and virus isolation on cell culture are also applied. In case of some positive patient, sequential sampling is performed in order to monitor the progression of infection, activation of immune responses, and possible genetic changes in virus genome. By 12 August of 2022, of 166 patients examined, 61 tested positive for MPXV (37 %). Besides skin lesions throughout the body, specific viral DNA were detectable from pharyngeal, anogenital, urine, serum, plasma and semen samples as well. Highest viral loads – even 5.92E+08 copies mL^{-1} sample - can be observed in skin lesions and rectal samples (64% and 24% from the 100 samples with highest load, respectively). The results from WGS show us that all of the 114 successfully sequenced samples (mostly complete whole genomes) from Hungary belongs to the West African clade of MPXV. In serological aspect, increased antibody level (1:80<="" p="" style="box-sizing: border-box;"> According to the given data, in terms of both qPCR and WGS the most relevant sample types are the skin lesions and rectal specimens, the correlation between higher viral load and clear, higher quality whole genomes is undeniable. Viral shedding with multiple bodyfluids is suggested due the positive results of other sample types. Interestingly MPXV DNA was detectable in case of a semen sample 40 days from symptom onset, although the presence of infective virions is questionable.
The success of virus isolation on cell culture is also dependent from sample type, as skin lesions proved to be the best. In conclusion, the data from the Hungarian MPXV cases are in a good correlation with international observations. However raising public awareness in risk groups is required, due to expected further increase in positive cases.

INTERACTION OF PROTOTYPE AND A45S VARIANT HPV-11 E7 PROTEINS WITH TUMOR SUPPRESSORS

ZSOLT BARNABÁS ÉLES, LEILA RAHMANI, JÓZSEF KÓNYA, ANITA SZALMÁS

Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

The mucosa-associated types of human papillomaviruses (HPV) - which are currently among one of the most common sexually transmitted pathogens - can contribute as a leading etiological agents to the development of both benign and malignant lesions of the anogenital tract and the head-neck region. The viral E7 oncoprotein is essential in the life-cycle and pathomechanism of HPV, which can change the stability and function of many cellular proteins. Among these interacting partners, we can find both the retinoblastoma-protein (pRB) and a recently discovered interacting partner, the PTPN14 cytoplasmic tyrosine phosphatase. PTPN14 can affect cell adhesion, cell proliferation and cell migration, thus, PTPN14 could be an important factor in natural life-cycle of HPV and also in the transformation of infected epithelial cells. Our current experiments are based on the fact that nucleotide polymorphisms of genomic regions encoding HPV proteins can contribute to changes in certain functions of viral proteins. Worldwide, the majority of HPV-11 positive clinical isolates represent the A2 subtype of HPV-11. This subtype characterized by an SNP in the E7 ORF region, which leads to an A45S amino-acid substitution in the E7 protein sequence compared to the reference HPV-11 genome. The goal of this study was to carry out a functional comparative study of the HPV-11 A45S E7 and the reference E7 proteins in relation to their interactions with pRB and PTPN14. First of all, specific primers with the appropriate mutation were designed and used for the construction of pCMV plasmid vectors expressing A45S E7 protein. For this site-directed mutagenesis studies we used an existing pCMV plasmid construct expressing the reference HPV-11 E7 protein. Then we transfected HPV-negative epithelial cells with pCMV plasmid constructs encoding prototype HPV-11 E7, HPV-11 A45S E7, and high-risk HPV-E7 proteins. Western-blot and pull-down assays were carried out to investigate the interactions between E7 oncoproteins and tumor suppressors. Our results indicate that the A45S amino-acid change in the HPV-11 E7 protein greatly changes the association of E7 with both retinoblastoma and PTPN14 proteins in the studied epithelial cell-lines. Moreover, the interactions seen with the HPV-11 A45S E7 protein show similarity to the associations of the E7 proteins of "high-risk" HPV types. Thus, genetic variants and genomic nucleotide polymorphisms of HPV-11 can change the E7 and its interactions with cellular proteins, which raises the possibility that the A45S amino-acid substitution can influence the prognosis and manifestation of HPV-associated diseases.

INVESTIGATION OF ERYTHRITOL PRODUCTION BY *YARROWIA* STRAINS

EDINA ESZTERBAUER, ÁRON NÉMETH

Department of Applied Biotechnology and Food Science, Faculty of Chemical Technology and Biotechnology, University of Technology and Economics, Budapest, Hungary

Due to the current lifestyle of the population, there are an increasing amount of people suffering from diabetes mellitus and obesity. Erythritol ($C_4H_{10}O_4$) is a naturally occuring sugar alcohol (polyol) that is widely used. Erythritol is 60-70% as sweet as table sugar (sucrose), but it has almost zero calories (0,2 kcal/g). The planned experiment in terms of erythritol production was performed in fermentation by *Yarrowia* strains. *Yarrowia lipolytica* is one of the best studied yeast species, both in basic research and applied biotechnology. For the fermentations 24 deepwell microtiter plate was used with a special sandwich cover providing desired aeration and hindering (cross) contamination. The ability of the strains (*Y. divulgata*, *Y. lipolytica* 594, *Y. lipolytica* 597) to produce erythritol and the conditions of erythritol fermentation were examined, in order to increase the efficiency of production, to achieve the highest possible erythritol concentration. While the strain *Y. lipolytica* 597 produced mannitol instead of erythritol, *Y. lipolytica* 594 produced low amount of erytritol, thus *Y. divulgata* was the best candidate with 44.38g L^{-1} erythritol concentration. Regarding media composition the main consideration was to compare 2 carbon sources (glycerol, glucose), 3 nitrogen sources (ammonium-nitrate, ammonium-sulfate, Na-nitrate) and 4 additives (Na-citrate, mannitol, ions supplement, polyethylene glycol) for erythritol production. Glucose is generally applied for *Moniliella* erythritol fermentations, but glycerol is more frequently used for *Yarrowia*, and we also found it better. While among nitrogen sources ammonium nitrate was the best, Na-citrate was found as best additive with *Y. divulgata*. However, the combination of the best media components was not so effective as expected, therefore we tried to find optimum setup with the help of artificial neural network based model analysing the observed fermentation dataset of more than 300 entries. Since the regression were high enough (over 0.9) the 4 output parameters can be estimated with 90% reliablity on the basis of the 17 inputs. Moreover, the change of individual output parameters as a function of time can be extracted from the program, so the time course of the fermentations can also be predicted. It can be read from the diagrams that erythritol production drops significantly above a certain glycerol concentration (approx. 40 g L^{-1}) due to substrate inhibition. The optical density (i.e. cell growth) also decreased above a glycerol concentration of approximately 25 g L^{-1}. In case of glucose, substrate inhibition of cell growth was obsereved only above a higher concentration of 100 g L^{-1}, however, glucose was not effective in terms of erythritol fermentation.

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

MILÁN FARKAS[1], DALMA MÁRTON[1], GERGELY MARÓTI[2, 3], ROLAND WIRTH[4], ÁKOS MALATINSZKY[5], NEVEEN MAJDI ALMALKAWI[1], ANDRÁS TÁNCSICS[1], BALÁZS KRISZT[1], MÁTYÁS CSERHÁTI[1]

[1]Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő; [2]Seqomics Biotechnology Ltd., Mórahalom; [3]Institute of Biochemistry, Biological Research Center, Eötvös Loránd Research Network, Szeged; [4]Department of Biotechnology, Faculty of Science and Informatics, University of Szeged, Szeged; [5]Institute for Wildlife Management and Nature Conservation, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

Drought is a major challenge for plant growth especially in dry grassland areas. Adaptation of plants to drought is highly dependent on the response of soil bacterial communities to moisture limitation. Bacteria that stimulate plant growth are often isolated from extreme environments such as arid regions and these bacteria are able to increase the survival of other types of plants during the dry period, not just those from which they have been originated. The objective of this study to reveal the bacterial communities associated to dominant plants such as *Stipa*, *Festuca* and *Poa* species from an open and a closed sand steppe in Hungary. In the future, we would like to investigate whether the isolated bacteria with plant growth-stimulating properties can improve the drought stress tolerance of agricultural plants. The diversity of bacterial community from the rhizosphere and bulk soil samples were investigatd by Illumina metagenome sequencing. Representatives of the Actinobacteria, Proteobacteria and Acidobacteria were the most abundant in both rhizosphere and soil samples. Among the Proteobacteria, the genera *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Devosia* were enriched on the root surface of all examined plants. The *Mesorhizobium* genus was more abundant at the open sand steppe area. *Variovorax* species increased on the rhizosphere of Festuca species both areas. The proportion of the genus *Pseudomonas* remained unchanged while the Actionobacterial *Rubrobacter*, *Pseudonocardia* and *Conexibacter* genera decreased on the surface of the roots. According to the genome-centric analysis of all samples, 76 bin genomes were assembled using the Metabat2 program, the evaluation of these result is in progress. A total of 149 strains were isolated from the sandy grasslands, of which 115 strains have been identified. The selected non pathogenic 48 strains was screened for plant growth promoting (PGP) traits, such as: osmotic stress tolerance, indole-3-acetic acid (IAA), exopolysaccharide (EPS), siderophore, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase production, nitrogen fixation and phosphate solubilization. Based on the results, 15 strains from genus *Pseudarthrobacter*, *Kocuria*, *Brevibacterium*, *Brevibacillus*, *Stenotrophomonas*, *Agrobacterium*, *Priestia*, *Pedobacter*, *Pseudomonas* and *Variovorax* possess multiple properties at the same time. The root colonization ability of the strains will be tested on 1 week old maize seedlings after labeling the bacteria with a GFP plasmid. So far, the plasmid was introduced into three *Pseudomonas* strains, of which SSZ104-1R isolated from *Stipa* was the most able to colonize the root surface of the seedling.

Acknowledgements: This research was funded by 2020-1.1.2-PIACI-KFI-2020-00020.

IMPROVE THE HEALTH OF THE INTESTINAL MICROBIOTA OF SWINE USING GREEN PHYTONUTRIENTS SUPPLEMENTATION

PÉTER FAUSZT, ANNA SZILÁGYI-RÁCZ, PÉTER DÁVID, LÁSZLÓ STÜNDL, JUDIT REMENYIK, MELINDA PAHOLCSEK

Faculty of Agriculture, Food Science and Environmental Management, University of Debrecen, Debrecen, Hungary

As the human population is expanding, there is an immense rising need for the agriculture to overcome efficiency problems, and to use resources in a greener way. It is a promising approach to improve the health of the animals by modulating the gastrointestinal tract microbiota. A feeding trial was carried out to investigate the phytonutrient-induced changes in the gastrointestinal tract microbiota of domestic swine (*Sus scrofa domesticus*). A basal diet (BD) was applied as a negative control (did not use any supplement), and the following dietary supplements were used at a 0,5% dose: anthocyanin (ANTH) enriched diet from the production of Hungarian sour cherry, synbiotics (SYN) from fermented corn, fermentable oligosaccharides (fOS) from Hungarian sweet red pepper seeds, and carotenoids (CAR) from Hungarian sweet red pepper pulps. And also a mixes of the above mentioned nutrients were also used in 0.5% (MIX1), and 1% (MIX2). These components are typical by-products in the food industry. Through the main growing period of the swine (period A, B, C, D), the gut contents of the animals were collected. Microbial DNA samples were isolated, and V3-V4 16S rRNA gene-based metagenomic sequencing was performed. Data analysis: i. Our main goal was to investigate the compositional changes in the core microbiome induced by the nutraceuticals enriched diet; ii. Correlations between microbiota families and body weight were investigated; iii. Alpha diversity metrices were checked through the growing stages of the animals, and due to different nutraceuticals enriched diet; iv. A network analysis was performed, to investigate the resilience and dynamics of complex microbial communities.

POSITION-CONSERVED SEQUENCE-UNIQUE STWINTRONS, RECENTLY GAINED SEQUENCE-SIMILAR "SISTER" STWINTRONS AND SUPER SYMMETRICAL "DAUGHTER" INTRONS IN *HYPOXYLON* SP. CO27-5

ERZSÉBET FEKETE, FRUZSINA PÉNZES, VIKTÓRIA ÁG-RÁCZ, NORBERT ÁG, MICHEL FLIPPHI, LEVENTE KARAFFA

Department of Biochemical Engineering, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

Spliceosomal U2 introns are widespread in nuclear transcriptomes (Eukarya). Alternative intron splicing can increase proteome diversity and provide means for (post)transcriptional regulation of expression. Intron RNA is usually rapidly turned over. Nonetheless, some intron RNAs are more resilient and accumulate, and carry out crucial functions in response to, e.g., nutrient exhaustion. Spliceosomal intron excision requires a ribonucleoprotein complex, the U2 spliceosome, and involves the terminal 5'-donor- and 3'-acceptor G's and the lariat branch point (BP) A of the intron. The genesis of new U2 introns is a vexing mystery.Various mechanisms of intron acquisition have been proposed in the literature. Priorly, we have described stwintrons, nested U2 introns which can only be removed properly by consecutive splicing reactions. In a [D1,2] stwintron, an internal intron is nested in the 5'-donor element of an external intron between the 1st and the 2nd nt (5'-G1|U2). Here, we have identified 117 genuine [D1,2] stwintrons in *Hypoxylon* sp. CO27-5. Transposon target site duplication was not observed and all but one stwintron were integrated seamlessly. The large majority of these stwintrons were sequence-unique albeit located at positions also occupied by [D1,2] stwintrons in the orthologous genes in 16 other Hypoxylaceae, suggesting they appeared in the lineage before the divergence of the 16 taxa. By contrast, 23 [D1,2]'s were sequence-similar. 22 of these "sister" stwintrons were unique to the narrow taxon of *Hypoxylon* strains CO27-5 and EC38. One striking distinction between the two groups of stwintrons involves the density of a terminal symmetry (40–50 nt), which is considerably higher in most sister stwintrons. Moreover, we found 10 canonical introns in CO27-5 which are sequence-similar to the termini of sister stwintrons but lack ~ 100 nt in the centre which separate two copies of a palindrome (5'-WTTCTAGAAA) at the edge of the terminal inverted repeat (TIR). This suggests that a deletion of that internal spacer along with one palindrome accidentally created a "cropped sister intron". These new canonical introns only consist of the two halfs of the TIR, and able to form a neat hairpin structure bringing the donor- and acceptor G's in very close proximity. We conclude that *Hypoxylon* sister stwintrons duplicate as [D1,2] stwintrons and that they can give rise to highly symmetrical canonical "daughter" introns, which once formed, propagate as introns. Our results suggest that the TIR is key to the (stw)intron propagation we inferred.

Acknowledgements: Supported by the Hungarian National Research, Development & Innovation Fund, grant K 138489 and prepared with the professional support of the Doctoral Student Scholarship Program, grant RH/527-3/2021 to V.Á-R. of the Co-operative Doctoral Program of the Ministry of Innovation and Technology financed from the National Research, Development & Innovation Fund.

ENVIRONMENTAL DNA SEQUENCING REVEALS DIFFERENTIAL RESPONSES OF PLANT PATHOGENIC FUNGI TO FORESTRY TREATMENTS

GABRIELLA FINTHA[1], PÉTER ÓDOR[2], CARLA MOTA LEAL[3, 4], ADRIENN GEIGER[3], GLODIA M. KGOBE[3, 4], RÉKA ASZALÓS[2], FLÓRA TINYA[2], BENCE KOVÁCS[2], JÓZSEF GEML[3, 4]

[1]Eszterházy Károly Catholic Univeristy, Eger; [2]Institute of Ecology and Botany, Centre for Ecological Research, Eötvös Loránd Research Network, Budapest; [3]ELKH – EKKE Lendület Environmental Microbiome Research Group, Eszterházy Károly Catholic Univeristy, Eger; [4]Doctoral School of Environmental Sciences, Hungarian University of Agricultural and Life Sciences, Gödöllő, Hungary

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 to study the effect of different forestry treatments on the richness and community composition. Samples were collected in 2020 October at the Pilis Forestry Systems Experiment (PFSE), a long-term ecological study etsablished in the Pilis Mountains that investigates the effects of forestry treatments on forest site, regeneration and multi-taxon biodiversity. The experiment was started in 2014 in a mature (>80 y) sessile oak–hornbeam (Carici pilosae-Carpinetum) forest and compares the forestry treatments of shelterwood (clearcutting, retention tree group, preparation cutting) and continuous cover forestry (gap-cutting) systems, which represent different severity of disturbance when compared to untreated control plots. Here, we present the first insights regarding the compositional dynamics of plant pathogenic fungi under the above forestry treatments. Richness and proportional abundance of plant pathogens were highest in clearcuts and gaps and correlated positively with herb cover and soil moisture.

Community composition of plant pathogenic fungi correlated strongly with treatment type, with significant differences observed in all forestry treatments when compared to the control and to each other. These differences in habitat preference were already evident at genus level, with *Fusarium*, *Periconia*, *Sarocladium* being dominant in gaps and clearcuts, *Verticillum* in control plots, and *Caliciopsis*, *Cephalosporium*, *Pestalotiopsis*, and *Seridium* in retention tree groups, among others. Finally,

the data presented here provide an unprecedented insight into the diversity and niche-based habitat partitioning of plant pathogenic fungi that is presumably driven in part by the altered abiotic conditions and changes in understory vegetation.

Acknowledgements: Supported by the Lendület Programme of the Hungarian Academy of Sciences and the Eötvös Loránd Research Network and by the OTKA K139387 grant from the National Research, Development and Innovation Office (NKFIH) to JG. Research infrastructure of the PFSE were funded by grants K111887, NKFIA K128441 from the NKFIH to PÓ.

NEW SETS OF SEQUENCE-SIMILAR [D1,2] SISTER STWINTRONS IN THE GENOMES OF TWO XYLARIACEAE SPECIES, *XYLARIA* SP. BCC_1067 AND *NEMANIA ABORTIVA*

MICHEL FLIPPHI, NORBERT ÁG, VIKTÓRIA ÁG-RÁCZ, FRUZSINA PÉNZES, LEVENTE KARAFFA, ERZSÉBET FEKETE

Department of Biochemical Engineering, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

Spliceosomal introns are ubiquitous in eukaryotic nuclear transcriptomes. Their precise excision from pre-mRNAs requires the action of the U2 spliceosome. The availability of complete genomes of > 1000 fungi provides opportunities to study intron gain amongst allied species. Fungal U2 introns are generally small – often < 150 nt. The short but ubiquitous intron donor, the BP element, and acceptor sequences are well defined in model genomes. Intervening sequences may 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. One can prove the existence of a stwintron splicing intermediate, an RNA species in which only the external U2 intron is still in place between the bounding exons, by targeted PCR. 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. For instance, 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'-G1|U2). These characteristics enabled the design of motif search models to predict stwintrons in whole genome sequences. On the accompanying poster we present the results of a [D1,2] stwintron search in the genome of *Xylaria longipes*. Amongst the identified stwintrons there were no sequence-similar stwintrons, only "uniquely occuring" stwintrons. However, when we used each of the 91 X. longipes stwintrons as queries in BlastN screens to identify orthologous stwintrons in related species in the Xylariaceae family, we found a few species featuring multiple [D1,2] stwintrons that were sequence similar to one certified *X. longipes* stwintron. The occurrence of such groups of "sister stwintrons" is indicative for ongoing stwintron propagation or recent stwintron duplication events in these other species of Xylariaceae. Here we present statistical analyses of the sequence-similar stwintrons found in *Xylaria* sp. BCC_1067 (GenBank accession no. SSCS00000000) and those in *Nemania abortiva* (JAJKKU000000000), their internal symmetry, their respective integration sites and the bounding exonic sequences, and compare the results with those of similar analyses for the originally defined 23 sister stwintrons in *Hypoxylon* sp. CO27-5, a species of Hypoxylaceae (another family in the Xylariales order).

Acknowledgements: Supported by the Hungarian National Research, Development & Innovation Fund, grant K 138489 and prepared with the professional support of the Doctoral Student Scholarship Program, grant RH/527-3/2021 to V.Á-R. of the Co-operative Doctoral Program of the Ministry of Innovation and Technology.

EFFECTS OF SOUR CHERRY FLESH EXTRACT ON LIVER FUNCTION AND MICROBIOTA IN HYPERCHOLESTEROLEMIC RABBIT MODEL

ILDIKÓ FORGÁCS[1], RÉKA MÁRIA SZEKERES[2], BÉLA JUHÁSZ[2], RITA KISS[2], MELINDA PAHOLCSEK[1], JUDIT REMENYIK[1]

[1]Institute of Food Technology, Faculty of Agricultural and Food Sciences and Environmental Management; [2]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Non-alcoholic fatty liver disease (NAFLD) is considered to be the most common non-infectious liver disease. It is related to metabolic syndrome, type 2 diabetes mellitus (T2DM), insulin resistance, and inflammation. According to „two-hit" theory, the liver becomes more susceptible to toxic effects after accumulation of lipids. The pathophysiology of NAFLD is complex and multifactor-driven. Morbidity is escalating globally, accounting for about 30% of total deaths, and the ratio is expected to be higher in 2030. Since the liver is directly connected anatomically and physiologically with the gut through the hepatic portal vein the proinflammatoric processes are developed in gut-liver axis in consequence of disbiotic intestinal microbiota. Due to the intestinal barrier disruption microbial endotoxins translocate into systemic circulation, which instigates low-grade metabolic inflammation. Oxidative stress plays the key role in progression from fatty liver to steatohepatitis, through inducing lipid peroxidation and increasing inflammation. Antioxidants nutrients are able to decrease the level of proinflammatoric cytokines (IL-8; TNFα), and liver enzymes (AST; ALT; GGT; ALP) in NAFLD. In addition antioxidants might influence microbial balance. In our investigation, 20 New Zealand White rabbits were randomized into four groups. First group was fed with normal chow for 44 weeks (C); second group was fed with normal chow for 32 weeks then treated with sour cherry flesh extract (PCE) for 12 weeks (C+PCE); third group was fed with atherogenic chow containing 1% cholesterol and 1% triglycerides for 44

weeks (HC); fourth group was fed with atherogenic chow for 32 weeks then treated with PCE for 12 weeks (HC+PCE). PCE was dissolved in water in 9 g kg^{-1} dosage. Blood samples were collected every third week in the 12 week-period, and faeces was collected at the last week. Plasma parameters were quantified and microbiota composition was analyzed at the end of the experiment. Levels of circulating liver enzymes were measured with Cobas c 311 analyser; IL-8, TNFα proinflammatoric cytokines were quantified with ELISA (Enzyme-linked immunosorbent assay) method. Targeted amplicon sequencing was used to compare the diversity of the intestinal microbiota of our experimental groups. Network analyses were performed to investigate treatment induced community shifts and to thoroughly investigate resilience of the core microbiota.

THE SHIFT OF FUNGAL PLANT PATHOGENIC COMMUNITY OF GRAPEVINE AMONG YEARS AND MICROHABITATS ARE GREATER THAN AMONG CULTIVARS, HEALTH STATES AND SEASONS

ADRIENN GEIGER[1, 2], CARLA MOTA LEAL[2], ANNA MOLNÁR[1], LUCA ANNAMÁRIA LEPRES[1, 2], GLODIA KGOBE[2], MARGOT MÜLLER[1], KÁLMÁN ZOLTÁN VÁCZY[1], JÓZSEF GEML[1]

[1]Institute of Biology, Eszterházy Károly Catholic University, Eger; [2]Doctoral School of Environmental Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

Grapevine trunk diseases (GTD) are one of the major fungal diseases of grapevine with several causing agents belonging to different genera. Esca is one of the most severe of them. Although GTD has been thoroughly studied in the past decades, the whole nature of the disease is still unknown. DNA-metabarcoding technique was used to reveal whether differenences in richness, abundance, and composition of plant pathogenic fungi exist among below- and aboveground microhabitats, among asymptomatic and Esca symptomatic grapevines, among seasons, years and cultivars. Soil, bark, and perennial wood samples were collected from asymptomatic and Esca symptomatic grapevines from four different cultivars (Kékfrankos, Leányka, Cabernet sauvignon, Chardonnay) in February and September of 2020 and 2021 in the Eger wine region. Richness and rarefied abundance of GTD-associated and non-GTD plant pathogenic fungi differed among microhabitats but not between health states. Larger compositional differences in plant pathogenic fungi were found within grapevine plants than among them. GTD pathogens were dominant in bark and wood samples while non-GTD pathogens were mainly restricted to soil. The plant pathogenic fungal community did not differ among cultivars or among seasons.
In 2021, the abundance and richness of plant pathogenic fungi were lower than in 2020. This can be due to the changes of certain environmental factors among the examined years, such as temperature and/or humidity. Our results suggest that pathogens related to GTDs are the members of the core microbiome, some of which can act as opportunistic pathogens on stressed plants. The role of the enviromental factors seems to be important, and studies are needed to investigate the abiotic conditions on fungal compositional dynamics in Esca-affected plants.

COMPARISON OF FUNGAL COMMUNITIES IN *BOTRYTIS*-INFECTED NOBLE ROT AND GREY ROT GRAPES

JÓZSEF GEML[1, 2], ÁDÁM HEGYI[1], JÚLIA HEGYI-KALÓ[1], MARGOT MULLER[2], KÁLMÁN ZOLTÁN VÁCZY[1]

[1]Institute of Biology, Eszterházy Károly Catholic University; [2]ELKH-EKKE Environmental Microbiome Research Group, Eötvös Loránd Research Network, Eger, Hungary

Botrytis cinerea is a well-known pathogen of several agricultural crops, causing grey rot in grapevine. However, under certain microclimatic conditions, *Botrytis* infection results in noble rot, an essential process in the production of the sweet and aromatic botrytized wines, such as the Tokaji aszú in Hungary. While the traditional view of noble rot and grey rot focuses on the dominant role of *B. cinerea*, former culture-based studies found that several plant pathogenic and saprotrophic fungi also inhabit noble rot grape berries, e.g., *Alternaria*, *Aureobasidium*, *Cladosporium*, *Rhodotorula* species. However, many microbes cannot be cultured using current methods and there are important gaps in our knowledge regarding the composition of the pathobiome of noble rot and grey rot. In this study, we generated and analyzed fungal ITS rDNA sequences with Illumina Novaseq to fully characterize the fungal community associated with healthy, noble rot and grey rot grape berries. Fungal community associated with noble rot and grey rot grape berries strongly differed in diversity and composition from those found in asymptomatic berries. In addition, even though noble and grey rot berries shared many fungal species, we also found several fungi specific to each rot type. Overall, berry type accounted for 49.53% of compositional variance among samples, with *Debaryomyces*, *Fusarium* and *Malassezia* characteristically found in asymptomatic berries and *Botrytis* dominating noble and grey rot communities, with relatively high abundance of *Aureobasidium* also observed. In addition, we found singificant compositional differences between the two sampling months, accounting for 13.34% of variance. In summary, this study provides the first detaled insights into the pathobiome composition grey rot and noble rot grapes. Although our results confirm the dominance of *B. cinerea* in grey and noble rot, there are several other fungi may play important roles in the botrytization process of grapes.

Acknowledgements: TKP2021-NKTA-16 (NKFIH), Lendület Programme No. 96049 (ELRN and HAS).

ABIOTIC FACTORS DRIVE HABITAT PARTITIONING OF ECTOMYCORRHIZAL FUNGI IN PANNONIAN FORESTS

JÓZSEF GEML[1, 2], CARLA MOTA LEAL[2], RICHÁRD NAGY[1], JÓZSEF SULYOK[3]

[1]Institute of Biology, Eszterházy Károly Catholic University; [2]ELKH-EKKE Environmental Microbiome Research Group, Eötvös Loránd Research Network; [3]Bükk National Park Directorate, Eger, Hungary

Ectomycorrhizal (ECM) fungi are among the most diverse and dominant fungal groups in temperate forests and are crucial for ecosystem functioning of forests and their resilience towards disturbance. We carried out DNA metabarcoding of ECM fungi from soil samples taken at 62 sites in the Bükk Mountains in northern Hungary. The selected sampling sites represent the characteristic Pannonian forest types distributed along elevation (i.e. temperature), pH and slope aspect gradients. We compared richness and community composition of ECM fungi among forest types and explored relationships among environmental variables and ECM fungal alpha and beta diversity. The DNA sequence data generated in this study indicated strong correlations between fungal community composition and environmental variables, particularly with pH and soil moisture, with many ECM fungi showing preference for specific zonal, topographic or edaphic 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. Despite the relatively high proportions of compositional variance explained by the tested environmental variables, a large proportion of the compositional variance remained unexplained, indicating that both niche (environmental filtering) and neutral (stochastic) processes shape ECM fungal community composition at landscape level. Our work provides unprecedented insights into the diversity, landscape-level distribution, and habitat preferences of ECM fungi in the Pannonian forests of Northern Hungary.

Acknowledgements: OTKA K21 139387 (NKFIH), Lendület Program no. 96049 (HAS and ELRN), Bükk National Park Directorate.

MICROBIOLOGICAL EXAMINATION OF EARLY MODERN GRAPE SEED FINDS AND COMPARISON WITH MODERN SAMPLES

GERDA GEORGINA GOTTSCHALL[1], ANDRÁS TÁNCSICS[2], FERENC GYULAI[3], BALÁZS KRISZT[1]

[1]Department of Environmental Safety; [2]Department of Molecular Ecology, Institute of Aquaculture and Environmental Safety; [3]Doctoral School of Environmental Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

Wine-producing grapes have played a major role in human society and culture since the earliest times, however, various pathogenic fungi and bacteria can cause great damage to the plant. The contemporary samples come from the beginning of aszú wine making, i.e. from the middle of the 17th century. During the excavation of the Tokaj-Hegyalja region, the grape seed samples were extracted from a depth of 2.5 m together with a layer of soil. The recent samples come from Tállya, from a winery that makes natural wine and have similar varietal characteristics, so they are suitable for testing. The purpose is to compare past and recent microbial communities that play a role in the fattening process, as well as reveal the possible causes of similarities and possible differences. The samples were spread on the surface of an R2A agar plate using the limiting dilution method, and after differentiating the individual colonies, pure cultures were created from them, which were identified at the species level using Sanger sequencing based on the 16S rRNA region. Based on the 16S rRNA gene sequences, we could isolate relatively few strains from the cultured archaeological samples on the R2A medium. Genus distribution of the identified strains: 38% *Streptomyces*, 31% *Cohnella*, 13% *Pilimelia*, 6% *Ralstonia*, 6% *Williamsia* and 6% *Bacillus*. A new species was identified among the determined strains, closest relatives: *Pilimelia columellifera* subsp. *pallida* 98.2%; and showed more than 97% homology with several *Streptomyces* strains (*S. corynorhini*, *S. laculatispora*, *S. brevispora*, *S. beijiangensis*, *S. paludis* and *S. pulveraceus* 97.4%). The exact definition of the new bacterial species is still in progress, with the necessary tests: genomic DNA analysis, phenotypic and chemotaxonomic tests, determination of cell membrane fatty acid components, respiratory and lipoquinones, and polar lipids. The current samples have been collected from Tállya and their examination is in progress. As a result of the research, the Tokaj-Hegyalja, as well as the country's earliest known microbial community related to grape growing, will be known, described and entered into the database. The results are expected to provide new data for the history of viticulture in the Carpathian Basin.

INVESTIGATION OF MICROAEROBIC BENZENE-DEGRADING BACTERIAL COMMUNITIES IN GROUNDWATER ENRICHMENT CULTURES AND SEDIMENT SLURRY MICROCOSMS

ANNA HARKAINÉ BEDICS[1], SINCHAN BANERJEE[1], PÉTER HARKAI[2], ANDREA CSÉPÁNYI[1], MÁRTON PÁPAI[1], BALÁZS KRISZT[2], ANDRÁS TÁNCSICS[1]

[1]Department of Molecular Ecology; [2]Department of Environmental Safety, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

The increasing level of petroleum hydrocarbon pollution significantly damages the ecosystem or even the human health. Simple aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (BTEX) are the most common contaminants of the groundwater and can therefore easily contaminate drinking water due to their relatively high water solubility. Benzene is an important industrial chemical and due to the discharge or disposal of product, it can pass into air, water and soil as well. Aromatic hydrocarbons decompose most rapidly and completely under aerobic conditions and the bioremediation has proven to be the most successful in solving problems 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 contaminated soil decreases rapidly. However, the availability of dissolved oxygen has a key role in the biodegradation because benzene, para- and ortho-xylene can be persistent contaminants under anaerobic conditions. Consequently, exploration of the bacterial communities of aromatic hydrocarbon contaminated, hypoxic environments has a current importance. To reveal those bacteria, which have a key role in the benzene biodegradation in hypoxic environments, microaerobic groundwater enrichment cultures and microaerobic and aerobic sediment slurry microcosms, degrading solely benzene, were set up. Groundwater and sediment samples originated from the "Siklós" BTEX-contaminated area. The bacterial community structure of the sediment slurry microcosms and enrichment cultures were investigated through 16S rRNA gene Illumina amplicon sequencing. Enrichments were dominated by members of the genus *Rhodoferax* followed by *Pseudomonas* and *Acidovorax*, while the abundance of other genera (e.g. *Sediminibacterium*, *Xanthobacter* and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*) were found only marginal. In contrast, *Azovibrio* and *Zoogloea* were the most common genera in microaerobic sediment slurry microcosms, while in case of the aerobic microcosms, genera of the family Burkholderiaceae and members of the genus *Pseudomonas* were the most dominant community members. Besides the most abundant genera, members of *Thaura*, *Sulfuricurvum*, *Acidovorax*, *Geobater*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* were also detectable with significant abundance in both microcosms. Overall, distinctly different benzene-degrading bacterial communities evolved under microaerobic and aerobic conditions.

SEPARATION OF SURFACTIN VARIANTS FROM THE FERMENT BROTH OF A *BACILLUS SUBTILIS* STRAIN

ZSÓFIA HEGEDÜS[1, 2], DÁNIEL HERCEGFALVI[1], CSABA VÁGVÖLGYI[1], ANDRÁS SZEKERES[1]

[1]Department of Microbiology; [2]Doctoral School of Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Surfactins are cyclic lipopeptides 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, they are potential candidates for therapeutic and environmental applications. 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 recently. However, there are only limited 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 various 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 normal phase flash chromatography. As a result of the flash chromatographic purification, surfactins could be separated into two groups, both contained two types of surfactin variants with different chain lengths. Further separation of the different variants in the two group were carried out by reverse phase flash chromatography and preparative HPLC. The collected fractions were analysed by thin layer chromatography and the determination of surfactin variants in the fractions during purification were carried out by HPLC-HESI-MS

Acknowledgements: Supported by the Hungarian Scientific Research Fund (OTKA K-128659).

METATRANSCRIPTOMIC ANALYSIS OF THE PROCESS OF NOBLE ROT

ÁDÁM ISTVÁN HEGYI[1], MARGOT OTTO[2], JÓZSEF GEML[2], JÚLIA HEGYI-KALÓ[1], KÁLMÁN ZOLTÁN VÁCZY[1]

[1]Institute of Biology, Eszterházy Károly Catholic University; [2]ELKH-EKKE Environmental Microbiome Research Group, Eötvös Loránd Research Network, Eger, Hungary

Botrytis cinerea, can lead to the formation of noble rot (NR) of grape berries under certain environmental conditions, resulting in favored metabolic and physical changes necessary for produc- ing highly regarded botrytized wines. The functional genes involved in the textural and biochemical processes are still poorly characterized. We generated and analyzed metatranscriptomic data from healthy (H) berries and from berries representing the four stages of NR from the Tokaj wine

region in Hungary over three months. A weighted gene co-expression network analysis (WGCNA) was conducted to link *B. cinerea* functional genes to grape berry physical parameters berry hardness (BH), berry skin break force (F_sk), berry skin elasticity (E_sk), and the skin break energy (W_sk). Clustered modules showed that genes involved in carbohydrate and protein metabolism were significantly enriched in NR, highlighting their importance in the grape berry structural integrity. Carbohydrate active enzymes were particularly up-regulated at the onset of NR (during the transition from phase I to II) suggesting that the major structural changes occur early in the NR process. In addition, we identified genes expressed throughout the NR process belonging to enriched pathways that allow *B. cinerea* to dominate and proliferate during this state, including sulphate metabolizing genes and genes involved in the synthesis of antimicrobials.

Acknowledgements: Funded by Thematic Excellence Program (grant no. TKP2021-NKTA-16). J.G. and M.O. are supported by the Lendület Programme (grant no. 96049) ofthe HAS nad ELRN. K.Z.V. supported by János Bolyai Research Scholarship of the HAS. Á.I.H. and J.H.-K. supported by the National Talent Programme (grant no. NTP-NFTÖ-21-B-0112 and NTP-NFTÖ-21-B-111 respectively). We thank the owners of the Szepsy Ltd. for the supporting vineyard, and Szabina Lengyel, Nikoletta Szalóki, Adrienn Geiger and Richard Golen, who helped in the collection, measurements and in the DNA extraction.

HUMAN RICKETTSIAL INFECTIONS IN HUNGARY

JUDIT HENCZKÓ[1, 2], KATALIN DECSOV[3], ENIKŐ KÁDÁR-HÜRKECZ[3], BERNADETT PÁLYI[1], ZOLTÁN KIS[1], ZSUZSANNA KIENLE[3]

[1]National Biosafety Laboratory, National Public Health Center; [2]Doctoral School of Health Sciences, Semmelweis University; [3]Department of Bacteriology, Mycology and Parasitology, National Public Health Center, Budapest, Hungary

Rickettsial infections, in particularly tick-borne rickettsioses are one of the most important emerging diseases in Europe. *Rickettsia conorii* subsp. *conorii* the aetiological agent of Mediterranean Spotted Fever (MSF) is the most frequent in Europe, however MSF-like and *Dermacentor*-borne necrosis erythema, lymphadenopathy/tick-borne lymphadenopathy/ Scalp Eschar and Neck Lymphadenopathy After Tick Bite cases caused by *Rickettsia* spp. have been identified in recent years. Several factors influence the rise in the number of cases, including global warming, the changes of the bird migration routes, transovarian and/or transtadial transmission between ticks, etc. In most cases the symptoms are characterized as non-specific, including headache, fever, muscle- and joint pain, maculopapular or papular rash, eshar ("tache noire") or ulcerative skin lesion may also appear. Nevertheless, none of these manifestations are pathognomonic and direct diagnostic methods to confirm the species are always required. Serology is still the gold standard technique. Genomic approaches, including real-time polymerase chain reaction (q-PCR) and whole genome sequencing, including metagenomics are the most powerful tools in the identification. Most of the species are biosafety level 3 pathogens, therefore all living procedures should be performed at specialised laboratories, according to the national regulations. At the National Public Health Center, the listed diagnostic methods are available since 2016. Until now, 286 human specimens were admitted to our laboratory. Human sera were tested by indirect immunofluorescence assay (Focus) samples were considered to positive while IgM/IgG titers were higher than 1:128 and/or fourfold increase of IgG antibodies. Only 27 samples were positive, of these presumably 26 were positive to spotted fever group and 1 sample was positive to typhus group by serology. Q-PCR were performed in 120 cases and 5 samples (whole blood and lesion samples) were positive, one lesion sample was suitable for whole genomic analysis. Purified DNA was shotgun sequenced on Illumina MiSeq instrument with Nextera XT (Illumina) library preparation kit. Genomic analysis was performed using CLC Genomics Workbench (Qiagen). The genomic analysis showed that the patient was suffered from *R. africae*, the African tick-bite fever which is the second most frequent cause of fever after malaria in travellers returning from sub-Saharan Africa. According to the literature *R. helvetica* and *R. slovaca* are presented in the Hungarian tick populations, but so far no human cases have been found. Acute infections are often difficult to diagnose due to mild and non-specific symptoms, transient bacteraemia phase of illness, poor sensitivity of serology early in disease, and use of effective empirical antibiotics. Our data suggest that, continued and improved *Rickettsia* spp. diagnostics is required to determine the travel-related and possible autochton infections in Hungary.

VECTOR-BORNE BACTERIAL AND PROTOZOAN PATHOGENS EMERGING OR DETECTED FOR THE FIRST TIME IN HUNGARY

SÁNDOR HORNOK

Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary

During the past 15 years, research at the Department of Parasitology and Zoology of the University of Veterinary Medicine focused on vector-borne (especially arthropod-borne) pathogens. The results include the discovery of two bacterial species new to science, in particular from genera of high veterinary-medical importance, i.e., *Candidatus* Rickettsia hungarica and *C.* Mycoplasma haemovis which were later also identified in other countries. It was proved for the first time that one of the five important groups of piroplasms, the so-called Western *Babesia* group occurs in the Eastern Hemisphere. Several bacterial and protozoan pathogens were discovered for the first time in Europe, including *Babesia crassa*, Far-Eastern *Babesia* genotypes,

Hepatozoon felis (2nd genogroup), *R. africae* and an *Ehrlichia chaffeensis*-like agent. Further vector-borne pathogens were newly detected in either Central Europe or in Hungary. When tick species new to the Hungarian fauna were identified (*Hyalomma rufipes*, *Ixodes kaiseri*, *Rhipicephalus sanguineus*), this implied the potential presence of relevant tick-borne pathogens. In other countries, the new bat tick species discovered in Hungary, *I. ariadnae* was shown to be an important carrier of borreliae. Bacterial and protozoan pathogens were also newly detected in certain blood-sucking arthropods, as exemplified by *Anaplasma marginale* in *Tabanus bovinus*; *R. helvetica* in *Haemaphysalis inermis* (with higher prevalence than in its known vector, *I. ricinus*); three *Theileria* spp. in *Stomoxys calcitrans*; *Babesia* sp. badger type-A in *I. canisuga* and type-B in *I. kaiseri*; as well as *A. ovis* in keds. First time molecular evidence for the infection of certain vertebrates with vector-borne pathogens suggests their involvement in the transmission cycle as reservoirs. In this context, the red fox was proved to be indirectly a carrier of *Ehrlichia canis* and birds to harbor *R. helvetica*. Bats appear to be susceptible for some piroplasms of ruminants, dogs and humans, as well as cattle for *A. ovis*. Epidemiological risks of acquiring vector-borne pathogens are influenced by both spatial and temporal factors. Considering the latter, *D. reticulatus* was shown to carry *B. canis* almost exclusively in the spring. The former include forested freeway resting areas as hotspots for Lyme-disease, ectoparasites of rodents harboring *A. phagocytophilum* and *R. helvetica* indoors, hedgehog and bat fleas as carriers of bartonellae and *R. helvetica* (even in winter in the city center, when and where its known vector, *I. ricinus* is not present). The prevalence of *A. phagocytophilum* is significantly higher in Budapest than in the countryside, owing to abundant urban carriers (such as Turdidae). The prevalence of this *I. ricinus*-borne pathogen was high in forest-dwelling game but it was absent in buffaloes grazing grassland. On the other hand, infection rates with fly-borne hemoplasmas appeared to be host-size-dependent. It is also interesting to note that ticks can be used as sentinels: *Coxiella burnetii* was detected in ticks one year earlier at the place of the largest Q-fever outbreak in Hungary. In summary, molecular evidence based on two genetic markers or long enough DNA fragment of a certain pathogen in the blood/tissues of a vertebrate host is a strong indicator of its susceptibility. If questing ticks or off-host (unfed) vectors are PCR-positive, this suggests their vector potential. However, molecular analyses of even blood-fed, engorged ticks (vectors) can be informative on the reservoir role of a host, when: (1) engorged tick larvae are PCR-positive for a pathogen lacking transovarial transmission, or (2) when strictly host-specific ectoparasites (e.g., lice) contain DNA of a pathogen from another host. Last but not least, molecular analyses of engorged ticks can also raise the possibility of an *a priori* carrier state or a vector role if (3) different genotypes are identified in the tick and in the blood of its host, or (4) when PCR-positivity is strongly associated with one species of engorged tick *vs* another on the same bacteremic or parasitemic host.

SURVEY OF ASYMPTOMATIC MENINGOCOCCAL CARRIAGE IN HUNGARY AMONG UNIVERSITY AND HIGH SCHOOL STUDENTS

ANNAMÁRIA HUBER[1], ESZTER KOVÁCS[1], ANDREA HORVÁTH[1], JUDIT SAHIN-TÓTH[1], EMESE JUHÁSZ[2], KATALIN KRISTÓF[2], ORSOLYA DOBAY[1]

[1]Institute of Medical Microbiology; [2]Institute of Laboratory Medicine, Faculty of Medicine, Semmelweis University, Budapest, Hungary

Epidemic meningitis caused by *Neisseria meningitidis* is a rare infection but can be associated with very high mortality and it is most common in early childhood and young adulthood. Asymptomatic carriage of the pathogen in the nasopharynx is not uncommon and plays an important role in the spread of the disease. In this study we have assessed the prevalence and risk factors of asymptomatic carriage of *Neisseria meningitidis* among young adults, for the first time in Hungary. We have collected samples from the posterior pharyngeal wall with a cotton swab. Altogether 610 samples were taken, 307 from secondary school children and 303 from university students. DNA was extracted from the swabs using QIAamp BiOstic Bacteremia DNA Kit. The presence of the pathogen was detected by qPCR, targeting the species-specific *sodC* gene, following the CDC guidelines. In case of the *sodC* positive samples, the serogroup (A, B, C, X, W, Y) was also determined. We have assessed the risk factors of colonization (e.g. smoking, dormitories, going to festivals, taking antibiotics) with the help of a questionnaire. Unfortunately the study subjects often did not always have reliable data about their own vaccination status. *Neisseria meningitidis* isolates were found in 201 of the 610 samples. The average carriage rate was found to be 33.0% and it was significantly higher among high school students (47.2%) than university students (18.5%) and it peaked at 17-18 years of age. Carriage was higher among men (41.4%) compared to women (32.3%). Most of the detected meningococci were non-typeable (93.5%), we only identified 9 type C, 3 type B and 1 type Y isolates. As for the risk factors we found that taking antibiotics in the previous 2 months lowered the risk of carriage: if taking antibiotics, the carriage rate was 29.0%, if not taking it was 36.5%. Similarly, both active or passive smoking decreased the risk for colonization. We could not demonstrate the role of any other risk factor. In conclusion, asymptomatic carriage of *Neisseria meningitidis* is high among young adults in Hungary. The high proportion of non-typeable strains correlates well with international data. The most common identifiable serotypes were B and C. According to data of the National Center for Epidemiology, type B has constantly been most prevalent in Hungary among invasive meningococcal infections in Hungary between 1988-2013, representing 74% of all cases, while type C accounted for several major outbreaks. Both types are associated with high case fatality rates. Meningococcal vaccination is not (yet) obligatory in Hungary, but these results show that immunization would be important for this age group, especially against types B and C.

PHENO- AND GENOTYPIC DIVERSITY IN THE PROBIOTIC *SACCHAROMYCES CEREVISIAE* VAR. *'BOULARDII'* YEAST

ALEXANDRA IMRE, ISTVÁN PÓCSI, WALTER PFLIEGLER

Department of Biotechnology and Microbiology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

The probiotic yeast, i.e. *Saccharomyces 'boulardii'*, belongs to the "European Wine" clade of the *S. cerevisiae* species. These fungi have been used for decades to alleviate gastrointestinal conditions, e.g. *Clostridium difficile* infection or diarrhoea. At the same time, *S. 'boulardii'* can also cause opportunistic infections, so research into its virulence and pathomechanism is timely. In the case of this yeast and other yeast species involved in probiotic development, usually only one or a very small number of isolates are tested for phenotype, safety and virulence factors. In our study, we investigated how different *S. 'boulardii'* isolates found in products and in human samples differ in genome structure variations (aneuploidy, segmental duplications, loss of heterozygosity) and in minor genomic mutations. We also investigated whether, based on their unique variations, the individual isolates may be considered significantly different genetic backgrounds despite their close kinship. In addition to our isolates collected in Hungary and sequenced by us (4 product and 10 clinical samples), we also compared the genomes of probiotic yeasts examined by previous studies and found that aneuploidy is rare in the *S. 'boulardii'* subclade. Conversely, loss-of-heterozygosity events are particularly common in certain genomic regions and can occur between two batches of the same product. Gene copy number variations affect very few genes within the subclade. The effects of SNPs and indel mutations within the subclade were assessed after de novo assembly and annotation of the genome of a product isolate, using the new genome as a reference. These variants affected genes important in various cellular functions (codon change, sometimes loss-of-function). As we expected, the existence of genomic variations resulted in the fact that our tested isolates behaved as different genetic backgrounds during phenotypic characterization, and furthermore, their phenotypes changed in different ways after gene knockout. Genetically modified probiotics and biotherapeutics will play an increasingly important role in the future. Our results highlight that even within a relatively uniform subclade, such as that of the probiotic yeast, the different genetic background can lead to unexpected phenotypic properties and can have a considerable influence on the properties of strains obtained by genetic modifications in an unpredictable way.

THE PHYLOGENOMICS OF YEASTS FOUND IN ANCIENT AND RECENT HUMAN SHOTGUN METAGENOMIC SAMPLES

ALEXANDRA IMRE, HANNA VIKTÓRIA RÁCZ, VALTER PFLIEGLER

Department of Biotechnology and Microbiology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

The research of the human mycobiome, the diversity of the fungi living in and on us, and their roles in health and disease is increasingly studied, but still lags behind the study of the bacterial microbiome. Several species of yeasts are members of the human mycobiome and there are many well-known opportunistic pathogenic species as well among these. Another important aspect of the human mycobiome is the transient or sometimes more established presence of yeasts used in food and beverage production. These include species that have been proposed for probiotic use as well. Despite the recent advancements in sequencing throughput and cost-effectiveness, shotgun metagenomic analyses still rarely focus on members of the mycobiome including the zymobiome (they yeast mycobiome). The status of several ubiquitous species is also not yet fully settled. The best example for such a yeast is the wine, ale and baker's yeast: Saccharomyces cerevisiae. It is often reported as a true or transient member of the microbiome, as well as a rare opportunistic pathogen, while at the same time, it is also used in probiotics (as *S. 'boulardii'*). However, many food items contain significant amounts of baker's yeast DNA even without live cells, complicating the evaluation of its role in the human body based solely on sequence data. In our current study, we aimed to analyze stool and vaginal shotgun metagenomic datasets to gain information on the prevalence of *S. cerevisiae* and altogether 50 other yeast species in various human cultures, and also, to compare the genomes extracted from metagenomes to the various clades and subgroups of the species using phylogenomic methods. We first created a curated list of 6000 human metagenomic samples from various ethnic groups, and a list of all currently available ancient metagenomic datasets, stretching back to Neanderthal fecal samples that are 40 thousand years old.
We were able to extract several relatively high coverage genomes of yeasts from these previously sequenced metagenomes. This allowed us to phylogenomically compare ancient and current yeasts from metagenomic samples with contemporary sequenced ones, including isolates actually cultured and sequenced from human hosts.

SURVIVAL FACTOR GENES OF *MUCOR LUSITANICUS*

OLIVÉR JÁGER[1, 2], GÁBOR NAGY[1, 2], MÓNIKA VARGA[1], RITA SINKA[3], CSABA VÁGVÖLGYI[1], TAMÁS PAPP[1, 2]

[1]Department of Microbiology; [2]ELKH-SZTE Fungal Pathomechanisms Research Group, Department of Microbiology; [3]Department of Genetics, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

In recent years, incidence of mucormycosis (i.e., invasive fungal infections caused by members of the order Mucorales, such as *Mucor lusitanicus*) 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. Genome of *M. lusitanicus* contains two genes encoding survival factor 1 (SVF1), which were named as *svf1a* and *svf1b*. Aim of the present study was to examine the expression and reveal the function of these genes, first in a Mucorales fungus. We demonstrated that SVF1 proteins are required for survival under conditions of oxidative and cold stress in *Mucor*. Knock-out of *svf1a* caused increased sensitivity to oxidative stress when compared to the wild-type strain. The sphingolipid metabolism of the knock-out strains was also investigated with HPLC techniques. We found that Svf1b affects sphingolipid biosynthesis, its absence altered the accumulation of phytosphingosine and its downstream metabolites. In both *svf1a* and *svf1b* knock-out mutants, conidial germination was delayed, vegetative growth was reduced and spore forming ability was impaired. We have studied the expression of the genes after culturing the fungus under different conditions by real-time quantitative reverse transcription PCR. 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. Furthermore, recognition and internalization of the fungal spores by macrophages were affected by the disruption of the *svf1* genes in *M. lusitanicus*.

Acknowledgements: Supported by project NKFI K131796 and grants ITM NKFIA TKP-2021-EGA-28 and ELKH 2001007.

TRANSCRIPTIONAL RESPONSE OF *CANDIDA AURIS* BIOFILMS TO FARNESOL AND TYROSOL TREATMENT

ÁGNES JAKAB[1], NOÉMI BALLA[1, 2], FRUZSINA KOVÁCS[1, 2], CSABA NAGY-KÖTELES[3], LÁSZLÓ MAJOROS[1], RENÁTÓ KOVÁCS[1]

[1]Department of Medical Microbiology, Faculty of Medicine; [2]Doctoral School of Pharmaceutical Sciences, Faculty of Pharmacy; [3]Department of Molecular Biotechnology and Microbiology, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

Candida auris is an emerging fungal pathogen that causes outbreaks in health care facilities with a high mortality rate. The conventional antifungal agents have limited effects against the majority of clinical isolates. Furthermore, multidrug-resistance is typical in case of *C. auris* biofilms; thus, innovative therapies are urgently needed. Farnesol and tyrosol are two fungal quorum-sensing molecules with opposite effect in terms of *Candida* morphogenesis. Moreover, they have a remarkable antimicrobial effect at supraphysiological concentrations. Our aim was to investigate genome-wide gene transcription changes induced by these compounds against *C. auris* biofilm using total transcriptome sequencing (RNA-Seq). We found that farnesol and tyrosol exposures significantly reduced the biofilm mass produced by *C. auris* and resulted 587 up- and 1851 down-regulated genes with significant differential expression, respectively ($P<0.05$). Following farnesol treatment, 138 and 199 genes showed increased (>1.5-fold change) or decreased (<-1.5-fold change) transcription level, respectively; while tyrosol resulted 686 up-regulated (>1.5-fold change) and 662 down-regulated (<-1.5-fold change) genes compared to control. Farnesol-induced genes involved in ribosomal small and large subunit biogenesis, RNA metabolic process and iron-sulfur cluster binding were up-regulated. Tyrosol resulted the up-regulation of genes involved in ribosomal small and large subunit biogenesis, RNA metabolic process, translation, unsaturated fatty acid biosynthesis as well as iron-sulfur cluster binding. Moreover, tyrosol decreased the expression of carbohydrate catabolic process, ergosterol biosynthesis, fatty acid beta-oxidation, response to endoplasmic reticulum stress, peroxisome and vacuole associated genes. Our study provides novel clues for future studies in terms of understanding of quorum-sensing molecules-related effect on *C. auris* biofilms.

Acknowledgements. R.K. is a János Bolyai Research Scholarship grantee of the HAS. This research was supported by the Hungarian National Research, Development and Innovation Office (NKFIH FK138462).

SEROPREVALENCE STUDY OF HUMAN POLYOMAVIRUSES

KRISZTINA JELES, MELINDA KATONA, ESZTER CSOMA

Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

The number of species within the Polyomaviridae family has increased since 2007. Currently, 14 human polyomaviruses are classified within the family, but only five of these viruses are associated with diseases. The human polyomaviruses do not result in obvious clinical symptoms in healthy individuals, but in immunocompromised patients they can cause disease, even severe one. Merkel cell polyomavirus, as a tumour virus is a causative agent of Merkel cell carcinoma. To reveal the significance of the other, not well known viruses, seroprevalence studies are essential. Detection of the antibodies against the viruses in different patient groups will answer some basic questions: the time of the primary infection, the rate of population infected by

the viruses, the possible geographical differences, and the risk factors for infections. Our aim was to develop an ELISA method by which we are able to detect IgG against the immunodominant antigen of the polyomaviruses, the major capsid protein VP1. The antigens, the polyhistidine-tagged VP1 proteins were produced in bacterial protein expression system. The VP1 coding sequence (codon optimized or not) was cloned into pTriEx™-4 Neo vector, and expression was carried out in Origami™ B(DE3) pLacI or Rosetta-gami™ B(DE3)pLacI bacteria. Protino® Ni-TED column was used to purify the proteins. VP1 proteins were analysed qualitatively and quantitatively by Western-blot and Coomassie Brilliant Blue staining, then used as an antigen in an indirect ELISA optimized. Seropositivity against eight human polyomaviruses, the age related optical density was examined in serum samples from children and adults (545-1030 samples/virus). Based on our results most of the human polyomaviruses are ubiquitous in the population, while for some polyomaviruses (e.g. Human polyomavirus 10, 12) the observed overall seropositivity was < 50 %. Childhood primary infection and infection increasing with ages was detected. Geographically different distribution of the viruses was not detected, and our result are in accordance with data of other research teams or with data published from Europe.

Acknowledgements: Supported by the National Research, Development and Innovation Office (NKFIH, FK18, FK128533). E. Cs. also supported by the János Bolyai Research Scholarship from the Hungarian Academy of Sciences.

INTRODUCTION OF A NEW AND 'CUTTING EDGE' MOLECULAR MICROBIOLOGICAL DIAGNOSTIC LABORATORY - ELTE BIOTECHNOLOGY FIEK

IMRE KACSKOVICS

ELTE Biotechnology FIEK, Faculty of Natural Science, ELTE - Eötvös Loránd University, Budapest, Hungary

The 'cutting edge' 440-square-meter molecular microbiology laboratory, created with an investment of almost HUF 1 billion, started operating at the beginning of summer 2022 on the Lágymányos Campus of ELTE. The capacities of the ultramodern laboratory will be available as a service to interested industrial partners, the clinic, the pharmaceutical industry, as well as domestic health insurance and the public. The infrastructure of the laboratory and the high level of professional training of the workers there can support clinical tests (this includes institutes conducting clinical tests and drug development and manufacturing companies), and diagnostic tests of suitable quality for the general public. In the accredited microbiological diagnostic laboratory, various diagnostic methods were first introduced in connection with COVID-19, primarily the direct detection of SARS-CoV-2 virus nucleic acid using the real-time PCR method from nasopharyngeal samples. Furthermore, it became important to implement a serological method suitable for detecting the amount of antibodies produced in the body as a result of the virus or vaccinations. We can carry out all EU approved molecular microbiological diagnostic tests. We are also keen to develop new methods with interested partners. (Please contact: Phone: +36 30 9385699; E-mail: microbiologia.laboratorium@fiek.elte.hu)

Acknowledgements: Project no. FIEK_16-1-2016-0005 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the FIEK_16 funding scheme.

MANGANESE EFFECT REVISITED: THE IMPACT OF STEEL QUALITY IN THE BIOREACTOR

LEVENTE KARAFFA

Department of Biochemical Engineering, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

High-yield citric acid production by the filamentous Ascomycete fungus *Aspergillus niger* requires a combination of extreme nutritional conditions, of which maintaining a low manganese(II) ion concentration (<5 mg L^{-1}) is a key feature. Technical-scale production of citric acid predominantly uses stainless-steel tank fermenters, but glass bioreactors used for strain improvement and manufacturing process development also contain stainless steel components, in which manganese is an essential alloying element. In this lecture I will demonstrate that during citric acid fermentations manganese(II) ions are leaching from the bioreactor into the growth media, resulting in altered fungal physiology and morphology, and significant reduction of product yields. The leaching of manganese(II) ions is dependent on the fermentation time, the acidity of the culture broth and the sterilization protocol applied. High concentrations of manganese(II) ions during early cultivation led to a reduction in citric acid yield. However, the effect of manganese(II) ions on the reduction of citric acid yield diminished towards the second half of the fermentation.

DESCRIPTION OF TEN NOVEL MYCOVIRUSES BELONGING TO THE TOTIVIRIDAE FAMILY IN SIX DIFFERENT *UMBELOPSIS* STRAINS

TÜNDE KARTALI[1], ILDIKÓ CSERNETICS-NYILASI[2], ORSOLYA NÉMETH[2], GERGŐ SÁVAI[2], SÁNDOR KOCSUBÉ[2], LÁSZLÓ BODAI[3], NÓRA ZSINDELY[3], GÁBOR NAGY[3], CSABA VÁGVÖLGYI[2], TAMÁS PAPP[1, 2]

[1]ELKH-SZTE Fungal Pathomechanisms Research Group, Department of Microbiology; [2]Department of Microbiology; [3]Department of Biochemistry and Molecular Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Mucoromycota is a less explored group in respect of virus-harbouring, compared to Asco- and Basidiomycota. Recently, more and more mycoviruses are identified from the so-called early-diverging lineages of fungi. Most mycoviruses have linear double stranded RNA (dsRNA) genome and most of them are tipically symptomless (cryptic). However, in some cases the presence of the virus causes smaller or greater changes in the phenotype of their fungal host as well as they can reduce or enhance the fungal virulence. Five genera belong to the Totiviridae family, among them, fungal viruses are generally found the *Totivirus* and the *Victorivirus*. In this work, we have determined novel dsRNA viruses in six different *Umbelopsis* strains with next-generation sequencing (NGS). We have done the molecular characterization of ten mycoviruses, and all of them are members of the Totiviridae family. In two *Umbelopsis ramanniana* strains (CBS 478.63 and CBS 243.58) we have identified four viruses: *Umbelopsis ramanniana* virus 6 to 8. In *Umbelopsis gibberispora* CBS 109328 isolate we have described *Umbelopsis gibberispora* virus 1 and 2, in *Umbelopsis angularis* CBS 603.68 strain one virus was observed (*Umbelopsis ramanniana* virus 6b), *Umbelopsis dimorpha* virus 1a and 2 was determined in *Umbelopsis dimorpha* CBS 110039 isolate, while *Umbelopsis versiformis* CBS 473.74 strain contain *Umbelopsis dimorpha* virus 1b. The identified genomes contain two open reading frames (ORF) encoding the coat protein (CP) and the RNA dependent RNA polymerase (RdRp). In all of the newly identified viruses, it is predicted that translation results in a fusion protein via a rare +1 (or-2) ribosomal frameshift, which is different from the that of the most Totivirus genus members. Based on the phylogeny inferred from the RdRp sequences, eight viruses (UrV7, UrV8a, UrV8b, UgV1, UgV2, UdV1a, UdV1b, UdV2) belong to the genus *Totivirus*, while two mycoviruses (UrV6a, UrV6b) are placed into a yet unclassified but well-defined Totiviridae-related group. Northern blotting was used to identify the dsRNA molecules containing the virus genes. In those strains, which contained several dsRNA fragments, we also examined, which dsRNA fragment can correspond to a given viral genome.

Acknowledgements: Supported by project NKFI K131796 and grants ITM NKFIA TKP-2021-EGA-28 and ELKH 2001007.

ANTIBIOTIC RESISTANT BACTERIA IN SURFACE WATERS WITH SEWAGE AND MICROPLASTIC LOAD

EDIT KASZAB[1], DONGZE JIANG[1], EMÍLIA LAURA DZSUDZSÁK[1], BENCE PRIKLER[2], ADRIENN MICSINAI[2], SÁNDOR SZOBOSZLAY[1], ISTVÁN SZABÓ[1], BALÁZS KRISZT[1]

[1]Department of Environmental Safety, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő; [2]Wessling Hungary Ltd., Budapest, Hungary

Sewage is a verified source of antibiotic-resistant (AR) microorganisms, antibiotic resistance genes (ARGs), and antibiotics. These types of contaminations cannot be entirely removed by traditional wastewater treatment processes therefore, sewage discharge can play a role in the spread of AR microorganisms in the microbial ecosystem of surface water recipients. Simultaneously, sewage is a verified emitter of microplastic particles serving as potential abiotic surfaces for biofilm-forming AR bacteria. In Hungary, the consequences of the simultaneous spread of microplastics and AR bacteria are not deeply investigated. The aim of this work was the preliminary evaluation of some chosen Hungarian streams with sewage loads to isolate and identify AR bacteria with traditional cultivation methods. In 2022, five chosen Hungarian streams were sampled in three locations (upstream, at the point of sewage inlet, and downstream). Physical and chemical parameters and microplastic contamination were detected. The same water samples were analysed with traditional cultivation for the selective isolation of colistin (COL) and carbapenem (CRE) resistant microorganisms using Chromatic medium. *Pseudomonas aeruginosa* was isolated with a three-step protocol using asparagine enrichment, cetrimide agar, and acetamide broth. Isolates were identified with 16S rDNA sequencing. By the time of writing, 112 isolates were isolated and identified using the protocol mentioned above verifying the overwhelming dominance of carbapenem-resistant *Pseudomonas* and *Aeromonas* species among COL/CRE resistant microorganisms. *P. aeruginosa* was not only detectable in the location of the sewage inlet, but it was common in the downstream (but not upstream) samples verifying its sewage origin and its persistence in surface water receivers. Besides the commonly detectable *Serratia* and *Sphingomonas* isolates with intrinsic resistance to colistin, the most common COL-resistant taxa were *Allorhizobium* and *Pannonibacter*. Still, their occurrence did not show a correlation with the sewage load. Our results serve basic information about the frequency, occurrence, and survival of AR (colistin and carbapenem-resistant) bacteria in surface water environments under sewage pressure. Further investigations are required to identify the main AR genes or mechanisms that play a role in the phenotypically detectable resistance. Furthermore, the undergoing adherence assays can clarify the potential role of the isolated AR microorganisms in the colonization of microplastic particles.

Acknowledgments: Supported by project no. 2020-1.1.2-PIACI-KFI-2021-00239 and by the TKP2020-NKA-16. E. K. was supported by the János Bolyai Research Grant of the Hungarian Academy of Sciences.

DNA AND SEROPREVALENCE OF KI, WU, MALAWI AND SAINT LOUIS POLYOMAVIRUSES

MELINDA KATONA, KRISZTINA JELES, ESZTER CSOMA

Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

KI and WU polyomaviruses (KIPyV and WUPyV) were described in 2007 from respiratory samples. Although the respiratory transmission of both viruses is possible, and KIPyV and WUPyV DNA and antigens have been detected in respiratory samples, their pathogenic roles have not been clarified. It is suggested that KIPyV and WUPyV might be respiratory pathogens causing respiratory disease or might be co-infectious, opportunistic agent. Malawi and Saint Louis polyomaviruses (MWPyV and STLPyV) were described from stool samples in 2012 and 2013. Both of them have been found is respiratory samples, in adenoids, and transmission via respiratory secretions is also suggested for them. Seroprevalence studies revealed that KIPyV, WUPyV, MWPyV and STLPyV are widespread viruses, but the seropositivity rates in the healthy, adult population published are different. Our aim was to assess the antibody against KIPyV, WUPyV, MWPyV and STLPyV in immunocompetent individuals to reveal the seroprevalence in different age groups. We also studied the DNA prevalence of these viruses in respiratory samples from patients with or without SARS-CoV-2 infection. For the seroepidemiological study the major capsid protein (VP1) of the polyomaviruses were expressed in bacteria. An in-house, indirect ELISA was developed using the VP1 proteins as antigens. Seropositivity against KIPyV, WUPyV, MWPyV and STLPyV, the age related optical density was examined in serum samples from children and adults (619-705 samples/virus). DNA prevalence was examined in a total of 1030 nasopharyngeal samples using multiplex, real-time PCR. KIPyV, WUPyV, MWPyV and STLPyV were detected in respiratory samples, MWPyV showed the highest prevalence. Association between SARS-CoV-2 and polyomavirus infections was not found. Seroprevalence study revealed high seropositivity in the adult population for all the viruses studied, however the rates are different. The overall seropositivity was 93 %, 89.2 %, 45.6% and 73.3 % for KIPyV, WUPyV, MWPyV and STLPyV. The seropositivity increased significantly with age for all viruses studied, although > 45 % of children < 6 years was seropositive for KIPyV, WUPyV and STLPyV. Seroprevalence difference in sexes was not detected.

Acknowledgements: Supported by the National Research, Development and Innovation Office (NKFIH, FK18, FK128533). E. Cs. was also supported by the János Bolyai Research Scholarship from the Hungarian Academy of Sciences.

LESSONS TO BE LEARNED: ARTHROPOD-BORNE DISEASES IN EUROPE AND HUNGARY

GÁBOR KEMENESI[1], GÁBOR FÖLDVÁRI[2], FERENC JAKAB[1], KORNÉLIA KURUCZ[1]

[1]Szentágothai Research Center, Institute of Biology, Faculty of Sciences, University of Pécs, Pécs; [2]Institute of Evolution, Centre for Ecological Research, Eötvös Loránd Research Network, Budapest, Hungary

Arthropod vectors and arthropod-borne diseases are expected to become one of the most significant public health and animal health problems in the near future as a result of climate change. This is especially true for Europe, where the problem caused by invasive biting mosquitoes or newly emerging vector species is already showing itself at the level of human health. The dynamics and epidemiological significance of pathogens spread by arthropod vectors are influenced by many factors, from population immunity, ecological and vector characteristics of the vectors to climate. The key to effective defense is a holistic approach to the problem, thus examining and understasnding all these factors together. In the presentation, the problems that most affect our country will be presented and, through a few examples (*Hyalomma* ticks, Invasive mosquitoes, West nile virus, *Dirofilaria* parasites), we will present the ongoing efforts and researches that aim to follow this holistic approach for a deeper understanding of the problem. The results of the genetic and disease-ecological study of the invasive biting mosquito species and the study of the Korean mosquito (*Aedes koreicus*) show that there is an urgent need for a change of attitude in the protection against vectors and close cooperation between the research units and executive bodies dealing with the subject. The lessons learned from our entomological outbreak analysis and human disease investigations carried out in the last 10 years are also presented together with the lessons that can be learned.

ROOTSTOCK GENOTYPE INFLUENCES PHYSIOLOGICAL RESPONSE AND MICROBIOME COMPOSITION AND DIVERSITY ON GRAPEVINE

GLODIA KGOBE[1, 2], ZSOLT ZSÓFI[3], ADRIENN GEIGER[1, 2, 4], CARLA MOTA LEAL[1, 2], ADRIENN TÓTH[3], SZABOLCS VILLANGÓ[3], LILI MÉZES[4], JÓZSEF GEML[1, 2, 4]

[1]Doctoral School of Environmental Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő; [2]ELKH-EKKE Lendület Environmental Microbiome Research Group; [3]Institute of Viticulture and Oenology; [4]Center for Food Science and Wine Science, Eszterházy Károly Catholic University, Eger, Hungary

Environmental factors have long been found to have significant effects in viticulture. Soil water availability is among the major elements that affect the growth and productivity of grapevine. The first organs to sense water deficiency and signal for the initiation of several responses to this abiotic stress are usually root systems. The use of commercial rootstocks in the vine industry is essential due to their capacity to adapt and deliver preferable traits to the scion. Environmental aspects that alter plant physiology may also influence plant-associated microbiome, including pathogenic, symbiotic, and commensal fungi that could directly affect plant health. Therefore, it is crucial to understand factors that have an impact on the structuring and function of plant microbiome. The main objective of this study was to assess the influence of rootstock and related changes in scion leaf physiology on the composition and diversity of fungi in grapevine leaves. To achieve this, we conducted fungal DNA metabarcoding of leaf samples taken in August and September 2020 from the Nagy-Eged hill terroir in Northern Hungary. Composition, richness, and abundance of fungal functional groups were compared among the rootstocks and the sampling months using ANOVA and NMDS ordination. The results showed significant differences in both the physiological measurements and leaf microbiome composition among rootstocks and months. Temporal variation illustrated more significant differences in the composition, richness and abundance of fungal communities compared to the different rootstocks analysed in one month. The interaction between rootstock and month explained the highest proportion of variance for the composition of the fungal community. Further analyses to determine the correlation between microbial composition and the rate of transpiration, rate of assimilation, and stomatal conductance on leaves will provide insightful knowledge on the relationship between physiological response and leaf microbiome under chronic summer drought conditions.

TRANSMISSION OF PLANT PATHOGENS BY VECTORS - AN OVERVIEW

BALÁZS KISS

Plant Protection Institute, Centre for Agricultural Research, Eötvös Loránd Research Network, Budapest, Hungary

Plant pathogens rely on vectors to various degrees in infecting healthy plants. For some, transmission by vectors is just an option, while others depend unavoidably on their highly adapted relationships with a single vector species. In the simplest cases, non-specific vectors, e.g. pollinators, can carry the pathogens mechanically on their body to new infection sites. In more specific pathogen-vector interactions, pathogens are transmitted by phylogenetically closely related vectors or by only one vector species. Apart from animal vectors, some root parasitic fungi also transmit soil-born plant viruses where zoospores can carry the virus particles externally or within the fungal cytoplasm. A surprising number of plant viruses are transmitted by insects with chewing mouthparts. The key feature of the specificity of beetle-transmitted viruses is their ability to rapidly move in the plant's vascular system, which permits the virus particles to escape from insect regurgitant at the wound surface. The majority of specific vectors of plant pathogens belong to arthropods with piercing-sucking mouthparts, namely hemipteran insects, thrips and acari. The diameter of their stylets permits the puncture of individual plant cells and the tapping of the vascular system without seriously hurting the plant tissues. Electropenetrography allows real-time monitoring of probing and feeding behaviour, i.e. the visualisation of stylet activities like pathway building, cell puncture, phloem or xylem feeding or salivation, which play a major role in the transmission of pathogens. Aphids are notorious vectors of plant diseases in temperate climates, mainly due to their extreme ability to transmit stylet-born viruses. These viruses are typically acquired and transmitted to epidermal plant cells during gustatory probes. Phloem feeder hemipterans (aphids, whiteflies, most leafhoppers, psyllids etc.) transmit several phloem-limited circulative pathogens. For instance, phytoplasmas are vectored by leafhoppers, planthoppers or psyllids. Only a few hemipterans specialised in xylem feeding; among them, *Philaenus spumarius* became the key vector of *Xylella fastidiosa*, a devastating bacterial disease introduced from North America to Europe. Mesophyllum feeder hemipterans (e.g. Typhlocibinae, Tingidae) feed destructively, which doesn't favour the transmission of plant pathogens. A dozen thrips species are the only vectors of the members of Tospovirus genus. Despite the apparent similarity of the mouthpart, thrips are phylogenetically far from hemipterans, while Tospovirus is the only plant pathogen genus in the Bunyaviridae family. Plant pathogen vectors are also known in two families of acari (Eriophyidae, Tetranychidae). Apart from arthropods, ectoparasitic phytonematodes also transmit specific plant viruses. Nematod's stylet can penetrate the individual cells of plant roots and pump cytoplasm without killing the cell. The transmission is non-circulative; virus particles are retained for a longer time in different parts of the oesophagus. The number of revealed interactions of plant pathogens and vectors continuously increases, and the establishment of alien pathogens and vectors generates new vector relations.

FUNCTIONAL ANALYSIS OF bZIP TRANSCRIPTION FACTORS AtfA AND AtfB IN *ASPERGILLUS NIDULANS*

BEATRIX KOCSIS[1, 2, 3], MI-KYUNG LEE[4], JAE-HYUK YU[5, 6], TIBOR NAGY[7], LAJOS DARÓCZI[8], GYULA BATTA[9], ISTVÁN PÓCSI[1, 3], ÉVA JULIÁNNA LEITER[1, 3]

[1]Department of Molecular Biotechnology and Microbiology, Faculty of Science and Technology; [2]Doctoral School of Pharmaceutical Sciences, Faculty of Pharmacy; [3]ELRN-UD Fungal Stress Biology Research Group, Faculty of Science, University of Debrecen, Debrecen, Hungary; [4]Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Jeongeup-si, Republic of Korea; [5]Department of Bacteriology, Food Research Institute, University of Wisconsin-Madison, Madison, USA; [6]Department of Systems Biotechnology, Konkuk University, Seoul, Republic of Korea; [7]Department of Applied Chemistry; [8]Department of Solid State Physics; [9]Department of Genetics and Applied Microbiology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

The eukaryotic bZIP type transcription factors AtfA and AtfB, regulating secondary metabolism, sexual development and stress responses, play critical roles in the organismal response to the environment. To examine the physiological functions of these bZIPs we constructed and phenotypically studied deletion and overexpression mutants of *atfA* and *atfB* in all combination (*ΔatfA*, *ΔatfB*, *ΔatfAΔatfB*, *ΔatfAatfBOE*, *ΔatfBatfAOE*, *atfAOE*, *atfBOE* and *atfAOEatfBOE*) in *Aspergillus nidulans*. We studied the stress sensitivity of the mutants with stress agar plate assays, in the presence of oxidative (2.0 mM diamide, 0.8 mM tert-butyl hydroperoxide or 0.08 mM menadione sodium bisulfite), hyperosmotic (2.0 M sorbitol or 1.5 M NaCl), heavy metal (300 μM cadmium chloride) and cell wall stress (54 μM CongoRed) generating agents. *ΔatfAatfBOE*, *ΔatfAΔatfB*, *atfAOEatfBOE* mutants showed increased sensitivity to the oxidative stress inducing agent diamide. Only one mutant, *ΔatfA* was sensitive to MSB, while the overexpression of *atfB* compensated this sensitivity in *ΔatfAatfBOE* mutant. *atfAOE*, *atfBOE*, *atfAOEatfBOE* showed increased tolerance to tBOOH meanwhile *ΔatfA* as well as *ΔatfAΔatfB* were sensitive to tBOOH. *atfAOEatfBOE* mutant showed increased tolerance to NaCl. The growth of *ΔatfB* mutant significantly reduced in the presence of NaCl, however this mutant was the most tolerant to sorbitol. After heavy metal stress treatment the growth of *ΔatfAatfBOE* mutant was slightly reduced but in *atfBOE*, *atfAOEatfBOE* mutants showed sensitivity to $CdCl_2$. The cell wall stress inducing CongoRed affected only the *ΔatfA* mutant, moderate tolerance was observed. Quantitative determination of the sterigmatocystin production was carried out by HPLC analysis from the point-inoculated surface cultures incubated for 5 days at 37°C. The production of this mycotoxin was reduced in *ΔAtfAatfBOE* and *ΔatfBatfAOE* and *AtfAOEatfBOE* mutants. The deletion of *atfA* led to the loss of sterigmatocystin production while *ΔatfAΔatfB* was able to synthesize this compound. We also determined the size of conidiospores. Based on light and scanning electron microscopy images, *atfBOE* mutant can be characterized by larger spore size compared to that of the control strain. We also tested the viability of the conidiospores under 50°C thermal stress for 10 min. *ΔatfAatfBOE* and *ΔatfBatfAOE* showed increased viability, meanwhile conidia of the *ΔatfB* showed reduced viability compared to the control strain. Conidiospore production was also quantified in all mutants. In *ΔatfA*, *ΔatfAΔatfB*, *atfBOE* mutants reduced conidiospore formation was observed, while in *ΔatfBatfAOE* the number of asexual spores increased compared to the control. We are also planning bimolecular fluorescence complementation experiments (BiFC) for the confirmation of the possible AtfA-AtfB heterodimer formation in vivo.

ETIOLOGIC AND PATHOGENIC ROLES OF PERSISTENT VIRAL INFECTIONS

JÓZSEF KÓNYA

Department of Medical Microbiology, Faculty of Medicine, University of Debrecen

The course of a persistent infection is classified as either latent or chronic or slow infection. During the persistent infectious course, the host suffers limited but either prolonged or repeating exposures from the microorganism in concern. As a result, a disease can or cannot develop and even if a disease manifested, typically a long term infectious course with accumulating injuries will eventually lead to the pathologic condition. This is to discuss the etiologic and pathogenic roles in three different virus-disease associations, namely, human papillomaviruses in cervical carcinogenesis, herpes viruses in periapical periodontitis, and measles virus in otosclerosis. The methodological approach applied in the projects was based on molecular Koch postulates involving cirteria for disease associations, consistency, time factor, reversibility and plausibility with a final goal to build evidences for the roles of infections in the investigated diseases.

IMPACT OF N-ACYL-HOMOSERINE LACTONE, QUORUM SENSING MOLECULE, ON *CANDIDA AURIS* ISOLATES

FRUZSINA KOVÁCS[1, 2], NOÉMI BALLA[1, 2], ZOLTÁN TÓTH[2], ÁGNES JAKAB[2], LÁSZLÓ MAJOROS[2], RENÁTÓ KOVÁCS[2]

[1]Doctoral School of Pharmaceutical Sciences, Faculty of Pharmacy; [2]Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Candida albicans-Pseudomonas aeruginosa mixed infections and/or colonization are commonly found in various clinical cases. This mixed species cooperation is well studied; however, the number of data focusing on *P. aeruginosa*–non-albicans species (as *C. auris*) are limited. *P. aeruginosa* significantly affects the morphological and virulence-related properties of *C. albicans*. This effect was mediated primarily by N-3-oxo-C12 homoserine lactone (3OC12HSL), a molecule studied for its role in cell-cell signalling. In this study, we examined the effect of 3OC12HSL on *C. auris* growth and invasion ability by epithelial transmigration assay with Caco-2 human epithelium model. In growth-related experiments, *C. auris* pre-cultures were grown in 5 mL yeast peptone dextrose (YPD) medium at 37ºC for 18 hours, diluted to an optical density of 0.1 (OD640) with YPD then grown further at 37ºC and at 2.3 Hz shaking frequency. Following a 4-hour incubation period, some cultures were supplemented with 3OC12HSL (200 μM and 400 μM), and microbial growth was followed by measuring changes in optical density. In invasion-related experiments, the effect of 200 and 400 μM 3OC12HSL were tested using Transwell cell culture insert. To obtain monolayers, 6×10^4 Caco-2 cells of the same passage number were seeded into Transwell cell culture inserts with 8 μm pore size, 1×10^5 pores per cm^2 density and 0.33 cm^2 area, polycarbonate membrane, and placed in 24 well plates. In all cases, the volume of the apical compartment was set to 200 μL and the basolateral was set to 1250 μL. Before the infection, *C. auris* strains grown overnight at 30 °C in YPD were washed with PBS and resuspended in the cell culture medium in 1×10^6 cells mL^{-1} concentration and were put into the apical compartment and incubated at 37°C in a humidified atmosphere of 5% CO_2. The medium in the apical and basolateral compartments were changed daily without disturbing the developing yeast layer. At 12, 24, 48 and 72 hours quantitative culturing was performed to determine the number of migrated *Candida* cells. Growth was significantly inhibited within 2 hours after the addition of 3OC12HSL as assessed by observed absorbance values (1.17±0.02, 1.05±0.007 and 0.97±0.01 for untreated control 200 μM-exposed and 400 μM-exposed cells, respectively), at OD640) ($p<0.05$) and quantitative culturing. The 3OC12HSL significantly enhanced the invasion of *C. auris* cells in concentration dependent manner compared to untreated control and *C. albicans* SC5314 reference strain ($p<0.01$-0.05). Our results help to understand the cell-cell relationship between *C. auris* and *P. aeruginosa*.

Acknowledgements: R. K. was supported by the Janos Bolyai Research Scholarship of the Hungarian Academy of Sciences. Research was supported by the Hungarian National Research, Development and Innovation Office (NKFIH FK138462).

DEVELOPMENT OF PCR FOR THE SPECIFIC DETECTION OF *BOTRYTIS CINEREA*

MÓNIKA KOVÁCS, ÁGNES BELÁK, ANDREA TACZMAN-BRÜCKNER, ANDREA POMÁZI

Department of Food Microbiology, Hygiene and Safety, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary

Botrytis cinerea is considered as one of the most important plant pathogenic fungi due to its broad host specificity and high degree of damage causing ability. Detecting the presence of the fungus before the appearance of symptoms is therefore very important, but very challenging. Most of the PCR-based diagnostic methods developed so far target different regions of the rRNA gene cluster, but results have also been achieved with primers designed for other genes. The aim of this study was to develop a PCR method based on gene sequences different from the previous ones, which enables the specific detection of *B. cinerea*. Based on data from data banks and publications, we selected three genes for development: *BMP1* and *BOS5*, which encode kinases (MAPK, MAPKK) that play an important role in intracellular signaling processes and through this in pathogenesis, and the *BcLCC2* gene, which encodes a laccase and whose role in pathogenicity can also be assumed. We designed primers for the three selected *B. cinerea* genes (*BMP1*, *BOS5* and *BcLCC2*). Since greater diversity can be expected in the case of introns, we designed the primers for the conservative regions of the exon sequences. During the planning, we took into account that the length of the sequence to be amplified should not exceed two hundred base pairs. The primers were characterized based on secondary structure and expected specificity. We designed and tested a total of 40 primer pairs for the three genes, and then selected those with the appropriate parameters. For *BMP1* one (Bmp1), for *BOS5* two (Bos-X1, Bos-X2), while for *BcLCC2* three (Lcc-X1-1, Lcc-X1-2, Lcc-X4) sets seemed suitable for further experiments. As a first step, we performed and, if necessary, optimized the PCR reactions by adding *B. cinerea* genomic DNA to the reaction mixture, and determined the appropriate annealing temperatures in terms of specificity. With the exception of Bos-X2, a specific product was formed in all cases, but in some reactions, despite the optimization, non-specific extra products were also generated. Among the reactions performed, the Bmp1, Bos-X1 and Lcc-X1-2 were deemed suitable for further experiments, in which we also performed the reactions with the involvement of other fungal species. Based on the results we found that the Lcc-X1-2 primer pair produces an amplicon only in the case of *B. cinerea* (the specific product is about 150 bp in size), thus the primer pair designed for the *BcLCC2* laccase gene is specific for *B. cinerea* species, and so we plan to continue our real-time PCR tests with these oligonucleotides.

LIBERATION OF PHENOLIC COMPOUNDS FROM SORGHUM GRAIN SAMPLES BY ZYGOMYCETES FUNGAL ENZYMES

TAMÁS KOVÁCS[1], ÍTALO JOSÉ LEMOS DE OLIVEIRA[1], BETTINA VOLFORD[1], DÓRA ANNA PAPP[1], MÓNIKA VARGA[1], BERNADETT MIHÁLY-LANGÓ[2], ANDREA PALÁGYI[2], CSABA VÁGVÖLGYI[1], MIKLÓS TAKÓ[1], JUDIT KRISCH[3]

[1]Department of Microbiology, Faculty of Science and Informatics, University of Szeged; [2]Cereal Research Non-profit Ltd.; [3]Institute of Food Engineering, Faculty of Engineering, University of Szeged, Szeged, Hungary

Sorghum bicolor (L.) Moench is the fifth most produced grain crop worldwide. There is growing interest towards sorghum as human food since it has favorable nutritional profile and contains bioactive compounds. Phenolics are important secondary metabolites of plants and have beneficial effects on human health through their antioxidative, antimicrobial, anti-inflammatory, anti-proliferative and anti-diabetic properties. In plant these metabolites mainly can be found in carbohydrate-glycoside or carbohydrate-ester forms, which reduce their bioavailability. Treatment with cellulase and lipase enzymes can be an eco-friendly strategy to liberate these bound/conjugated phenolic compounds. Although many zygomycetes are great extracellular enzyme-producers, their ability to enrich phenolics from grain samples is rarely investigated. In this work, we tested an enzymatic approach using cellulolytic and lipolytic cocktails from *Rhizomucor miehei*, *Gilbertella persicaria* and *Mucor corticolus* to liberate phenolics from grain of broom, GK Emese and Farmsugro 180 sorghum plants. Enzyme cocktails were produced in a wheat bran-based solid-state fermentation system and were partially purified by gel filtration before use. The obtained crude enzyme extracts diluted in acetate buffer (pH 6.0) had detectable cellulase and lipase activities. During liberation experiments, 1 g of grinded grain substrate was treated with 10 ml crude enzyme extract, and the reaction mixtures were incubated at 30°C (in case of *Gilbertella* and *Mucor* enzymes) or 50°C (in case of *Rhizomucor* enzyme) for 7 hours. Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity, i.e., free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl, ferric reducing antioxidant power, cupric ion reducing antioxidant capacity and oxygen radical absorbance capacity, measurements were carried out in the samples taken at predefined intervals. Results show that the enzymatic cocktail of *R. miehei* increased significantly the TPC, TFC and the antioxidant activity in all three sorghum grain samples. Peak values of the above-mentioned activities were observed at the early phases of the treatments thus a short incubation time is enough. The cocktails of *M. corticolus* and *G. persicaria* caused significant increases in case of the broom sorghum sample, however no or non-significant increases were observed in the other grains. According to our results, the cellulase/lipase treatment had a positive effect on the release of antioxidative phenolics from sorghum grain samples and *R. miehei* is a promising candidate for further experiments.

Acknowledgements: Supported by the grants NKFI FK134886 and ITM NKFIA TKP-2021-EGA-28.

ENHANCING THE STABILITY OF FUNGAL LIPASES BY IMMOBILIZATION ONTO ACCUREL MP 1000 SUPPORT

ALEXANDRA KOVÁCS-KOTOGÁN, TAMÁS PAPP, CSABA VÁGVÖLGYI,MIKLÓS TAKÓ

Department oif Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Lipases (E.C. 3.1.1.3) are versatile biocatalysts in the industry because they can catalyze different types of reactions, such as lipolysis as well as trans- and interesterification. The main limitation of the application is their moderate stability and activity at harsh conditions (e.g., high temperature, presence of organic solvents). Immobilization of enzymes can improve their stability, allow their reusability in multiple reactions, and facilitates the separation of the catalyst from the product. Therefore, enzyme-support complexes can be used more economically in industry than free enzyme preparations. Among the today's immobilization techniques, a well-applicable method for lipase entrapment is the adsorption onto a hydrophobic matrix, such as to Accurel MP 1000 (particle size < 1500 μm) polypropylene porous carrier. In this study, physical adsorption of commercial lipases from the fungi *Rhizopus oryzae*, *Rhizopus niveus*, *Aspergillus niger*, *Rhizomucor miehei* and *Candida rugosa* was carried out using Accurel MP 1000 support. After optimization of the binding, biochemical properties of the immobilized enzymes were analyzed with particular attention to the stability of the complex. Effect of glutaraldehyde treatment on the stability of created complexes was also examined. During the reusability tests, in general, the complexes retained more than 50% of their lipolytic activity for 3-6 cycles. Residual activity of the glutaraldehyde-treated complexes increased by 10-50%. The thermal stability of the immobilized lipases improved by 20-40%, which was further increased by 10-30% by glutaraldehyde treatment at 60°C. The enzyme-support complexes retained 60-70% of their activity after 24 hours incubation at pH 5.5, 7.0 and 8.5. Adsorption resulted in an increase in pH stability for *R. oryzae*, *R. miehei* and *C. rugosa* immobilized lipases compared to free enzymes. The glutaraldehyde treatment increased the pH stability of *A. niger* immobilized enzyme by 30-40%. Organic solvent stability of the immobilized enzymes was investigated after 4 hours incubation in the presence of solvents with different Log Pow values. The immobilized lipases were the most stable in hexane with 75-100% residual activity (Log Pow: 3.5). Glutaraldehyde treatment improved the stability of *R. oryzae*, *A. niger* and *R. miehei* enzyme preparations in organic solvents by about 10-20%. The solution of free enzymes was completely inactive after 3 months of storage at 5 and

25°C and it showed only 5-15% of the initial activity when stored at -20°C. At temperatures of -20,5 and 25°C, the immobilized enzymes retained 55-100% of their initial activity after 3 months of storage.

Acknowledgements: Supported by the grants NKFI FK134886 and ITM NKFIA TKP-2021-EGA-28.

ANTICANCER ACTIVITY OF SYMMETRICAL SELENOESTERS ON BREAST CANCER CELL LINES

ERZSÉBET KRISTÓF[1], BÁLINT RÁCZ[1], NIKOLETTA SZEMERÉDI[1], ENRIQUE DOMÍNGUEZ-ÁLVAREZ[2], GABRIELLA SPENGLER[1]

[1]Institute of Medical Microbiology, Albert Szent-Györgyi Medical School, University of Szeged, Hungary; [2]Spanish National Research Council (CSIC), Madrid, Spain

Breast cancer is a leading cause of cancer related death in women. The therapy for breast cancer besides radiotherapy and surgery involves the use of anthracyclines and taxanes. However, resistant tumors and metastatic tumors show poor response rates to the available therapeutic alternatives. Selenium (Se) and its derivatives have demonstrated promising anticancer activity, additionally in several previous in vitro studies selenoesters showed chemosensitizing activity. In the present study we evaluated the cytotoxic, antiproliferative, and chemosensitizing activity of novel symmetrical selenoesters with different functional groups on doxorubicin sensitive and doxorubicin resistant breast cancer cell lines. The cytotoxic and antiproliferative activities of Se-compounds were assessed by the thiazolyl blue tetrazolium bromide (MTT) assay on the doxorubicin sensitive MCF-7 human breast cancer cell line and its doxorubicin resistant KCR subline. To study the interaction of Se-compounds and the chemotherapeutic drug doxorubicin a checkerboard microplate method was used on the sensitive and resistant breast cancer cell lines. The apoptosis inducing activity of symmetrical selenoesters was evaluated using Annexin V-FITC/PI double staining on the doxorubicin sensitive MCF-7 and on the doxorubicin resistant KCR human breast cancer cell lines. All the evaluated compounds showed significant cytotoxic and antiproliferative effects, the most cytotoxic and most potent inhibitors of proliferation proved to be the Se-compounds with methyl-ketone moieties. On the KCR cell line except for one compound, all of the selenoesters demonstrated synergistic interaction with doxorubicin. Concerning apoptosis induction only one of the methyl-carbonyl containing selenoesters had activity similar to the positive control 12H-benzophenothiazine regarding early and late apoptosis on the KCR cell line, furthermore only one methyl-cyano selenoester induced late apoptosis in MCF-7 cells. In our study the symmetrical selenoesters displayed potent cytotoxic, antiproliferative, and apoptosis inducing activity, along with synergistic interaction with doxorubicin on sensitive and resistant breast cancer cell lines.

DNA-BASED COMPARISON OF LEAF- AND WOOD-ASSOCIATED FUNGAL COMMUNITIES BETWEEN GRAPEVINE AND WOODY ROSACEAE

LUCA ANNAMÁRIA LEPRES[1, 2], ANNA MOLNÁR[1], ADRIENN GEIGER[1, 2], KÁLMÁN ZOLTÁN VÁCZY[1], JÓZSEF GEML[1, 3]

[1]Institute of Biology, Eszterházy Károly Catholic Univesity, Eger; [2]Doctoral School of Environmental Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő; [3]ELKH-EKKE Lendület Environmental Microbiome Research Group, Eszterházy Károly Catholic University, Eger, Hungary

Grapevine (*Vitis vinifera*) is one of the major cultivated plants worldwide and is naturally colonized by commensal, beneficial or pathogenic microorganisms. Many of these can influence plant health condition and disease incidence and severity. Grapevine microbiome studies have primarily focused on grapevine plant parts and rhizosphere, while the influence of semi-natural ecosystems on the composition of the grapevine microbiome is practically unknown. There have been records of several plant pathogenic fungi being shared among grapevine and stone fruit and pome fruit trees, but we lack the systematic knowledge of possible interactions among fungal communities in these crops at landscape-scale. Here, we compared possible connections between grapevine microbiome and that of wild and cultivated woody Rosaceae in a landscape. Specifically, we generated and analyzed DNA metabarcoding data to assess the compositional overlap of leaf- and wood-associated fungi associated with grapevine, apricot (*Prunus armeniaca*), pear (*Pyrus communis*), dogrose (*Rosa canina*) and blackthorn (*Prunus spinosus*). Dominant genera of fungi included: *Cladosporium*, *Filobasidium*, *Aotearoamyces*, *Knufia*, *Nothophoma*, *Didymella*, *Buckleyzyma*, *Macrophomina* etc.

We found that both sampling source (leaf vs. wood) and host identity had strong influence on fungal community composition, explaining ca. 20% and 26.6% of compositional variance, respectively. The observed compositional overlap among grapevine and wild and cultivated Rosaceae fruit species living near vineyards suggests that a landscape-level approach is needed to better understand the microbiomes of grapevine and fruit trees, with implications for integrated crop protection.

Acknowledgements: Supported by the TKP2021-NKTA-16, National Research, Development and Innovation Office grant to the Eszterházy Károly Catholic University" and the Lendület Programme No. 96049 (ELRN and HAS).

DETECTION AND ANALYSIS OF THE GENETIC BACKGROUND OF RARE B-LACTAMASE GENES OF *BACTEROIDES* STRAINS

BAKHTIYAR MAHMOOD, ELISABETH NAGY, JÓZSEF SÓKI

Institute of Medical Microbiology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary

β-lacatamase production by β-lacatamase genes is well-known among *Bacteroides* spp. among which there are frequent and more significant (*cepA*, *cfiA*) and rare and less-characterized ones. However, all may be important contributors to β-lacatam resistance and genetics in cases of these bacterial species. Our aim in this study was to assess the prevalence and charcaterize of some of these genes described earlier. We detected the *cblA*, *crxA*, *blaHGD1 oxa347* and *pbbA* genes in the collections of *Bacteroides* strains from clinical (n=400) and faecal (n=184) samples by RT-PCR and used molecular methods for their characterization. *cblA* was *B. uniformis*-specific and all *B. uniformis* strains contained it in both collections. *crxA* was *B. xylanisolvens*-specific causing carbapenem resistance and could be found in 6 and 3 *B. xylanisolvens* strains from faecal and clinical samples, respectively. *crxA* was not clonal among *B. xylanisolvens* strains (contrary to *cfiA*) which implied a rate of mobility/emergence by independent evolutionary events. *blaHGD1* was *B.* (*Phocaeicola*) *vulgatus*-specific and could be found among all the *B. vulgatus* isolates from faecal (n=37) and clinical samples (n=26). No *oxa347* carrying isolate could be found in our collections despite its presence in a multidrug-reistant *B. fragilis* strain from Denmark. *pbbA* was mobile between *Bacteroides* species also causing β-lacatam/β-lacatamase inhibitor combination resistances in 3 clinical isolates. As exemplified by our results rare β-lacatamase genes should not be overlooked in β-lacatamase resistance of *Bacteroides* spp.

ALGAL-BACTERIAL INTERACTIONS AND THE INTERPLAY OF MACROMOLECULE ACCUMULATION AND FERMENTATION ON BIOHYDROGEN PRODUCTION

GERGELY MARÓTI[1, 2], PRATEEK SHETTY[1], ATTILA FARKAS[1], BERNADETT PAP[1], GABRIELLA HUSZÁR[1]

[1]Institute of Plant Biology, Biological Research Center, Eötvös Loránd Research Network, Szeged; [2]Seqomics Biotechnology Ltd., Mórahalom, Hungary

Past empirical research has demonstated that bacterial interaction is essential for enhancing algal growth, algal biohydrogen generation, and bioremediation. However, little is known about the mechanisms and microalgal functions activated under bacterial associations. To investigate this, different bacterial species were co-cultivated with *Chlamydomonas reinhardtii* cc124 green algae. Bacterial species were isolated from diverse environments, including biogas sludge, soil, and commercial biostimulants. Pairwise algal-bacterial combinations were cultivated for five days in synthetic wastewater. The accumulated biohydrogen was recorded daily and the specific algal growth rate was determined based on the variation in algal cell concentration. Successful bacterial candidates were identified by high algal biohydrogen production and algal biomass. We have investigated the effect of bacterial phylogenetic relationship and growth rate on algal growth, nutrient intake, and biohydrogen production. All bacterial interactions contributed to an increase in algal biomass. Importantly, we detected a species-specific influence of the Bacillaceae family members on the enhancement of algal biohydrogen generation. This impact of enhanced biohydrogen generation was also found when members of Bacillaceae family were co-cultivated with other algae, such as *Chlorella* and *Micractinium* sp.

THE RELATIONSHIP BETWEEN STAINLESS STEEL QUALITY AND PRODUCT YIELD DURING *ASPERGILLUS NIGER* CITRIC ACID FERMENTATIONS

ALEXANDRA MÁRTON, VIVIEN BÍRÓ, KATICA KRAMCSÁK, ANDREA KUN, ERZSÉBET FEKETE, LEVENTE KARAFFA

Department of Biochemical Engineering, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

The filamentous ascomycete fungus *Aspergillus niger* is frequently used as a platform organism in the fermentation industry: it is a prolific producer of organic acids, enzymes, and other metabolites. Most *A. niger* strains growing in liquid medium with high sugar content are superior producers 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. Citric acid production requires several specific technological/nutrient conditions, such us high sugar concentration (>10%, w/v), low pH (<2), high dissolved oxygen levels (>30%), suboptimal concentration of phosphate and some other trace elements. Among the metal ions, the concentration of manganese (II) ions (Mn) has the greatest effect on citric acid yield. Mn concentrations > 5 μg L^{-1} have been shown to drastically reduce citric acid yield due to altered fungal morphology and physiology. While an Mn-limited growth medium is crucial for high product yield, the medium can not remain completely Mn-

free either as this metal ion is often acts as a co-factor by a wide range of enzymes. A variety of methods have been used to remove the excess Mn from the culture broth: cation exchange of the growth medium, precipitation with ferrocyanide and chelation by phytic acid. The manganese limited culture environment does not last during the entire fermentation process: in fact, by the end of the process, Mn-concentration may exceed 30 µg L^{-1}. Industrial-scale production takes place in stainless steel bioreactors, but laboratory scale glass fermenters also contain stainless steel components. Corrosion of the surface may lead to metal ion leaching, which depends on the culture broth pH, sterilization protocol and fermentation time. Here we analyse the mechanism and kinetics of Mn-leaching from the stainless steel components of 6 L-scale glass bioreactors during optimized *A. niger* citric acid fermentations, and discuss its impact on product accumulation.

Acknowledgements: Supported the Hungarian National Research, Development & Innovation Fund, grant K 138489.

DIFFERENT FATES OF THE ACQUIRED ALTERNATIVE OXIDASE *aoxB* PARALOGOUS GENE IN STRAINS AND ISOLATES OF *ASPERGILLUS NIGER*

ALEXANDRA MÁRTON, VIVIEN BÍRÓ, KATICA KRAMCSÁK, ANDREA KUN, ERZSÉBET FEKETE, MICHEL FLIPPHI, LEVENTE KARAFFA

Department of Biochemical Engineering, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

Ubiquinol:oxygen oxidoreductase [non-electrogenic] (EC 1.10.3.11) is a terminal oxidase in branched mitochondrial electron transport. The enzyme transfers electrons from ubiquinol directly to molecular oxygen, producing ubiquinone and water, while releasing the chemical energy associated with these electrons as heat instead of using that energy to generate proton motive force. This "alternative oxidase" (AOX) thus bypasses the electrogenic cytochromes downstream in the respiratory chain, decelerating the build-up of the proton gradient over the inner membrane. It contributes to sustained recycling of reduction equivalents (NADH, NADPH & $FADH_2$) into their oxidized forms without excessive ATP production through oxidative phosphorylation, dissipating surplus reducing power whilst decreasing oxidative stress, an inevitable byproduct of the action of the electrogenic components of the respiratory chain. AOX is near ubiquitous in the fungal kingdom. Notably it is absent from the ascomycete yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, generally considered genetic "model" species. However, our comparative survey established the widespread presence of multiple paralogous aox genes within the Aspergillaceae family (Ascomycota; Pezizomycotina; Eurotiomycetes; Eurotiales). Different duplication events gave rise to an aox paralogous gene in *Aspergillus terreus* (section Terrei) and *Aspergillus niger* (section Nigri), species that are used to produce organic acids at industrial scale. The second *aox* gene in *A. niger* and *A. welwitschiae* is unique within all three taxonomical series in the section Nigri for which representative species have been genome sequenced but it also occurs in two species in the subgenus Nidulantes (members of different sections) and in one *Penicillium* species. In these four fungi, the *aoxB* paralog is orientated divergently from a paralog of an otherwise ubiquitous gene for a type-II alternative NADH dehydrogenase (NADH:ubiquinone oxidoreductase [non-electrogenic]: EC 1.6.5.9). This is a non-proton pumping enzyme that bypasses complex I of the respiratory chain, complementing one of the effects of non-electrogenic AOX. Interestingly, the acquired aoxB gene in genome-sequenced strains of *A. niger* features genetic variation at a level not detected in the ubiquitous *aoxA* gene. We found four mutations that inactivate the encoded enzyme directly or, at the least, terminally modify the *aoxB* gene product. One of these is a deletion that removes exon 1 and intron 1 from the *aoxB* gene but also includes some 60 % of the coding regions of the divergently orientated alternative NADH dehydrogenase paralogous gene. We could thus subdivide the formal species *A. niger* into five groups (variants; cryptic species; species complex) according to their *aoxB* allele.

Acknowledgements: Supported the Hungarian National Research, Development & Innovation Fund, grant K 138489.

COMPARISON OF SOIL BACTERIAL COMMUNITIES UNDER DIFFERENT AGRICULTURAL LAND USES

DALMA MÁRTON[1], MILÁN FARKAS[1], NEVEEN MAJDI ALMALKAWI[1], GERGELY MARÓTI[2, 3], ROLAND WIRTH[4], ANDRÁS TÁNCSICS[1], TAMÁS SZEGI[5], BALÁZS KRISZT[1], MÁTYÁS CSERHÁTI[1]

[1]Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő; [2]Institute of Biochemistry, Biological Research Center, Eötvös Loránd Research Network, Szeged; [3]Seqomics Biotechnology Ltd., Mórahalom; [4]Department of Biotechnology, Faculty of Science and Informatics, University of Szeged, Szeged; [5]Institute of Environmental Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

Agricultural soil management practices have an important effect on soil fertility. Unfortunately, soil microorganisms are not used as everyday indicators of soil quality, but should be considered by the landowners in the future, because they play a critical and important role in ecological processes of agricultural systems. The indigenous ecosystem diversity was reduced by last 50 years agricultural activity (especially arable cultivation). Different types of vegetation and intensive agricultural practices can

highly influence the composition and activity of the soil microbial community and soil physicochemical properties. Very little information is available of soil bacteria characterization connected to the minor natural soil types of the Carpathian-basin. Our research is trying to fulfil these lacks, and collect the missing information from the main soil types. In the present study we aimed to describe the bacterial communities from different agricultural land use soils. Precision agriculture crop management were used on Boldog and Székesfehérvár sites. The first site is 20 ha arable area, which is portioned into 13 management zones according the soil parameters. It is a perfect example how the micro-geographical soil diversity shapes the microbial community. The effect of main different land-use types: arable land, pasture land and forest on the same soil types were investigated at Galgahévíz and Hort areas. Illumina 16S rDNA amplicon sequencing was used to reveal the bacterial community composition of the precision farming sites, while Illumina metagenom sequencing was used to assess precisely the microbial community diversity of arable-, pasture land and forest. Furthermore, we also examined the physical and chemical properties of the soil samples of the sites. The soil samples were dominated by microorganisms belonging to Actinobacteria, Proteobacteria and Acidobacteria. Generally, the results indicate that soil type, $CaCO_3$, humus content and pH play a key role in shaping bacterial communities of the investigated agricultural lands.

Acknowledgements: Funded by 2020-1.1.2-PIACI-KFI-2020-00020. D. M. was supported by ÚNKP-22-3, New National Excellence Program of The Ministry For Innovation And Technology.

ISOLATION AND IDENTIFICATION OF BACTERIAL STRAINS CAPABLE OF DECOMPOSING DRUGS

TAMÁS MIREISZ[1, 2], RÓZSA FARKAS[1], FLÓRA BOGLÁRKA HORVÁTH[1], GORKHMAZ ABBASZADE[1], ERIKA TÓTH[1]

[1]Department of Microbiology, Faculty of Science, ELTE-Eötvös Loránd University; [2]Budapest Waterworks Inc., Budapest, Hungary

Organic micro-pollutants have been targeted by science and the media, as medicines used in large quantities (such as anti-inflammatory drugs) are now endangering our drinking water. Quantitative detection of organic micropollutants and investigation of their effects in natural environments is a serious challenge due to their significant sorption, accumulation properties and very low concentrations. During our research, selective enrichment was carried out in drug-containing microcosms (ibuprofen, diclofenac, acetylsalicylic acid) derived from Danube water samples. Bacteria were isolated on culture media containing the given drug as the sole source of carbon. Isolated bacteria (62 strains) were determined based on their 16S rRNA gene and then classified into 25 different bacterial species. Based on literature data, the analysis of the genome of the type species were done, and gene clusters involved in the degradation of aromatic compounds were examined. Among the identified species, we found only one Gram-positive bacterium (belonging to the Firmicutes: *Paenibacillus latus*), all other bacteria are Gram-negative belonging to Proteobacteria phylum. In the case of samples loaded with diclofenac, members of the genus *Aeromonas*, *Klebsiella* and *Rahnella* (Gammaproteobacteria) were isolated in the largest number. In the case of samples loaded with ibuprofen, members of the genus *Klebsiella* (*Klebsiella quasivariicola*, *Klebsiella pneumoniae*, *Klebsiella huaxiensis*, *Klebsiella michiganensis*) were dominant, but *Pseudomonas* (*Pseudomonas aeruginosa*, *Pseudomonas nitroreducens*, *Pseudomonas nitritireducens*) species were also present. In the acetylsalicylic acid samples, in addition to the most typical *Burkholderia* genus, other Betaproteobacteria and Alphaproteobacteria were also found. During the tests, only *Sphingobium yanoikuyae* and *Klebsiella michiganensis* were able to grow both on the media containing diclofenac and ibuprofen. During the examination of the aromatic metabolic genes, 7 different metabolic pathways were identified, of which the catechol branch of the beta-ketoadipate pathway as well as the salicylate and gentisate catabolism are the most significant. The genes of the catechol branch of the beta-ketoadipate pathway are found in 78 % of the examined bacteria, while the genes encoding salicylate and gentisate catabolism are found in 70 %. Due to the functioning of the aromatic metabolic genes identified during the research, the microorganisms are presumably able to use the studied pharmaceutical molecules, thereby reducing the concentration of organic micropollutants in the environment, but it needs further studies.

PRODUCTION OF MUSHROOM COMPOST USING DIFFERENT RAW MATERIALS

ANDRÁS MISZ[1], AMANDA SÁNDORNÉ SZŐKE[1], NÓRA BAKOS-BARCZI[1], MARIANNA VISNYEI[1], HENRIETTA ALLAGA[2], LÁSZLÓ KREDICS[2], CSABA VÁGVÖLGYI[2], CSABA CSUTORÁS[1]

[1]Uj Champignons Ltd., Budapest; [2]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

The basis for the effective and economical cultivation of button mushroom (*Agaricus bisporus*) is the provision of high-quality propagating material and mushroom compost. The mushroom compost covered with a casing layer and interwoven with the mycelium of the mushroom must meet a number of chemical, physical and biological parameters to ensure the expected crop yield. The button mushroom compost has an appropriate structure, is rich in nutrients, free of pathogens and pests, and is a selective growing medium for *Agaricus*, the main ingredient of which is wheat straw with a high cellulose and hemicellulose content. The latter also plays a key role as a carbon source in the proper fruiting body formation. During our experiments, we examined the effect of different compost materials from the commonly used ones on the process of growing button mushroom.

In addition to the wheat straw used in the traditional technology, we produced compost from the straw/stem residues of other cereals (rye, rapeseed), or their combinations. We examine the physical, chemical and microbiological changes that take place during the composting process in the composts produced in this way, using classical and molecular methods. The composition of the microbial communities in the composts with changed composition is examined by metagenome analysis, and their practical applicability is evaluated in cultivation experiments.

Acknowledgements: Supported by the Cooperative Doctoral Program Doctoral Student Scholarship program (A. M.) and by Hungarian Ministry for Innovation and Technology (2020-1.1.2-PIACI-KFI-2020-00111).

UNDERSTANDING RELATIONSHIPS AMONG GRAPEVINE CHEMICAL AND PHYSIOLOGICAL PARAMETERS AND LEAF AND BERRY MYCOBIOME COMPOSITION

ANNA MOLNÁR[1], JÓZSEF GEML[1, 2], ADRIENN GEIGER[1, 3], CARLA MOTA LEAL[2, 3], GLODIA KGOBE[3], ADRIENN MÁRIA TÓTH[4], SZABOLCS VILLANGÓ[4], LILI MÉZES[1], ATTILA MÁRK CZEGLÉDI[1], GYÖRGY LŐRINCZ[4], ZSOLT ZSÓFI[4]

[1]Food and Wine Research Institute, Research and Development Center; [2]ELKH–EKKE Lendület Environmental Microbiome Research Group, Eszterházy Károly Catholic University, Eger; [3]Doctoral School of Environmental Sciences, Hungarian University of Agricultural and Life Sciences, Gödöllő; [4]Institute for Viticulture and Enology, Faculty of Natural Sciences, Eszterházy Károly Catholic University, Eger, Hungary

Improving our knowledge on biotic and abiotic factors that influence the composition of the grapevine mycobiome is of great agricultural significance, due to potential effects on plant health, productivity, and wine characteristics. Here, we assessed the influence of scion cultivar on the diversity and composition of fungal communities in the berries and leaves of three different cultivars. We generated DNA metabarcoding data, and statistically compared the richness, relative abundance, and composition of several functional groups of fungi among cultivars, which are partly explained by measured differences in chemical composition of leaves and berries and physiological traits of leaves. Fungal communities in leaves and berries show contrasting patterns among cultivars. The richness and relative abundance of fungal functional groups statistically differ among berry and leaf samples, but less so among cultivars. Community composition of the dominant functional groups of fungi, i.e., plant pathogens in leaves and saprotrophs in berries, differs significantly among cultivars. We also detect cultivar-level differences in the macro- and microelement content of the leaves, and in acidity and sugar concentration of berries. Our findings suggest that there appears to be a relatively diverse set of fungi that make up the grapevine mycobiome at the sampled terroir that spans several cultivars, and that both berry and leaf mycobiomes are likely influenced by the chemical characteristics of berries and leaves, e.g., pH and the availability of nutrients and simple carbohydrates.
Finally, the correlation between fungal community composition and physiological variables in leaves is noteworthy, and merits further research to explore causality. Our findings offer novel insights into the microbial dynamics of grapevine considering plant chemistry and physiology, with implications for viticulture.

Acknowledgements: Supported by the grant TKP2021-NKTA-16, NRDI Office to Eszterházy Károly Catholic University and the Lendület Programme No. 96049 (Eötvös Loránd Research Network and Hungarian Academy of Sciences).

EXPLORING THE EFFECTS OF TERROIR, SEASON, AND VINTAGE ON THE GRAPEVINE PATHOBIOME

CARLA MOTA LEAL[1], ADRIENN GEIGER[2], ANNA MOLNÁR[3], GLODIA KGOBE[4], LUCA LEPRES[5], MARGOT MÜLLER[2], KÁLMÁN VÁCZY[3], JÓZSEF GEML[2]

[1]Doctoral School of Environmental Sciences, Hungarian University of Agricultural and Life Sciences, Eger; [2]ELKH – EKKE Lendület Environmental Microbiome Research Group; [3]Food and Wine Research Centre, Eszterházy Károly Catholic University, Eger, Hungary

The terroir concept is widely used and known to explain some of the differences in sensory and chemical characteristics of grapes and wines. The concept itself partly is based on known or presumed spatial differences in edaphic and mesoclimatic factors. These environmental differences likely affect plant-associated microbes also, with possible implications for plant health as well as crop yield and quality. In this study, we compared the compositional dynamics of plant pathogenic fungi in three different sampling sources (microhabitats): soil, woody tissue, and bark of the grapevine cultivar Furmint in late winter and late summer in 2020 and 2021, plus three different terroirs in the Tokaj wine region. DNA sequence data were generated by Illumina NovaSeq at BaseClear (Leiden, the Netherlands) with fungal-specific primers targeting the rDNA internal transcribed spacer (ITS) region. The data analysis was all performed in R. Of the 123 different plant pathogenic fungal genera found, *Diplodia*, *Phaeomoniella*, and *Fusarium* showed the highest richness for each source. Although both richness and proportional abundance of plant pathogenic fungi differed significantly among microhabitats, proportional abundance differed only among terroirs and health types (symptomatic and non-symptomatic), and richness differed between vintages and seasons. When

samples from the three different microhabitats were analyzed separately, we found significant compositional differences among terroirs, season, and vintage in all microhabitats, with terroir explaining from 14.46% to 24.67%, season 1.84% to 2.98%, and vintage with 3.67% to 6.39% of the variance in the community composition of plant pathogenic fungi. The compositional difference between health types was only significant in wood and bark samples. In the case of terroirs, some of the observed differences likely are caused by environmental filtering caused by differences in edaphic and mesoclimatic conditions, while differences in weather as well as management practices, e.g., differences in canopy management and application of fungicides, may partly explain the observed seasonal and vintage dynamics of fungi.
We provide here novel information on the compositional dynamics of plant pathogenic in different microhabitats among terroirs, different seasons, and vintages with implications for plant health studies.

DIFFERENCES IN EXPRESSED FUNCTIONAL GENES OF FUNGI IN NOBLE ROT AND GREY ROT GRAPES

MARGOT MÜLLER[1, 2], JÓZSEF GEML[2], ÁDÁM HEGY[1], JÚLIA HEGYI-KALÓ[1], JÓZSEF KUN[3], ATTILA GYENESEI[3], KÁLMÁN ZOLTÁN VÁCZY[1]

[1]Food and Wine Research Centre, Eszterházy Károly Catholic University; [2]ELKH-EKKE Lendület Environmental Microbiome Research Group, Eötvös Loránd Research Network, Eger; [3]Szentágothai Research Centre, University of Pécs, Pécs, Hungary

Botrytis cinerea is a necrotrophic fungus causing grey rot (GR) with crucial economic losses in fruit crops but can also cause noble rot (NR) in grape berries under certain microclimatic conditions, leading to desired physico-chemical changes required for producing botrytized wines. In addition to *B. cinerea*, many other fungi and yeasts are metabolically active in NR and GR grape berries, but the functional roles of these are still scarcely understood. We generated and analyzed metatranscriptomic data from healthy (H), NR and GR grape berries collected in the Tokaj wine region in Hungary. Based on previous, culture-based studies and our DNA sequence data from the same samples, RNAseq reads were aligned the reference genomes of the dominant fungal species in the sampled grapes: *Alternaria alternata*, *Botrytis cinerea*, *Epicoccum nigrum*, *Aureobasidium pullulans* and *Rhodotorula graminis*. To gain insight into the functional roles of these fungi and yeasts in terms of metabolism and textural changes during NR and GR, differential expression and functional enrichment analyses were conducted. Our study shows that all fungi and yeasts are most active in NR, followed by GR and H berries. Beside *B. cinerea*, several functional genes of all fungal and yeast species expressed during NR were linked to the well-known physico-chemical changes that occur in NR berries. The identification of antagonistic microbe interaction genes from all filamentous fungi and yeast species only during NR highlights provides additional explanation for the production of certain secondary metabolites as well as ethanol during NR development. Finally, the identification of virulence genes in only *B. cinerea* during GR confirms that it is mainly responsible for the onset of the disease.

Acknowledgements: Supported by TKP2021-NKTA-16 (NKFIH), Lendület Programme No. 96049 (ELKH, HAS).

WEST NILE VIRUS INFECTIONS IN HUNGARY – CURRENT EPIDEMIOLOGICAL SITUATION AND PHYLOGENETIC ANALYSIS OF THE HUNGARIAN VIRUS STRAINS

ANNA NAGY, ANDRÁS HORVÁTH, ORSOLYA NAGY, ANITA KOROKNAI, NIKOLETT CSONKA, ESZTER MEZEI, MÁRIA TAKÁCS

Division of Virology, National Public Health Center, Budapest, Hungary

West Nile virus (WNV) is one of the most important human pathogenic flaviviruses in Hungary. Altogether 485 human infections were diagnosed between 2004 and 2021. Most of the patients presented with moderate or severe neurological involvement. A particular remarkable increase in human and animal WNV infections was observed in 2018, while the number of reported cases significantly decreased during the COVID-19 pandemic. Whole blood and urine samples from patients with acute WNV infection and WNV strains isolated on cell culture or animal inoculation were investigated by reverse transcription PCR assays. Genome sequencing was carried out by Sanger-method, following by next-generation whole genome sequencing. Between 2014 and 2019, altogether 82 WNV PCR positive human samples were partially sequenced, targeting the NS3 region of the genome. During a retrospective study a further 15 WNV isolates, and 10 PCR positive clinical specimens were investigated by whole genome sequencing. Maximum likelihood phylogenetic analysis revealed that the Hungarian WNV strains belong to two subclades of lineage 2: one of them is related to the Balkan subclade, while the other contains strains from the Southern and Western European countries. Based on our sequence data, the 2018 WNV outbreak was more likely caused by certain environmental factors and endemic WNV strains, rather than recently introduced novel WNV variants. WNV viruses of genetic lineage 2 emerged in the last 15 years and became widespread in Central Europe and the Mediterranean Basin. In Hungary, the presence and co-circulation of multiple lineage 2 WNV strains could be identified in the last few years. In the light of the 2018 WNV outbreak in Europe, the European Centre for Disease Prevention and Control anticipates the need of

multi-country outbreak investigations through sequence-based typing. Besides whole blood, the collection of urine samples can highly support the viral RNA detection, molecular typing and virus isolation.

HERPES SIMPLEX VIRUS IS YOUNGER THAN IT WAS KNOWN EARLIER: NEWS OF MICROBIAL ARCHEOGENETICS

KÁROLY NAGY

Semmelweis University, Budapest, Hungary

Coanalysis of host and pathogen genomes offers rich opportunities to address questions such as how long ago they have infected humans, how diseases they induced may have changed, what are the patterns of viral diversification, how these may mirror the interactions and behaviors of their hosts. Humans are hosts to a large number of viruses. Herpes simplex virus 1 (HSV-1), affects at least two-thirds of the human population. Phylogeographic analysis has suggested the virus codiverged with human migrations out of Africa, more than 50.000 years ago. Recent study uncovered and sequenced ancient herpes genomes from teeth of European samples from the 3rd and 17th centuries indicate, that its origin was much more recent: around 5.000 years ago, during the Bronze Age. Archeogenetic studies revealed the role of *Y. pestis* in the first plague in the Roman Empire at the 6th century („Justinian plague"), and also proved, that at the 14th century epidemic („Black Death") which killed 60% of population in parts of Eurasia was initiated by *Y. pestis* evolved at the modern day Kyrgyzstan. Collapse of Aztec society was caused by catastrophic *Salmonella* outbreak. Archaic DNA (aDNA) analysis confirmed the presence of *smallpox* virus in population around the fall of the Western Roman Empire, and also in Vikings. Recently it was reported that the *measles virus* - thought to have emerged in humans around the ninth century - might have jumped to people in the first millennium B.C. and hepatitis B virus had been infecting humans since the Bronze Age, 5,000 years ago. We earlier uncovered and analyzed polymorphism of the HIV coreceptor gene CCR5 in isolated aDNA samples of 18th century mummies of Vác, and proved their occurrence before the HIV/AIDS epidemic. aDNA studies are yielding new information about pathogens past and present. New technologies allow the isolation of DNAs long enough for sequencing, but several factors - including Millard's effect - still influence quality of the isolated DNA. Technological limitations mean that we are currently able to sequence only the genetic material of pathogens that contain double-stranded DNA, excluding many important RNA viruses.

PHYLOGENETIC ANALYSIS OF DENGUE VIRUS ISOLATES FROM IMPORTED INFECTIONS BETWEEN 2017-2022

ORSOLYA NAGY[1, 2], ANNA NAGY[2], ANITA KOROKNAI[2], NIKOLETT CSONKA[2], MÁRIA TAKÁCS[1, 2]

[1]Institute of Medical Microbiology, Faculty of Medicine, Semmelweis University; [2]National Reference Laboratory for Viral Zoonoses, National Public Health Center, Budapest, Hungary

Dengue virus (DENV) is one of the most frequently imported tropical arbovirus infections in Hungary. Between 2017-2022 altogether 87 imported DENV infections were diagnosed by complex microbiological methods at the National Reference Laboratory for Viral Zoonoses of National Public Health Center, Hungary. Almost half of the infections (41 cases) were identified in 2019, while during the SARS-CoV-2 pandemic case numbers dropped significantly due to travel restrictions. Sera, anticoagulated whole-blood- and urine samples were collected at the acute phase of the infection from laboratory confirmed dengue infected patients. Virus isolation was attempted on Vero E6 cell lines, successful isolation was confirmed by visible CPE and increasing viral loads detected by real-time PCR technique. DENV isolates were further analyzed using Sanger sequencing for identification of different serotypes and genetic variations. Virus isolation proved to be successful in case of 12 clinical specimens (10 sera and 2 anticoagulated whole-blood samples), the most commonly isolated serotype was Dengue-1. Although Ct values of acute phase sera and anticoagulated whole blood specimens were not significantly different during laboratory diagnosis of DENV infections using real-time PCR, the most suitable sample type for virus isolation proved to be serum. Due to the rising of temperatures and changes in climate conditions, introduction of tropical arbovirus infections to new geographical areas could be expected, resulting in growing annual case numbers of imported infections. Virus isolation from clinical specimens and characterization of isolated DENV variants help the identification of the most frequent circulating DENV types that can further result in autochthone transmission even in Europe.

FUNCTIONAL STUDY OF HUMAN PAPILLOMAVIRUS TYPE 33 LONG CONTROL REGION SEQUENCE VARIANTS

ESZTER ORAVECZNÉ GYÖNGYÖSI, BRIGITTA LÁSZLÓ, ANITA SZALMÁS, JÓZSEF KÓNYA, GYÖRGY VERESS

Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

High-risk human papillomaviruses (HPVs) are responsible for the development of cervical and other anogenital cancers. It is known, that intratypic sequence variants of high-risk HPVs (such as HPV 16, 18, 31) have different oncogenic potential, partly due to natural variants of viral long control region (LCR). The control region of the HPV genome (LCR) has a very important role in the regulation of the viral replication and transcription. In the case of HPV 33, which is a widespread oncogenic HPV type, there are no data on the phylogenetic and functional differences of LCR in Hungary. So, the purpose of this study was to see the natural genetic variants of HPV 33, and to investigate the functional differences between them. DNA from HPV 33 positive cervix samples was amplified by PCR reaction with HPV 33 LCR specific primers. After sequencing the LCR variants, multiple sequence alignment and phylogenetic analysis were carried out. Representative HPV 33 LCR sequence variants were selected for cloning and functional analysis. After transient transfection of HeLa cells, luciferase reporter assays were used for functional analysis of different HPV 33 LCR sequence variants. Several HPV 33 variants were identified with single nucleotide changes and a 79 bp deletion. According to the constructed phylogenetic tree, our variants belong to the A lineage (A1 and A2 sublineages) which is the most frequent lineage in Europe. In functional analysis, we found that variants belonging to A2 sublineage have significantly lower transcriptional activity than samples belonging to A1. This phenomenon was also observed in the case of deletion constructs which contain only the 3' part of the HPV 33 LCR. Nucleotide changes in the LCR can affect transcription factor binding sites and result in altered transcriptional activity of the regulatory region. Our results can help to understand the correlation between LCR polymorphism and oncogenicity of HPV 33.

DEVELOPMENT OF A BACTERIAL CONSORTIUM FOR SIMULTANEOUS DICLOFENAC, IBUPROFEN AND CARBAMAZEPINE BIODEGRADATION

MÁRTON PÁPAI[1], ANDRÁS TÁNCSICS[1], DAOOD HUSSEIN[2], GERGELY MARÓTI[3], BALÁZS KRISZT[4], OFIR MENASHE[5], TIBOR BENEDEK[4]

[1]Department of Molecular Ecology, Institute of Aquaculture and Environmental Safety; [2]Laboratories of Food Analysis, Institute of Horticultural Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő; [3]Institute of Plant Biology, Biological Research Center, Eötvös Loránd Research Network, Szeged; [4]Department of Environmental Safety, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary; [5]Water Industry Engineering Department, Achi Racov School of Engineering, Kinneret Academic College on the Sea of Galilee, Emek Ha'Yarden, Israel

Among pharmaceutical residues diclofenac (DIC), ibuprofen (IBU) and carbamazepine (CBZ) are the most frequently detected in aquatic ecosystems. As it was determined earlier, at environmentally relevant concentrations these compounds may have ecotoxicological effects on aquatic organisms such as fish, crustacean and mussels. Since conventional wastewater treatment facilities are inefficient in the elimination of these compounds, new alternatives or supplementary elimination possibilities need to be developed. For the elimination of such compounds biotechnological approaches are the most promising from a sustainability point of view. In this study a bacterial consortium containing *Stenotrophomonas*, *Rhizobium*, *Nocardioides* and *Brevundimonas* isolates was established and tested for simultaneous DIC, IBU and CBZ biodegradation (conc. 1.5 ppm each) in Bushnell-Haas medium, in a natural water sample and in wastewater effluent sample. The pharmaceutical biodegradation capacity of the consortium in natural water and wastewater effluent samples was tested also in macroencapsulated state using the Small Bioreactor Platform technology (SBP). The results indicated that the highest pharmaceutical biodegradation by the consortium could be recorded in co-metabolic settings when the test media contained as additional carbon source glucose (3 ppm) and yeast extract (0.3 ppm), as well as ammonium-nitrate (1 ppm). In mineral salts solution the concentration of DIC, IBU and CBZ was reduced by 72%, 100% and 30%, respectively.
In natural water samples, in the presence of the autochthonous bacterial community the concentration of DIC and IBU was reduced by 25% and 94%, respectively, no CBZ biodegradation was recorded.

CHEMOTAXONOMIC ANALYSIS OF *PSEUDOMONAS* SPECIES

DÓRA ANNA PAPP, KITTI TARI, THU HUYNH, SÁNDOR KOCSUBÉ, ANDRÁS SZEKERES, ZSUZSANNA HAMARI, CSABA VÁGVÖLGYI, MÓNIKA VARGA

Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Phytopathogen fungi are capable of producing secondary metabolites that pose health hazards to animals and humans. Different practises have been applied to control plant diseases. Using microorganisms or enzymes to inhibit some plant diseases is a well-known promising and reliable alternative to reduce the need for agrochemical pesticides. Rhizosphere bacterial species such as *Bacillus*, *Streptomyces* and *Pseudomonas* have been proved to be efficient biocontrol agents to combat plant diseases. Our aims were to isolate fluorescent pseudomonads capable of suppressing *A. flavus* growth, or/and AB1 biosynthesis, or/and degrading the toxin. We have selected 60 fluorescent *Pseudomonas* strains and additionally we have isolated 5 new *Pseudomonas* strains from corn rhizosphere. The bacterial isolates were identified with 16S rRNA gene sequence analyses, *rpoD* and *rpoB* housekeeping gene sequencing. Beside PCR-based molecular method, chemotaxonomic techniques were applied such as cellular fatty acid profile and secondary metabolite profile in order to compare the different taxon identification methods. Fatty acid methyl esters (FAME) were identified with gas chromatography using Sherlock microbial identification System (Microbial Identification Inc.) Little is known about secondary metabolite based identification of *Pseudomonas* species. First we optimized culture conditions and extraction processes to widen the metabolite profile of the extracts. Secondary metabolite profiles were determined with LC-HMRS method.
Our results showed that molecular and fatty acid based analyses are equivalent methods. Identification based on the secondary metabolites proved to be promising, some of the metabolites were characteristic to specific species.

Acknowledgements: Supported by NKFI K139312 project.

IN VITRO EXAMINATION OF THE ANTIVIRAL EFFECT OF SYNTHETIC AND SEMI-SYNTHETIC COMPOUNDS AGAINST SARS-COV-2 IN THE NATIONAL VIROLOGICAL LABORATORY

HENRIETTA PAPP, ANETT KUCZMOG, MÓNIKA MADAI, FERENC JAKAB

National Laboratory of Virology, University of Pécs, Pécs, Hungary

On the 11th of March, 2020 the World Health Organization declared a global pandemic caused by a newly emerged coronavirus, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The scientific community demonstrated an unprecedented collaboration around the globe. Different disciplines worked together to develop vaccines, and antiviral therapies and to understand the pathomechanism of SARS-CoV-2. At the National Laboratory of Virology at the University of Pécs several studies were conducted in collaboration. Amongst those, numerous synthetic, natural, and semi-synthetic compounds were tested in vitro against SARS-CoV-2. After a microscopic evaluation, the effective candidates were analyzed. Viral genomic copy number was ascertained using droplet digital PCR and the 50% inhibitory concentration was determined. In collaboration with Cebina GmbH, azelastine-HCl was found to be promising based on in silico screen and in vitro tests. Two major projects were carried out with different research groups from the University of Debrecen. One of the projects, which was led by Péter Bai, was focused on registered poly-ADP ribose polymerase inhibitors where rucaparib proved to inhibit viral entry. The other project was led by Anikó Borbás and aimed to test different glycopeptide antibiotics and their newly synthesized apocarotenoid conjugates. We found that glycopeptide antibiotic derivatives have the potential as a lead molecule in drug development campaigns. It is also worth noting that bixin, a natural apocarotenoid, alone exerts antiviral activity. In collaboration with Péter Buchwald from the University of Miami, we found that the methylene blue, which is used to treat methemoglobinemia can be repurposed for the treatment of coronavirus disease- 19 (COVID-19). Our in vitro results established a valuable base for further anti- SARS-CoV-2 drug developments. Azelastine-HCl containing nasal spray has already entered clinical trials. A diverse set of effective drugs against mild-, moderate and severe COVID-19 can help to contain the further SARS-CoV-2 waves, thus antiviral studies are still relevant and advantageous to come to a halt in the global pandemic.

PRODUCTION OF A NOVEL DEFENSIN FROM *SOLANUM LYCOPERSICUM* L. IN FUNGAL EXPRESSION SYSTEMS

REBEKA PAPP[1], GYÖRGYI VÁRADI[2], ZOLTÁN KELE[2], GÁBOR RÁKHELY[1, 3], PÉTER POÓR[4], LÁSZLÓ GALGÓCZY[1, 5], LILIÁNA TÓTH[1]

[1]Department of Biotechnology, Faculty of Science and Informatics; [2]Department of Medical Chemistry, Albert Szent-Györgyi Medical School, University of Szeged; [3]Institute of Biophysics, Biological Research Centre, Eötvös Loránd Research Network; [4]Department of Plant Biology, Faculty of Science and Informatics, University of Szeged;[5]Institute of Biochemistry, Biological Research Centre, Eötvös Loránd Research Network, Szeged, Hungary

Plant defensins are small, highly stable, cysteine-rich peptides. They are part of the plant innate immune system against fungal pathogens. They show different antifungal mode of action and antifungal spectrum. Considering the above mentioned features, the high antifungal activity, and the natural origin, they can be applied as potential biofungicides to treat or prevent plant infections and crop contaminations caused by phytopathogenic fungi. As the plant host produces them only small amount upon

fungal infection, their bulk production by a fermentable and generally recognized as safe microbial expression system is necessary for practical use. According to the literature fungal-based expression systems are applicable. Based on our recent investigations, a *Penicillium chrysogenum*- and a *Pichia pastoris*-based heterologous expression systems could be applicable for extracellular production of a novel *Solanum lycopersicum* L. antifungal defensin (K4CBP6). In addition of the small amount of the full-length mature protein, a variant of K4CBP6 lacking an arginine residue at the N-terminus was identified in the ferment broth of the *P. chrysogenum*-based expression system. The *P. pastoris*-based expression system was able to produce correctly maturated K4CBP6 in high amount; however, several different variants in length containing extra amino acids from the signal sequence at the N-terminus were detected.
These results suggest that the *P. pastoris*-based expression system is more appropriate for the bulk K4CBP6 production than the *P. chrysogenum*-based one; however, the latter one supports better the correct maturation.

Acknowledgements: L.T. was financed by the Hungarian National RDI Office - NKFIH, FK 134284 project.

FULL-LENGTH METABARCODING ANALYSIS OF HUNGARIAN SILAGE AND HAYLAGE SAMPLES

KATALIN PAPPNÉ MURVAI[1], ENIKŐ HORVÁTH[1], KADMIEL PEREIRA[1], ALEXANDRA IMRE[1], HANNA V. LOVÁSZNÉ RÁCZ[1], BÉLA BÉRI[2], ISTVÁN PÓCSI[1], WALTER P. PFLIEGLER[1]

[1]Department of Molecular Biotechnology and Microbiology, Institute of Biotechnology, Faculty of Science and Technology; [2]Department of Animal Husbandry, Institute of Animal Science, Biotechnology and Natural Conservation, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Debrecen, Hungary

Metagenomic tools have been widely used in research, such as determining the composition of the human microbiome or the bacterial biodiversity of samples from different environments. Assessment of bacterial diversity through sequencing of 16S ribosomal RNA (16S rRNA) genes has been an approach widely used in environmental microbiology, and increasingly in agricultural microbiology as well, particularly since the advent of high-throughput sequencing technologies. The 16S rRNA is highly conserved between prokaryote species but it has hyper-variable regions which can be used for genus identification, as well as species-level identification with the newest, long-read sequencing methods. In the course of our work, the bacterial composition of various haylage and silage samples were investigated. We analysed the bacterial composition of the samples using full-length 16S metagenomic barcoding performed with the Oxford Nanopore long-read sequencer. The data from the sequencing were processed with the software Emu, that works with an expectation-maximization algorithm to generate taxonomic abundance profiles from full-length 16S rRNA reads. We focused on bacterial diversity of various haylage and silage samples at species and genus level, and on the predicted metabolic diversity based on abundance data. Based on the data from the samples, we were able to compare the bacterial composition of different feeds and the effect of the ensiling process, to our knowledge, in the first study of its kind in Central Europe. We also identified several previously rarely recorded bacteria from the samples, among others, *Eoetvoesia caeni*, a genus and species recently described from Hungary.

Acknowledgemenmts: Supported by Project no. 2018-1.2.1-NKP-2018-00002 implemented with the support provided from the National RDI Fund of Hungary, financed under the 2018-1.2.1-NKP funding scheme.

PRO-CONTRA EVALUATION OF LONG-READ SEQUENCING BASED METHODS IN BAKER'S YEAST GENOMICS

HANNA VIKTÓRIA RÁCZ, VALTER PÉTER PFLIEGLER

Department of Molecular Biotechnology and Microbiology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

Baker's yeasts play an important part in our everyday life: we utilize them in our homes on a smaller scale, and in food and beverage production on bigger scale, and even as nutritional supplements. Therefore, understanding their genomics and how they relate to each other is crucial. Previously, short-read sequencing with deep coverage provided useful insights. However, there remained some unanswered questions, partially due to the polyploid nature of these yeasts, to which long-read sequencing seems to be the solution. In our present work, we aimed to adapt Oxford Nanopore long-read sequencing for baker's yeast whole genome sequencing, assembly and phylogenomics. We adapted wet lab methods: high molecular weight DNA isolation, library preparation; along with hardware and software background: high-performance computer build, benchmarking assembly software pipelines; and phylogenomic approaches including variant-calling-based and alignment-and-assembly-free methods. Through these efforts, we were able to establish a stable sequencing protocol for our polyploid yeast strains. Based on these data and through monitoring software releases and efficiency, we assembled highly continuous genomes with outstanding quality (avg. N50 = 900,000, avg. BUSCO > 95%, avg. scaffolds: 17). We created two whole genome assembly pipelines, one long-read based and one long- and short-read based, both of which require less than 2 hours to run on an advanced PC. To be able to utilize these softwares, we built a high-performance computer, concentrating on memory size, GPU performance and

top-quality cooling system. Nowadays the accuracy of long-read sequencing is approaching the accuracy of short-read sequencing. Longer and more accurate reads are better for whole genome assembly, opening up new possibilities for which novel bioinformatic approaches may be necessary. Here, we describe a pipeline that effectively combines long-read sequencing and assembly with alignment-and-assembly free analysis and with variant-calling based phylogenomic network analysis, with an emphasis on polyploidy and admixed, heterozygous genomes. However, there are some limiting aspects as well. Computers with decent performance are rather expensive, faster processing costs more. Analyzing components of the genome that are circular (mitochondrium, plasmid) is also not easily feasible yet with current generalized long-read methods. In summary, using long-read sequencing to better understand baker's yeast genomics proves to be irreplaceable. In terms of value for money, the advantages outweigh the possible methodological problems, and novel pipelines, such as the one presented here, may give unprecedented insight into the genomic characteristics of polyploid baker's yeasts.

CHARACTERIZATION OF THE INTERACTION OF HPV E7 ONCOPROTEINS WITH THE MYOSIN PHOSPHATASE TARGET SUBUNIT (MYPT1)

LEILA RAHMANI, ZSOLT BARNABÁS ÉLES, JÓZSEF KÓNYA, ANITA SZALMÁS

Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Mucosal human papillomaviruses (HPVs) are the most common sexually transmitted infectious agents. About 80% of sexually active people are infected with at least one type of HPV during their lifetime, most often with HPV-16, which is also the most prevalent genotype in invasive anogenital carcinomas and precancerous lesions. The effect of HPV E6 and E7 oncoproteins is essential for the development and progression of HPV-associated tumors. These viral proteins can interact with cellular proteins that regulate host cell survival, proliferation, and migration. The mapping of these interacting partners may be of crucial importance in the early prognosis and targeted therapy of HPV-caused tumors. Recent studies aimed at identifying protein interactions have assumed a connection between the HPV E7 oncoprotein and the target subunit of myosin phosphatase, MYPT1 (Myosin phosphatase target subunit 1). This creates the possibility that MYPT1 and, through this connection, myosin phosphatase can play a role in the life cycle of the virus and in the development of HPV-associated tumors. We aimed to characterize these relationships during our experiments. Firstly, we performed a comparative analysis of the endogenous expression levels of MYPT1 protein in immortalized HPV positive (HeLa, CaSki) and negative (C33a, HEK-293, HaCaT) cell lines. Then, we investigated the effect of HPV E7 proteins from different genotypes (HPV-11, HPV-16, HPV-18, HPV-31) on the target protein in HEK-293 and C33a cell lines. Furthermore, we performed HPV E6/E7 specific siRNA treatment in HPV-18 positive HeLa cells and investigated the effect of gene silencing on MYPT1 protein expression. The interaction between the HPV E7 oncoproteins and the MYPT1 was confirmed by pull-down method in HEK-293 cell line. Moreover, we performed experiments on the expression and localization of MYPT1 protein in primary human keratinocyte cells to verify the results. Protein expression levels were studied by Western blot method. The results of our experiments show that the presence of HPV E7 proteins leads to reduced expression levels of MYPT1, thereby possibly inhibiting its antitumor effect. HPV-16 E7 had the most prominent effect on MYPT1 protein expression.
Moreover, we observed a very strong connection between MYPT1 and HPV-16 E7. These results can explain the much more frequent appearance of tumors formed by HPV-16 compared to other HPV types.

MRSA CARRIAGE AMONG BROWN RATS (*RATTUS NORVEGICUS*) IN HUNGARY

JUDIT SAHIN-TÓTH[1], ALEXANDRA JUHÁSZ[1], ERVIN ALBERT[2], CARLA LÓPEZ CAUSAPÉ[3], SARA CORTÉS LARA[3], MÁTÉ GUGOLYA[1], ANDREA HORVÁTH[1], ORSOLYA DOBAY[1]

[1]Institute of Medical Microbiology, Faculty of Medicine, Semmelweis University; [2]Department of Pathology, University of Veterinary Medicine, Budapest, Hungary; [3]The Health Research Institute of the Balearic Islands (IdISBa), Palma de Mallorca, Spain

In 20-30% of humans *Staphylococcus aureus* can permanently colonize the skin and mucus membranes, most particularly the nasal cavities. Its numerous virulence factors make it one of the most common human pathogenic bacteria. It can become an MRSA via the mutation of the penicillin binding protein, which can lead to serious hospital acquired (HA) infections like surgical site and blood stream infections or ventilation associated pneumonia. Due to its multi-resistance, MRSA is listed by the WHO as a "high priority" pathogen and became a global health threat. In addition to HA-MRSA, strains originating from both domestic or wild animals (livestock-associated or LA-MRSA) have been reported with increasing frequency in the recent years. Brown rats (*Rattus norvegicus*) are considered the most widespread pest species in urban areas where they live in close vicinity to humans. They colonize the sewage system and get in contact with human waste, frequently interacting with human faeces. This way they are exposed to anthropogenic antimicrobial residues and can acquire, carry, and spread multidrug resistant bacteria. In previous surveys, low MRSA prevalence (1.6-3.5%) among rats was reported, but MRSA lineages previously described in humans and livestock were identified. Rats can also play an important role in facilitating transfer of genetic elements (virulence factors and resistance genes) between Staphylococci and other bacterial species. Based on this information

the aim of this study was to survey *S. aureus* and MRSA carriage in rats for the first time in Hungary. 200 animals in the Budapest area, collected by rodent control companies in 2020-21, were screened and the isolated strains were further examined for resistance and virulence genes. The nose and the skin of the animals were sampled with cotton swabs. After cultivation on *S. aureus* Chromagar, catalase and Pastorex latex agglutination tests were done for phenotypic identification. To verify *S. aureus* strains, PCR was performed on the species specific *nucA* gene. MRSA strains were identified with PCR, detecting the *mecA* or *mecC* genes. The MRSA isolates were further analyzed with whole genome sequencing. *S. aureus* carriage was found to be 12.5% (25/200). Among the 25 strains, four proved to be MRSA and all four isolates were identified as *mecC*-MRSA. Three of these were typed as ST2676-t843, and one as ST2676-t20262. All carried the SCCmec type XI element. Beside the *mecC* gene, only the *blaZ* resistance gene was found, and gamma hemolysins and the *lukED* leucocidin virulence genes were detected. The first *mecC*-MRSA in Hungary was recently described by our work group from hedgehogs, but this is the first time that *mecC*-MRSA was isolated from rats in the country. This draws attention to the presence of the *mecC* gene in other animal hosts and that prompt diagnostic techniques are required when isolating MRSA strains.

Acknowledgements: This project received funding from the EU Horizon2020 programme, AmReSU ID:952491

IN VITRO ANTIFUNGAL ACTIVITY OF BIOSURFACTANT PRODUCED BY *BACILLUS* SPECIES AGAINST *ALTERNARIA ALTERNATA*

JESSE SAKIYO, ÁRON NÉMETH

Department of Applied Biotechnology and Food Science, Faculty of Chemical Technology and Biotechnology, University of Technology and Economics, Budapest, Hungary

With a favorable climate, fungal diseases may proliferate enormously and damage crops; pathogenic fungi can dwell within or on the surfaces of plants. The fungus generates spores carried to the plant by wind, water, or animals such as insects. When the spores land on the plant, they adhere to the surface, causing infect begin which destroys plants. There have been several negative consequences on the environment and human health related to using chemical fungicides that were less efficient and broad-spectrum. Biosurfactants as alternatives to chemical fungicides to combat resistance development and to lessen negative impacts on the environment and human health. We will evaluate and screen different compounds produced by *Bacillus licheniformis* DSM13, *Bacillus subtilis* DSM10, and *Geobacillus stearothermophilus* DSM2313 to evaluate their antifungal properties. *Bacillus* species have been proven to secrete rhamnolipids and lipopeptides, and they may have qualities that make them acceptable for specific applications depending on their structure and content. Biosurfactant structural and compositional diversity is unequivocally substrate dependent, employed in a wide range of commercial applications. Biosurfactants consist of classes of single molecules that perform a variety of tasks with molecular structures consisting of hydrophilic and a hydrophobic part; the use of microbial origin surfactants has become more important as an alternative strategy to reduce the usage of pesticides and to be sustainable for the environment. This study aims to investigate inhibitory activity against the mycelial growth using several bioassays that are well-known and widely utilized, including disk-diffusion, well diffusion, broth or agar dilution, and other in vitro antifungal susceptibility testing methods against *Alternaria alternata*.

DETECTION AND ISOLATION OF *ACTINOBACILLUS PLEUROPNEUMONIAE* FROM WILD BOARS

RITA SÁRKÖZI, LÁSZLÓ MAKRAI, LÁSZLÓ FODOR

Department of Microbiology and Infectious Diseases, University of Veterinary Medicine, Budapest, Hungary

Actinobacillus pleuropneumoniae is a major pathogen of swine, which can cause severe pleuropneumonia in pigs, but sometimes the disease can be generalized. Diseases caused by *A. pleuropneumoniae* are frequent all over the world, resulting in high losses among domestic pigs. However, our knowledge on the occurrence of *A. pleuropneumoniae* in wild boars and feral pigs is limited. We aimed to examine the carriage of *A. pleuropneumoniae* by hunted wild boars.
The presence of *A. pleuropneumoniae* was examined in tonsils of 68 hunted wild boars collected at a game processing unit. An in-house designed species-specific PCR test was used to detect the gene of Apx IV toxin, and the samples were inoculated on a modified selective medium. *A. pleuropneumoniae* was detected in 10 animals (14.7%) by PCR and one *A. pleuropneumoniae* serotype 12 strain was isolated. The antibiotic resistance pattern of the strain resembled field strains that were isolated from farmed pigs in Hungary. This is the first case for the detection of *A. pleuropneumoniae* not only using PCR or ELISA, but also its isolation, identification, and serotyping.

WHERE BARCODING NO LONGER HELPS: GENOME CHIMERIZATION AND NETWORK EVOLUTION OBSCURE PHYLOGENETIC AND TAXONOMIC RELATIONSHIPS

MÁTYÁS SIPICZKI

Department of Genetics and Applied Microbiology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

DNA barcoding is the process of identifying species through the sequencing of specific DNA segments (barcode regions) and their comparison with sequences deposited in DNA sequence databases. The barcode sequences can distinguish individuals (strains) of different species because their sequence variation between species exceeds that within species. Investigation of primary (D1/D2 domains and ITS segments of rDNA repeats) and secondary barcode sequences (ACT1, EF2, RPB2 and TEF1) of large numbers of isolates of the ascomycetous yeast genus *Metschnikowia* and the genome sequences of the type strains of species revealed that the analysis of the barcode sequences fails to differentiate species and reconstruct accurate phylogenetic trees when the strains have chimeric genomes composed of mosaics of different origins because the intragenomic diversity is comparable to or even higher than the interstrain diversity.
The incongruent phylogenetic relationships among the barcodes of a strain can be attributed to reticulate interactions (network-like evolution) of genomes that bring together genes of different phylogenetic histories.

EFFECT OF SOUR CHERRY ANTHOCYANINS ON HEALTHY HUMAN ORAL MICROBIOME

BOGLÁRKA EMESE SKOPKÓ[1], MELINDA PAHOLCSEK[2], ANNA ANITA SZILÁGYI-RÁCZ[2], PÉTER FAUSZT[2], PÉTER DÁVID[2], JUDIT RITA HOMOKI[2], MÓNIKA ÉVA FAZEKAS[2], PIROSKA BÍRÓNÉ MOLNÁR[2], LÁSZLÓ STÜNDL[2], JUDIT VÁRADI[3], GÁBOR VASVÁRI[3], RENÁTÓ KOVÁCS[4], KINGA ÁGNES BÁGYI[5], JUDIT REMENYIK[2]

[1]Biomaterials and Prosthetic Dentistry, Faculty of Dentistry; [2]Institute of Food Technology, Faculty of Agricultural and Food Sciences and Environmental Management; [3]Pharmaceutical Technology, Faculty of Pharmacy; [4]Department of Medical Microbiology, Faculty of Medicine; [5]Department of Operative Dentistry and Endodontics, Faculty of Dentistry, University of Debrecen, Debrecen, Hungary

The chewing gum usage is proven to have favourable effect on maintenance of proper oral hygiene by its physical actions and chemical composition. The sour cherry contains anthocyanins, which can have bactericide actions against oral bacteria. The experiment is based on earlier research of Homoki et al. [1], where they proved that chewing gum usage with anthocyanin rich sour cherry extract significantly reduces the amount of human salivary alpha-amylase and number of *Streptococcus mutans* counts in saliva of participants. The background of this phenomen that chewing stimulus liberates the biofilm embedded *S. mutans* cells at the beginning and with the continuous decreasing of human salivary alpha amylase by decreased starch breakdown can reduce the caries formation. Other investigations also showed the bactericide effect against (ciprofloxacin-resistant) *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* of sour cherry with agar diffusion method examining different salivary bacteria. A 3-week experiment included 20 people, who were first examined by cariological and basic periodontal examination. Patients were selected with good oral hygiene, lack of serious general diseases and respiratory infection treated with antibiotics in the past 2 months. Saliva sampling was made between 12:00 and 14:00 on the 1th, 4th and 7th days of the week through 3 weeks. The first week was a control period (C), when only saliva samples were taken. A full mouth scaling was made at beginning of the 2nd week, before starting to chew - daily 3 times, for 2 weeks – the gum with sour cherry extract; then 10 participants changed their toothbrush (BR), and 10 did not change it (NBR). The saliva samples were carried and processed in the Institute of Food Technology where they were stored at -80°C till the laboratory analysis. 16S rRNA sequencing was started with DNA isolation by Inhibitor Removal Technology, followed by sequencing with Illumina MiSeqSystem. Our study supported that the 2-week usage of the chewing gum with anthocyanin and toothbrush change is beneficial on the healthy human oral microbiome.

[1] Homoki et al (2018) Food Funct 9: 4008.

Acknowledgements. Supported by the GINOP-2.2.1-15-2017-00079 and EFOP-3.6.1-16-2016-00022 projects co-financed by the European Union and European Social Fund.

CHARACTERIZATION OF THE BEAUVERCIN PRODUCING ABILITY OF HUNGARIAN *FUSARIUM* SPP. WITH MOLECULAR BIOLOGICAL METHODS

ÁKOS SUHAJDA, INES AMARA, CSABA DOBOLYI, ZSÓFIA TISCHNER, RÓZSA SEBŐK, EDIT KASZAB, BALÁZS KRISZT, MÁTYÁS CSERHÁTI

Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

The view of moulds can be Janus-faced: first of all, they have beneficial properties, such as their important role in the production of food and pharmaceutical products, such as enzymes and organic acids, but they also responsible for very harmful effects, such as the mycotoxins they produce. Mycotoxins, i.e. secondary metabolites synthesised by moulds, often exhibit a wide spectrum of biological activities (e.g. cytostatic, cytotoxic, immunosuppressive, antibiotic, etc.) which could open up new horizons in the development of pharmaceuticals and plant protection products. Our view is that there might be a dual nature of the assessment not only of moulds but also of the bioactive substances they produce. Beauvericin is a cyclic hexadepside peptide of non-ribosomal origin that has antibiotic, antiviral, cytotoxic and insecticidal activities. It is this latter property that has made it a focus of our interest, as the European Union's Green Agreement currently being formulated, which already targets the restrictions of the usage of conventional pesticides, thus making the possibilities for insect control increasingly challenging. Beauvericin can also be synthesised by a wide range of fungal species, including saprophytic, human, insect and phytopathogenic fungi, typically *Beauveria*, *Fusarium* and *Isari* species. Based on literature data, the representatives of the *Fusarium incarnatum-equisetum* species complex (FIESC) are able to produce the highest amounts, that is our main reason why it became our objective to investigate the beauvericin producing capability of our departmental strain collection, currently consisting of 100 *Fusarium* strains. Our objective was also to identify the species of the „succesful strains" by the elongation factor region. The beauvericin producing ability of the strains was tested using a gene-specific primer (Beas_1, Beas_2) by conventional PCR technology. After revitalization of the cryopreserved strains, DNA was extracted from the juvenile mycelia and then our target sequences were amplified using the gene-specific primer with PCR reaction, after purification products were sequenced. From the 100 *Fusarium* strains tested, 24 strains were found to be capable of beauvericin production. Finally, the nucleotide sequences were also compared with beauvericin producing strains in international databases, which allowed us to infer the extent of beauvericin producing species complexes dominant in the Carpathian Basin.
In the future, it would be important to investigate the actual toxin-producing capacity of potential beauvericin-producing strains using chemical analytical methods.

Acknowledgements: Supported by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2020, National Challenges Subprogramme (TKP2020-NKA-16).

cotH GENES ARE NECESSARY FOR NORMAL SPORE FORMATION AND VIRULENCE IN AN OPPORTUNISTIC HUMAN PATHOGEN FUNGUS, *MUCOR LUSITANICUS*

CSILLA SZEBENYI[1, 2], SÁNDOR KOCSUBÉ[1, 2], KARINA KISS[1], YIYOU GU[3], ASHRAF S. IBRAHIM[3, 4], RITA SINKA[5], ROLAND PATAI[6], LÁSZLÓ BODAI[7], GÁBOR NAGY[7], SÁNDOR DOMINIK KOCSIS[1], BENCE RAFAEL[1], A. S. T. KHALIEFEH SILIMAN[1], CSABA VÁGVÖLGYI[1, 2], GÁBOR NAGY[1, 2], TAMÁS PAPP[1, 2]

[1]Department of Microbiology; [2]ELKH-SZTE Fungal Pathomechanisms Research Group, Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary; [3]Los Angeles Biomedical Research Institute at Harbor-UCLA Med Center, Torrance; [4]David Geffen School of Medicine at UCLA, Los Angeles, California, USA; [5]Department of Genetics, Faculty of Science and Informatics, University of Szeged; [6]Laboratory of Neuronal Plasticity, Molecular Neurobiology Research Unit, Institute of Biophysics, Biological Research Centre, Eötvös Loránd Research Network; [7]Department of Biochemistry and Molecular Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Clarification of the pathomechanism of mucormycosis, understanding the interaction of Mucorales fungi with their hosts, and the identification of potential virulence factors and new biomarkers are essential. 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, furthermore the mutant library of the kinase family has been extended by four new mutants (cotH9, cotH12, cotH13, cotH15). Nine 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. Possible changes in cell wall structure were monitored using the cell wall stressors Congo red and Calcofluor white (CFW) as well as fluorescence microscopy and flow cytometry analysis. Pathogenicity of the mutants was examined in *Drosophila melanogaster*, *Galleria mellonella* and a murine model of mucormycosis. Cell wall stressors affected differently the cotH mutants. 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. The CFW fluorescence intensity was higher for the cotH4 mutant compared to the control, which may be related to an increased chitin content or to changes in the cell wall structure that facilitate the access of the dye to chitin. Viability studies in DKA

mice demonstrated that deletion of either *cotH3* or *cotH4* genes attenuate the pathogenicity of *M. lusitanicus*. Importantly, the cotH3 mutant did not show reduced virulence in a *G. mellonella* model but did in a DKA mouse model with elevated GRP78 receptor expression, although the protein does not carry the characteristic motif determined earlier for *R. delemar*. Due to its sequence similarity to the *Rhizopus* CotH3 and the presence of the "CotH-motif", CotH4 could be a potential ligand for the GRP78 receptor, which requires further investigation. Given that CotH proteins are involved not only in pathogenicity but also in spore size and structure, it is important to consider the role of CotH protein family members not only as virulence factors but also in spore formation and other physiological roles.

Acknowledgements: Supported by the NKFI project K131796 and the grants ITM NKFIA TKP-2021-EGA-28 and ELKH 2001007. G.N. is grateful for the support of the Premium Postdoctoral Fellowship Program of the HAS (460050). C.S. is supported by the ÚNKP-20-4-I New National Excellence Program of the Ministry for Innovation and Technology.

REVERSAL OF MULTIDRUG RESISTANCE BY SELENOCOMPOUNDS IN 2D AND 3D TUMOR CELL CULTURES

NIKOLETTA SZEMERÉDI[1], SIMONA DOBIASOVÁ[2], JITKA VIKTOROVA[2], HELENA GBELCOVÁ[3], ENRIQUE DOMÍNGUEZ-ÁLVAREZ[4], GABRIELLA SPENGLER[1]

[1]Institute of Medical Microbiology, Albert Szent-Györgyi Medical School, University of Szeged, Hungary; [2]University of Chemistry and Technology, Prague, Czech Republic; [3]Comenius University, Bratislava, Slovak Republic; [4]Institute of Organic Chemistry, Madrid, Spain

Selenium-containing compounds have proven to be effective to prevent cancer and reduce drug resistance. It has been confirmed that selenocompounds can induce apoptosis, modulate autophagy, inhibit multidrug efflux pumps such as P-gp, inhibit cancer metastasis leading to decreased tumor drug resistance. Based on these antecedents, new selenoesters were synthesized and the antitumoral effects of these compounds were studied in vitro using 2D and 3D cell cultures of sensitive and resistant human ovarian and breast cancer cell lines. In our study, the effect of selenoesters on size and compactness of spheroids of ovarian and breast cancer cells was investigated. Human ovarian and breast cancer cells (HOC and MCF-7, parental and resistant, respectively) were seeded in ultra-low attachment 96-well plates at a cell density of 0.5×10^5 cells mL^{-1}. After 3 days of spheroid formation, the plates were carefully washed once with PBS and fresh DMEM medium (99 µL) was added to the samples. Using a binary compound dilution, the concentration range of compounds K3, K4, and K7 was prepared in new 96-well plates (0.16–10 mM). After the transfer of 1 µL compound, the final concentration range of compounds was 1.6–100 µM. After 72 h of incubation, 100 µL of CellTiter-Glo 3D Reagent (Promega) was added to each well. Spheroid plates were incubated for 25 min at room temperature. Subsequently, the luminescent signal was recorded by a detector (SpectraMax i3x Multi-Mode Microplate reader with MiniMax Imaging Cytometer, Molecular Devices). Ketone-selenoesters and cyano-selenoesters were evaluated for their anticancer and MDR modulation potential using 2D and 3D cultures. Selenoesters K1 and K2 caused the most significant decrease of viability in all tumor cell lines tested in 2D assays. Therefore, the anticancer and MDR modulation potential of selected selenoesters was tested on 3D ovarian and breast cancer cell models. The penetration of the tested compounds was much easier into the ovarian aggregates; thus, the formation of sensitive ovarian spheroids was highly affected by all tested compounds. Selenoesters K3 and K7 were effective against HOC/ADR spheroid growth predominantly in the first 24 h after the addition of the compounds. K3 showed the most significant cytotoxic activity on cells of both ovarian 3D models. The IC50 values of K3 for the 2D and 3D HOC cell cultures were almost the same, which would favor this compound as a promising anticancer agent. It can be concluded that the selected ketone-selenoesters are potent anticancer agents against sensitive and resistant ovarian and breast cancer cell lines. The mode of action of these derivatives is related to the production of reactive oxygen species ,additional studies are required.

TRANSCRIPTOMIC ANALYSIS OF *SEPTOGLOMUS CONSTRICTUM* INOCULATED TOMATO (*SOLANUM LYCOPERSICUM* L.) PLANTS EXPOSED TO HEAT SHOCK

VIKTOR SZENTPÉTERI[1, 2], ZOLTÁN MAYER[2], LEVENTE KONTRA[2], HONG DUC NGUYEN[2], KATALIN POSTA[1, 2]

[1]Agri-biotechnology and Precision Breeding for Food Security National Laboratory; [2]Institute of Genetics and Biotechnology, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

High temperature stress endangers plant growth and yield. In order to make plants more resilient to this threat it is necessary to better understand the mechanisms that take part in the mitigation of heat stress. The use of beneficial microbes like Arbuscular mycorrhizal (AM) fungi can be an effective tool to help plant overcome this obstacle. In previous works we compared different AM strains regarding their effectiveness in abiotic stress tolerance boosting and found that Septoglomus constrictum was one of the strains with the most promising result. We aimed to further investigate and understand this interaction between *Septoglomus constrictum* and tomato during heat stress. Tomato (*Solanum lycopersicum* L.) var. Moneymaker plants were inoculated with AM fungus *S. constrictum* and were grown for eight weeks. After the cultivation period an intense six hour long 42 oC heat shock was applied immediately before harvest. Heat stress induced physiological

changes were observed by measuring relative water content, leaf water potential, stomatal conductance, chlorophyll fluorescence (Fv/Fm). The level of stress was confirmed by measuring hydrogen peroxide (H_2O_2), malondialdehyde (MDA) content in leaves and the antioxidative enzymes: superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in leaves and roots of mycorrhizal and non-mycorrhizal plants. In order to better understand the symbiosis induced changes during the heat stress reaction, Illumina based RNA sequencing was also performed. Differently expressed genes (DEG's) in leaves and roots have been identified and main processes that participate in the heat stress resistance in mycorrhizal plants have been also described. The most notable changes is that beside the transcription factors and chaperons that take part in the heat stress reaction, we also found an enrichment of DEGs connected to sugar and lipid metabolic and transport processes during the combined presence of AM and heat stress.

Acknowledgements: Funded by the industrial research and development projects in Hungarian–Vietnamese cooperation, grant number 2019-2.1.12-TÉT_VN-2020-00001, and Agri-biotechnology and Precision Breeding for Food Security National Laboratory, grant number RRF-2.3.1-21-2022-00007.

INHIBITORY EFFECT OF ECDYSTEROIDS ON *CRYPTOCOCCUS NEOFORMANS* STRAINS

BETTINA SZERENCSÉS[1], MÁTÉ VÁGVÖLGYI[2], MÁRTON BENEDEK HÁZNAGY[2], ATTILA HUNYADI[2], ILONA PFEIFFER[1], CSABA VÁGVÖLGYI[1], MÓNIKA VÖRÖS[1]

[1]Department of Microbiology, Faculty of Science and Informatics; [2]Institute of Pharmacognosy, Faculty of Pharmacy, University of Szeged, Szeged, Hungary

Ecdysteroids are natural 6-ketosteroids, occurring both in the flora and the fauna. In mammals, ecdysteroids are nontoxic compounds with several beneficial pharmacological effects (e.g., non-hormonal anabolic and adaptogenic activities) [1], however, until now, their possible use as antimicrobial agents had been poorly investigated. In our current study, one natural ecdysteroid (20E; 20-hydroxyecdysone) and its semi-synthetic analogues (20E-EOx, 20E-ZOx and 20EL) were tested for their activities against human pathogenic yeast species *Cryptococcus neoformans*. *C. neoformans* is an opportunistic human pathogen yeast that can cause serious disease primarily in immunocompromised individuals. The infection starts with inhalation of the airborne basidiospores or dried cells [2]. The spores germinate in the lung, thereafter the cells disseminating by the blood stream can reach and colonize the central nervous system. Cryptococcosis concerns about 1 million persons in the world - most of them are HIV-infected - and causes the death of more than 600000 patients per year [3]. The minimal inhibitory concentration (MIC) of the compounds on *C. neoformans* strains IFM 5844 and IFO 410 was determined by microdilution method in RPMI medium. Two compounds, 20E-ZOx and 20E-EOx, inhibited the growth of both *C. neoformans* strains with a MIC of 2 mg $mL^{-1}$and 1 mg mL^{-1}, respectively. The two other compounds (20E and 20EL) proved ineffective against this yeast. The combination of the ecdysteroids with each other resulted additive interactions except 20E and 20EL combination. The interaction of these compounds with ion pump-inhibiting drugs (verapamil, indomethacin and quinidine) was checked by checkerboard titration method. None of the inhibitor effected the growth of C. neoformans strains alone in concentration range 100-12.5 µg mL^{-1}. However, verapamil exerted additive interaction with 20E and 20E-ZOx while the combination of verapamil with 20-EOx resulted synergistic interaction.

[1] Lafont and Dinan (2003) J Insect Sci 3:7.
[2] Kohler et al (2015) Cold Spring Harb Perspect Med 5: a019273.
[3] Warkentien and Crum-Cianflone (2010) Int J STD AIDS 21: 679.

Acknowledgements: Supported by UNKP-21-4-SZTE-281 (M. V.) grant of the New National Excellence Program.

INVASIVE ASPERGILLOSIS INDUCED MiRNA SIGNATURES IN ONCOHEMATOLOGY PATIENTS

ANNA ANITA SZILÁGYI-RÁCZ[1], GÁBOR FIDLER[1], PÉTER DÁVID[1], EMESE TOLNAI[1], LÁSZLÓ REJTŐ[2], RÓBERT SZÁSZ[3], SZILÁRD PÓLISKA[4], SÁNDOR BIRÓ[5], MELINDA PAHOLCSEK[1]

[1]Institute of Food Technology, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Debrecen; [2]Department of Haematology, Jósa András Teaching Hospital, Nyíregyháza;[3]Division of Haematology, Institute of Internal Medicine; [4]Department of Biochemistry and Molecular Biology; [5]Department of Human Genetics, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Invasive aspergillosis (IA) is an acute infection affecting patients who are immunocompromised, as a result of receiving chemotherapy for malignancy, or immunosuppressant agents for transplantation or autoimmune disease. The diagnosis of IA remains challenges, especially in the early stages of the disease due to the atypical clinical manifestations and lack of a gold standard diagnostic test. The application of blood biomarkers, like nucleic acid targets has become a research trend. Nowadays,

the connection between extracellular microRNA levels and several pathological processes, including different infections are increasingly recognized. MicroRNAs are class of small (19-24 nt), noncoding RNAs that can regulate gene expression post-transcriptionally. Numerous studies reported the potential of free circulating microRNAs as disease biomarkers in diagnosis and a reliable tool for future use. The prime aim of this study was to identify IA specific circulating miRNA signatures in the whole blood samples of oncohematology (OH) patients that could serve as good biomarkers for the prompt diagnosis of invasive aspergillosis. Therefore, we performed an analysis of high-throughput small RNA sequencing data obtained from 26 HO patients and 24 healthy controls. In silico bioinformatics analyses revealed 8 differentially expressed microRNAs (hsa-miR-191–5p, hsa-miR-106b-5p, hsa-miR-16–2-3p, hsa-miR-26a-5p, hsa-miR-15a-5p, hsa-miR-20a-5p, hsa-miR-106a-5p and hsa-miR-17–5p) with high specificity and sensitivity to discriminate the IA-infected and non-IA OH patients, which were also validated by qRT-PCR measurements. This pilot study is the first effort to understand the levels of circulating DEMs to identify stable, disease-specific diagnostic markers. Finding possible non-invasive biomarkers at early stages of disease progression is crucial for evaluation of high-risk patients to establish follow-up strategies. Invasive aspergillosis specific microRNAs have the potential to serve as good biomarkers for disease diagnosis and may also lead to a better understanding of IA pathogenesis.

STUDYING THE INTERACTION BETWEEN ORAL PATHOGENIC BACTERIA AND *CANDIDA* SPECIES AT THE LEVEL OF EXTRACELLULAR VESICLES

ZÓRA SZILOVICS[1], ÉVA VERES[1], KRISZTINA BUZÁS[2], CSABA VÁGVÖLGYI[1], ATTILA GÁCSER[3, 4]

[1]Department of Microbiology, Faculty of Science and Informatics, University of Szeged; [2]Synthetic and System Biology Unit, Biological Research Centre, Eötvös Loránd Research Network; [3]MTA-SZTE "Lendület" „Mycobiome" Research Group; [4]HCEMM-USZ Hungarian Centre of Excellence for Molecular Medicine, Szeged, Hungary

The human oral cavity is colonized by more than 700 microbes, such as bacteria, viruses, fungi, known as the oral microbiota. As a result of environmental effects, such as smoking or infections, the microbial composition may change, which can result in dysbiosis that may lead to diseases, such as oral candidiasis. Oral candidiasis is most commonly caused by *Candida albicans*, which can alter the bacterial diversity. To examine the nature of such fungal-bacterial interactions, we aim to investigate the interaction between *Candida* species and oral pathogenic bacteria at the level of extracellular vesicles (EV). For our experiments we used the *C. albicans* SC5314 and *C. parapsilosis* CLIB214 strains, along with *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* as pathogenic bacterial counterparts. We optimized the fungal and bacterial EV isolation protocol from liquid media. The characterisation of the EVs by NanoSight showed round shaped particles with diameters between 50 and 250 nm. We examined the effects of EVs released by *C. parapsilosis* and the yeast and hyphae form of *C. albicans* on the growth and biofilm formation efficiency of *S. aureus*, *P. aeruginosa* and *E. faecalis* and vica versa. As a results, we found that EVs from *C. albicans* and *C. parapsilosis* had different effects on the growth of *S. aureus*. Fungal EVs also affected the biofilm formation efficiency of bacterial species. Regarding the effect of bacteria, the bacterial EV treatment altered the biofilm formation efficiency of *C. albicans* and *C. parapsilosis* in a species dependent manner. Using fluorescence microscopy we found that fungal cells and bacterial EVs colocalize after 4 hours of incubation, and fungal cells form hyphae with various efficiency. Based on these results it can be assumed that there is an interaction between fungal and bacterial cells at level of extracellular vesicles.

THE EFFECT OF PHYTONUTRIENT-ENRICHED DIET ON MICROBIAL COMMUNITIES OF THE GASTROINTESTINAL TRACT OF POULTRY

ZSOMBOR SZŐKE, PÉTER DÁVID, PÉTER FAUSZT, ANNA SZILÁGYI-RÁCZ, LÁSZLÓ STÜNDL, FERENC GÁL, JUDIT REMENYIK, MELINDA PAHOLCSEK

Institute of Food Technology, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Debrecen, Hungary

In intensive animal farming, the appropriate microbiome composition of livestock plays a key role in the reduction and elimination of husbandry techniques related stress. The effects of nutrients on the gut microbiota are receiving increasing attention thanks to modern molecular biology techniques. Certain phytonutrient-enriched feeds may even replace the use of antibiotics. During our work, we applied targated 16S rRNA amplicon- and shot-gun metogenome sequencing techniques to thoroughly study the phytonutrient-enriched feeding protocols induced compositional changes in the gastrointestinal track microbiota of poultry. We investigated the effect of the feed protoype on the composition and diversity of the core microbiom and community resilience. By comparing the abundance of metabolic pathway encoding genes in our control and experimental groups functional metogenomics was applied.

Altough the use of antibiotics has decreased in most EU countires to a greater or lesser extent, Hungary is still ranked as the fifth largest user of antibiotics in terms of livestock production. Reducing and replacing the use of antibiotics is not only a sustainable, green approach, but may also help to pruduce safe and high qualitiy poultry meat.

ENZYME-ASSISTED PRODUCTION OF BIOACTIVE HYDROLYSATES FROM EDIBLE OILS

MIKLÓS TAKÓ, ZSÓFIA TERÉZIA FURKA, TAMÁS KOVÁCS, ALEXANDRA KOTOGÁN, TAMÁS PAPP, ERIKA BEÁTA KEREKES, CSABA VÁGVÖLGYI

Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Many types of valuable compounds can be obtained from biological residues using microbial hydrolases as a biocatalyst. Lipases, for instance, can release fatty acids and other bioactive molecules from plant and animal oil samples through hydrolysis of triacylglycerols and other ester molecules. In our previous experiments, fatty acid-enriched cocktails from olive, rapeseed, linseed, almond, peanut and grape seed vegetable oils and from menhaden fish oil were produced using a commercial *Rhizomucor miehei* lipase. The present work evaluates some of the bioactive properties, i.e., antioxidant and antimicrobial capacities, of the obtained hydrolysates. The antioxidant property of samples was studied by Folin-Ciocalteu and ferric reducing power assays before and after lipase treatments. Antimicrobial studies were performed with food-contaminating bacteria, i.e., *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas putida* and *Staphylococcus aureus* through broth microdilution method. Lipase treatment caused an increase in the antioxidant capacity of natural oils involved, which showed strong or moderate correlation with the amount of some fatty acids present. Hydrolysis resulted in increased antimicrobial activity for certain oils, but the oils and their hydrolysates inhibited the growth of bacteria differently depending on the microorganism tested. In general, high inhibitory activity was detected for grape seed, linseed and menhaden fish oil samples, and the *E. coli* and *S. aureus* were the most sensitive to the oils and hydrolysates. Bioactivity of the obtained cocktails can be utilized for development of natural food preservatives and/or sanitizers.

Acknowledgements: Supported by the grants NKFI FK134886 and ITM NKFIA TKP-2021-EGA-28.

IDENTIFICATION OF *BACILLUS* AND *CANDIDA* SPECIES BASED ON THEIR FATTY ACID PROFILES

HUHN THU[1], MÓNIKA VÖRÖS[1], FLÓRA BOHNER[1], GYÖRGY SIPOS[2], ATTILA GÁCSER[1], CSABA VÁGVÖLGYI[1], ANDRÁS SZEKERES[1]

[1]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged; [2]Functional Genomics and Bioinformatics Group, Research Center for Forestry and Wood Industry, University of Sopron, Sopron, Hungary

The Sherlock Chromatographic Analysis System (CAS), is developed and marketed by MIDI, Inc. for analyzing whole-cell FAs. The method using FAs 9-24 carbons in length and automated gas chromatography (GC) analysis analyzes qualitatively and quantitatively whole-cell FAMEs. FA profiles were automatically assessed by GC including a comparison of the peak retention times and the equivalent chain length of samples with those of known standards. The general CAS procedure contains the cultivation of microbes on appropriate medium and incubation conditions, the harvest and extraction of cell mass to collect whole-cell FAMEs, which are served as samples to the GC processing. In our study, CAS methods were developed for certain *Bacillus* and *Candida* species to perform routinely, user-friendly, and fast-automated identification as a taxonomic method. Several *Bacillus* species are already involved in Sherlock CAS and can be identified using a commercially available method and library. Using this available identification procedure, 107 isolates were identified out of 128 environmental *Bacillus* isolates including 4 strains as *B. atrophaeus*, 6 strains as *B. cereus*, 27 strains as *B. licheniformis*, 39 strains as *B. megaterium*, 4 strains as *B. pumilus*, 5 strains as *B. simplex*, and 18 strains as *B. subtilis*. After that, the identity of the unknown *Bacillus* strains was revealed using molecular tools as *B. endophyticus* (1 strain) and *B. velezensis* (20 strains). Then the fatty acid methyl ester (FAME) profiles of these molecularly identified isolates were analyzed and applied as samples (n=3) to develop a new CAS library including the new *Bacillus* species. Furthermore, a yeast method of CAS was applied to identify yeast species using commercially available libraries. However, these chemotaxonomical libraries have not contained the recently described *Candida auris* species, yet. Thus, using the collection of isolates belonging to this species, a novel library was built for their identification. As a result, the developed method can be applied to classify *C. auris* at both species and clades levels.

Acknowledgements: Supported by the Hungarian Scientific Research Fund (OTKA K-128659 and K-123952). The project received funding from the EU's Horizon 2020 research and innovation program (under grant agreement No. 739593).

FUNGI IN RAGWEED INFLORESCENCE: THEIR BIODIVERSITY AND DISPERSAL

ZSÓFIA TISCHNER[1], ORSOLYA UDVARDY[2], ÁRON BÉNI[3], MÁRTA DOBRÓNÉ TÓTH[4], FLÓRA SEBŐK[1], CSABA DOBOLYI[1], EDIT KASZAB[1], BALÁZS KRISZT[1], DONÁT MAGYAR[2]

[1]Department of Environmental Safety, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő; [2]National Public Health Center, Budapest; [3]Institute of Agrochemistry and Soil Science, Faculty of Agricultural and Food

Sciences and Environmental Management, University of Debrecen, Debrecen; [4]Institute of Environmental Science, University of Nyíregyháza, Nyíregyháza, Hungary

The common ragweed (*Ambrosia artemisiifolia*) inflorescence is characterised by high levels of allergenic pollen production and release. The most affected area in Europe is Hungary, where about 85% of agricultural land is threatened by ragweed. Almost 60% of allergic patients suffer from ragweed allergy in Hungary. Our previous samplings have shown that ragweed flowers and pollen have a high fungal diversity. To investigate the ragweed flower, as a microbial environment, the following aims have been established: 1. molecular identification of the fungal strains previously collected from the flowers, 2. investigate the effect of ragweed flower on the dispersal of fungi. From the sampling locations in Hungary, the isolated strains were placed at culture collection at Institute of Aquaculture and Environmental Safety, Gödöllő. We identified the strains from the collection with molecular methods using different loci and primers. Primarily, the ITS region of the ribosomal RNA gene cluster was used. For subsets of the isolates, a fragment of the TEF 1α gene and GAPDH gene were also amplified. Sanger-sequencing of the amplicons was performed on ABI 3130 Genetic Analyzer (Applied Biosystems, USA). To study the dispersal of fungi, laboratory experiments and field samplings were performed using Hirst-type air sampler. Based on our results, the most common fungi colonizing ragweed pollen and inflorescence were *Alternaria* and *Cladosporium*. We identified 19 strains belonged to the genus *Cladosporium*, 15 strains belonged to the genus *Alternaria* and other species: *Aureobasidium pullulans*, *Beauveria pseudobassiana*, *Coprinellus domesticus*, *Filobasidium magnum*, *Mortierella alpina*, *Peroneutypa scoparia*, *Stemphylum vesicarium*, *Talaromyces purpureogenus*, *Thyridium endophyticum*. Microscopical observations of air samples showed the colonization of pollen grains by different fungal taxa. Pollen grains infected by fungi became common at the end of the pollen season. These particles correlated positively with wind speed and airborne spores, but a negative correlation was found with temperature. Our observations showed that anemophilous flowers of ragweed stalks are suitable habitats for growth and sporulation of *Alternaria* and *Cladosporium*, but they also offer an optimal base for the aerosolization of large quantities of their spores. This wind-exposed habitat may be one of the factors that explains the high atmospheric concentration of *Cladosporium*. This seems to be an adaptation of cladosporia for a successful airborne dispersal strategy.

Acknowledgements: This research was supported by the project TKP2020-NKA-16 and the ÚNKP-22-4.

LLOVIU VIRUS, AN ENDEMIC FILOVIRUS IN EUROPE

GÁBOR TÓTH, TAMÁS GÖRFÖL, FERENC JAKAB, GÁBOR KEMENESI

Szentágothai Research Center, Institute of Biology, Faculty of Sciences, University of Pécs, Pécs

Only Marburg and Ravn viruses were isolated directly from bats so far and the role of *Rousettus aegyptiacus* as a reservoir is now clear. Lloviu virus (LLOV) is the only member of the Filoviridae family which was ever found in European bats. Since the initial appearance of the virus in 2002 Spain, the seroprevalence and RNA positivity were reported from Schreiber's bats (*Miniopterus schreibersii*) in Spain and Hungary. We established a monitoring system of Schreiber's bats in Hungary in 2013 and after the detection of viral RNA in 2016, we started an active and passive surveillance in multiple countries throughout Europe. Here we present the experiences and results of this surveillance spanning from 2016 to 2022. We operated a mobile laboratory unit on-site and screened the blood samples of these bats with LLOV-specific real-time RT-PCR. Neutralizing antibodies against LLOV were measured with pseudotyped virus neutralization assay and the viral genomic sequences were determined with amplicon-based sequencing technology, using the Nanopore sequencing platform. Positive animals were re-sampled at the collection site, these materials were used for in vitro isolation experiments at the Biosafety Level 4 laboratory, University of Pécs. Bat blood samples and ectoparasites of positive animals were positive for LLOV RNA. The LLOV seropositivity among live animals were varying between 10-20%. During the study period we occasionally found dead animals, exclusively after, or at a late stage of hibernation period. One-third (33%) of these animals were LLOV RNA positive, which was 1,14% in live animals. We isolated the virus from these bats multiple times from multiple locations. We examined more than 10 animals with histological methods to get a clearer picture about the pathology of the virus in these animals. Schreiber's bat has a wide geographic distribution in Europe and the Mediterranean region. By the successful infection of human cell lines, we demonstrated the zoonotic potential of the virus. Our results emphasize the importance of bats in the evolution of filoviruses and nominates Schreiber's bats as possible reservoirs for this virus. The identification of the virus in a different ecosystem compared to the tropics, raises several questions about the ecology, evolution and zoonotic risk of filoviruses.

GENES AND ENZYMES INVOLVED IN THE CATABOLISM OF PLANT-DERIVED AROMATICS IN THE FILAMENTOUS FUNGUS *ASPERGILLUS NIGER*

ADRIAN TSANG

Centre for Structural and Functional Genomics, Concordia University, Montreal, QC, Canada

Lignin is a complex polymer of aromatic compounds and is the second most abundant polymer on Earth, after cellulose, accounting for approximately 30% of all organic carbon. Many other aromatic compounds are found in plant biomass, including tannins, shikimate, quinate, phenylpropanoids, flavonoids, and coumarins. In microorganisms, nearly all plant-derived aromatic compounds that can be utilized as a carbon source are funneled into one of seven dihydroxylated aromatic intermediates, which then undergo ring fission and conversion to TCA cycle intermediates. The mechanisms by which aerobic bacteria catabolizes aromatics are well understood. Here, we used complementary approaches to reveal the genes and enzymes involved in the catabolism of aromatics in the filamentous fungus *Aspergillus niger*. First, we used functional annotation information from the manually curated genome of *A. niger* to identify genes possibly encoding the enzymes involved in aromatics utilization. We further refined the assignment of the pathway genes using the following approaches: whole transcriptome sequencing to reveal genes upregulated in the presence of aromatics; deletion of candidate genes to observe their ability to grow on aromatics; determination by mass spectrometry of the metabolites accumulated by deletion mutants; and enzyme assays of the recombinant proteins encoded by candidate genes. In this report, we used as an example the aggregate experimental evidence to assign the genes for the degradative pathway of protocatechuic acid. The results show that the protocatechuate-degradative pathway of *A. niger* is highly analogous to that of bacteria. However, we demonstrated that a gene essential for the catabolism of protocatechuic acid in *A. niger* is not present in the bacterial pathway. Likewise, we showed that the genes/enzymes involved in the catabolism of other aromatics in *A. niger* are similar, but not identical, to the known bacterial pathways.

RATIONAL DESIGN OF SYNTHETIC ANTIFUNGAL PEPTIDES

GYÖRGYI VÁRADI[1], ATTILA BORICS[2], GÁBOR BENDE[3], ZSÓFIA OLÁH[3], GÁBOR K. TÓTH[1, 4], GÁBOR RÁKHELY[3, 5], LÁSZLÓ GALGÓCZY[2, 3]

[1]Department of Medical Chemistry, Albert Szent-Györgyi Medical School, University of Szeged; [2]Institute of Biochemistry, Biological Research Centre; [3]Department of Biotechnology; [4]MTA-SZTE Biomimetic Systems Research Group, Faculty of Science and Informatics, Unversity of Szeged; [5]Institute of Biophysics, Biological Research Centre, Eötvös Loránd Research Network, Szeged, Hungary

As the consequence of an emerging number of fungal infections and contaminations caused by drug- or pesticide-resistant strains, there is an urgent need to develop new antifungal strategies for medicine and agriculture. Some features of the synthetic peptides spanning the γ-core motifs of antifungal proteins from filamentous fungi render them to be promising candidates. The γ-core motif consists of the consensus amino acid sequence GXCX3-9C, in which X can be any amino acid. The promising features are the prompt antifungal effect, the fungicidal mode of action, and the minimal potential for resistance development. However, the low antifungal efficacy, the narrow antifungal spectrum, and the structural instability could diminish the potential application of γ-core peptide derivatives as antifungal agents. In the present work, we investigated the influence of different amino acid substitutions on these unfavourable features. Increasing the positive charge and hydrophilicity of the γ-core motif elevated the antifungal efficacy and broadened the antifungal spectrum. The overall positive net charge proved to be the primary and the most important feature that determined the antifungal efficacy. Application of the S-tert-butyl protecting group on cysteine residues made the γ-core peptide derivative to be active against filamentous fungi but decreased the efficacy on yeasts. Cysteine-serine substitutions facilitated the structural integrity of γ-core peptide derivatives with a minor decrease in the antifungal efficacy. The structural integrity of the γ-core peptide derivatives did not influence the antifungal efficacy.

Acknowledgements: L.G. was financed by the Hungarian National RDI Office - NKFIH, FK 134343 project.

IDENTIFICATION OF A NEW, TYPE-1 PEROXISOME TARGETING SIGNAL IN FUNGI

GABRIELLA IMOLA VARGA, JUDIT ÁRON, SUREN NEMUUZAYA, CSABA VÁGVÖLGYI, ZSUZSANNA HAMARI

Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Peroxisomes are important compartments involved in many biological processes, such as oxidation of fatty acids and metabolization of hydrogen peroxide. The dominant peroxisome-related biological processes have already been revealed during the past decades, but there is a large gap in our knowledge regarding their species- or environment-specificity. Those peroxisomal proteins that are present in the peroxisomes in a quantity below the detection limit and/or are only detectable in the peroxisomes under special environmental conditions, cannot be identified by using routine proteomic methods. In silico

analysis of the N- and C terminal regions for the presence of peroxisomal targeting signal 2 or 1 (PTS-2 and PTS-1), respectively, is still a reasonable approach to predict the peroxisomal localization of a given protein. Henceforth, expansion of the recently available list of PTS motifs has high importance. Recently, five new plant PTS-1 motifs were identified by a peroxisome proteome analysis in *Arabidopsis thaliana*. Out of these, the SYM motif exists at the C-terminus of two proteins (AN1402 and AN5316) in *Aspergillus nidulans*. A thorough in silico analysis of the available fungal genomes and proteome databases revealed that occurrence of proteins with the SYM motif at the C-terminus is rare or even missing in the individual fungal proteomes. By fluorescent microscopy analyses of SYM-tagged Gfp and Gfp-tagged AN1402 and AN5316 proteins, we provided experimental evidence that the C-teminal SYM motif is a true PTS-1 signal in Aspergillus nidulans.

FUNCTIONAL CHARACTERIZATION OF TWO P-TYPE ATPASES FROM *ASPERGILLUS* SPECIES

ILDIKÓ VIG[1, 2], ZSIMOND BENKŐ[1], CSABA BARNABÁS GILA[1], ZOLTÁN PALCZERT[1], ISTVÁN PÓCSI[1, 2], TAMÁS EMRI[1, 2]

[1]Department of Biotechnology and Microbiology; [2]ELRN-UD Fungal Stress Biology Research Group, Institute of Biotechnology, University of Debrecen, Debrecen, Hungary

Although Fe^{2+} P-type ATPases are important virulence factors in certain human pathogenic bacteria (e.g. *Mycobacterium tuberculosis*, *Listeria monocytogenes*, or *Streptococcus pyogenes* [1-3]) surprisingly little is known on these proteins in fungi. Here, we studied the importance of two P-type ATPases, *Aspergillus nidulans* CrpA (AN3117) and *Aspergillus fumigatus* PcaA (Afu1g16130), in metal ion homeostasis. *A. nidulans ΔcrpA* gene deletion mutants, besides of their Cu^{2+} and Cd^{2+} sensitivity [4-5], showed increased Zn^{2+} sensitivity as well. Although the absence of CrpA did not influence Fe^{2+}, Fe^{3+} and the menadione sodium bisulfite elicited oxidative stress tolerance of the fungus, the *ΔcrpA* mutants were characterized with elevated sensitivity against the combined menadione Fe^{3+} stress. Expression of *A. fumigatus pcaA* in *Saccharomyces cerevisiae* increased the Cd^{2+} (as it was expected [6-7], Zn^{2+} but not Cu^{2+} or Fe^{2+} tolerance of the yeast. Menadione and Fe^{3+} antagonistically reduced the growth inhibitory effects of each other (like in the case of *A. nidulans*), however, the expression of *pcaA* did not cause alterations in menadion, Fe^{3+}, or combined menadion – Fe^{3+} stress sensitivity of *S. cerevisiae*.
According to these results none of the studied ATPases seem to be functioning as Fe*2+* pump. However, as potential Zn*2+* ATPases they can substantially interfere with iron metabolism.

[1] Patel et al (2016) J Biol Chem 291: 11529.
[2] Pi et al (2016) Mol Microbiol 100: 1066.
[3] VanderWal et al (2017) Infect Immun 85: e00091-17.
[4] Antsotegi-Uskola et al (2017) Front Microbiol 8: 912.
[5] Boczonádi et al (2020) J Basic Microbiol 60:574-584.
[6] Bakti et al (2018) Front Microbiol 9: 744.
[7] Kurucz et al (2018) J Basic Microbiol 58: 957.

Acknowledgements: Financed by the National RDI Office (Hungary) project K131767.

ANALYSIS OF THE *cwf14* GENE ENCODING THE G10 PROTEIN INVOLVED IN SPLICING IN *SCHIZOSACCHAROMYCES POMBE*

ILDIKÓ VIG[1], LAJOS ÁCS-SZABÓ[2], ZSIGMOND BENKŐ[1], LÁSZLÓ ATTILA PAPP[2], MATTHIAS SIPICZKI[2], IDA MIKLÓS[2]

[1]Department of Biotechnology and Microbiology; [2]Department of Genetics and Applied Microbiology, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

The *S. pombe cwf14* gene encodes a G10 protein which is a member of the Cdc5 protein complex. This complex plays an important role in the maturation of mRNAs. Consequently, any mutation in this gene results in mis-splicing of several RNAi factors and leads to the defective function of the RNAi-mediated processes, such as the pericentric heterochromatin assembly or the telomere scilencing mechanism. In order to better understand the function of the *cwf14* gene, the deletion mutant strain was subjected to phenotypic studies. Our results showed that the mutant strain was sensitive to changes in pH and temperature, presence of caffeine and congo red. In addition, bioinformatic studies were also performed, which showed that the Cwf14 protein has a high degree of evolutionary conservatism. In BLASTp analyses, the homologous proteins of several species showed more than 50% identity to the *S. pombe* Cwf14 protein. From these, we selected the genes encoding the *Candida albicans* and human homologous BUD31 protein for further experiments. These genes were cloned and interspecific complementation analysis was used to investigate whether, in addition to sequence similarity, functional conservativism was observed between the *S. pombe cwf14* gene and its homologues. Our results show that both *Candida albicans* and the human gene could partially complement the mutant phenotype of the *cwf14*Δ *S. pombe* strain.

DEEP LEARNING-GUIDED GENOME RESOLVED METAGENOME AND METATRANSCRIPTOME ANALYSIS OF MICROBIAL COMMUNITY IN THREE FULL-SIZE BIOGAS PLANTS

ROLAND WIRTH[1, 2], ZOLTÁN BAGI[3], PRATEEK SHETTY[2], MÁRK SZUHAJ[3], SALLY CHEUNG[3], KORNÉL L. KOVÁCS[3, 4], GERGELY MARÓTI[1, 2]

[1]Seqomics Biotechnology Ltd, Mórahalom; [2]Institute of Plant Biology, Biological Research Centre, Eötvös Loránd Research Network; [3]Department of Biotechnology, Faculty of Science and Informatics; [4]Department of Oral Biology and Experimental Dental Research, Faculty of Dentistry, University of Szeged, Szeged, Hungary

Anaerobic digestion is a microbe-driven process of biomass decomposition, which is an environmentally friendly model of bio-waste valorization and nutrient recycling. Rational management of the microbial communities in anaerobic reactors requires a thorough understanding of the anaerobic microbiome and its response to environmental and process disturbances. Microbial indicators of optimal performance and benchmark values for well-performing reactors will then help monitoring sustainable operation of the overall process. In this study, we have examined the microbial community of three Hungarian state-of-the-art industrial biogas plants, which have been utilizing distinct biomass compositions as main substrates for biogas production. In microbial communities many microbes are not cultivable via traditional microbiological methods. To overcome these limitations, cutting-edge in silico deep neural network-guided genome resolved metagenomics approach was used to reveal the members of the anaerobic microbial „dark matter". During the 1 year-long investigation we observed that specialized microbial communities were established in the biogas digesters. This microbiome had a high flexibility to adapt to the environmental conditions. The metagenome and metatranscriptome data showed that a stable core microbiome was present in the digesters, predominated by biopolymer decomposers and syntrophic bacteria, being in a strong connection with the hydrogenotrophic methanogen archaea. Among decomposers and syntrophic microbes several potentially new microbes were also detected that may substantially expand the existing repository of Biogas Microbiome (http://microbial-genomes.org/) genomes and our knowledge on anaerobic biogas-producing microbiomes.

Acknowledgements: Supported by National RDI Fund projects PD132145, FK 123902, FK123899, 2020-3.1.2.-ZFR-KVG-2020-00009.