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ABSTRACTS OF UNKP CONFERENCE (14 June 2019, Szeged)

Chitosan-induced changes of immune responses in tomato plants

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Fungal cell wall-derived elicitor chitosan (CHT) is one of the most commonly-used microbe-associated molecular patterns (MAMP) to examine plant defence reactions, hence its ability to activate pattern-triggered immunity by inducing stomatal closure as well as inhibiting the opening of stomata at dawn among the first defence reactions. Plant defence responses can be different in the light and dark, but the daytime- and light-dependent effects of CHT on the stomatal movements, generation of reactive oxygen species (ROS) and photosynthetic activity in guard cells compared to the mesophyll cells remained unexplored.

Plants were treated with CHT at different times of the day. To clarify the role of light in plant defence reactions, we kept some plants under light and simultaneously in the dark following the treatment in the morning. We found that CHT not only inhibited the light-triggered stomatal opening at dawn but also induced the closure of stomata in the morning, independently of the daytime of the treatments. Production of ROS was enhanced in the morning after CHT treatments, whereas significant nitric oxide accumulation was observed only in the late light phase in the guard cells. CHT decreased the actual quantum yield of the second photosystem (F_{PSII}) and the photochemical quenching coefficient (qP) at dawn both in mesophyll- and guard cells. Moreover, increased *PR1* (*Pathogenesis-related 1*) expression was found at dawn and in the morning after the application of the elicitor, which demonstrates the induction of plant defence responses, whose light-dependence was further confirmed by the artificial-darkening experiments. Based on our results, we can conclude that the presence or absence of light has an elemental role in the regulation of plant immune responses in case of elicitor treatments.

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Tight junction structural studies using superresolution microscopy

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The neurovascular unit is responsible for maintaining brain homeostasis and therefore neuronal function. This is provided by the concerted function of the endothelial and glia cells, pericytes and neurons constituting the NVU. One of the main tasks of the NVU is controlling the transport of solutes between the blood circulation and the brain parenchyma. This is achieved by the blood-brain barrier that on one hand blocks paracellular transport via the continuous tight junctions that very tightly connect adjacent brain endothelial cells and on the other hand by strictly regulating transcellular transport through numerous transporter proteins and by keeping pinocytosis and transcytosis at a low level. The main proteins of tight junctions are claudins that polymerize to form strands making an intricate net interconnecting the plasma membranes of adjacent cells.

The structure and composition of the tight junction exhibits changes in pathologies such as inflammation, brain injury and neurological disorders. Changes of tight junction structure and composition cause changes in barrier permeability often exacerbating diseases and causing oedema.

Based on freeze fracture electron microscopy, the grid size of the claudin net is too small to resolve by conventional microscopy but with superresolution microscopy it is achievable.

We studied the tight junction structure of brain endothelial cells - that is mainly constituted by claudin 5 - *in vitro* and e*x vivo* using superresolution STED microscopy. As tight junctions are universal in all endothelia and epithelia, we compared brain endothelial and intestinal tight junctions. We also developed methods to image the tight junction structure in the lateral

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membrane of cells.

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New therapeutic targets - Investigation of autophagy induction role of *Neosartorya fischeri* antifungal protein (NFAP) in the native producer

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Neosartorya fischeri NRRL 181 is able to produce a small, cysteine-rich, cationic antifungal protein, the NFAP with anti-mould activity. NFAP did not show toxic effect on mammalian cell lines (keratinocytes, intestinal epithelial cells, and leukocytes) suggesting specific fungal target(s) in its mode of action. Based on this, NFAP is a promising candidate as new antifungal compound. One of the limiting factors of its applicability as medical drugs is the poor pharmacokinetic property. However, extra- or intracellular targets of NFAP in sensitive-fungal strains or the native producer could be considered as bases to develop new antifungal compounds. The present work was aimed to investigate the antifungal effect, uptake and localisation of NFAP in *N. fischeri* NRRL 181; furthermore, to isolate its potential intracellular targets from this fungus for deducing its potential biological role.

Depending on the applied concentration, NFAP showed growth inhibitory activity itself on the native producer *N. fischeri* NRRL 181 *in vitro*, and evoked morphological aberrations of germlings and hyphae. Localization studies using a green fluorophore-labelled NFAP demonstrated that it is able to bind to the outer layers of *N. fische*ri NRRL 181 conidia and ascomata to exert these effect; and when these asexual reproductive spores start to form germ tube, NFAP enters in the cell and causes cell death. Target-screening experiments in *N. fischeri* NRRL 181 using the Random Oligopeptide Phage Display System (New England Biolabs) signed that NFAP possibly interacts with a regulatory factor of the autophagy induction (ATG11) in connected with programmed cell death during nutrient depletion. Our results suggest that ATG11 is a potential NFAP-specific target, and NFAP can induce autophagy in the native producer by specific binding to it. Far-Western blot analysis is in progress to confirm this finding.

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Size-dependent activity of silver nanoparticles on the morphological switch of *Candida albicans*

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Candida albicans is one of the most common opportunistic pathogenic fungi causing invasive infections especially in immune compromised patients. Several factors influence the pathogenicity of the opportunistic pathogens, among them secreted enzymes, heat tolerance, toxin production as well as dimorphic growth. During the dimorphic switch the yeast forms convert into virulent hyphae, which promote the adhesion and invasion of the fungal cells and the development of biofilm. In the biofilms the cells are surrounded by extracellular matrix, which protects them from antifungal agents, thus the surviving biofilm-resident cells are intrinsic sources of recurrent infections.

Silver nanoparticles (AgNPs) are the most frequently used metal nanoparticles due to their antibacterial, antiviral and antifungal activity. The toxic effect of AgNPs are caused by the intracellular release of silver ions, which leads to the generation of reactive oxygen species resulting in mitochondrial disfunction and apoptosis. Nevertheless, the efficacy of the applied AgNPs might largely depend on their size, shape and surface charge. Although the antifungal activity of AgNPs has already been proven

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on numerous species, the impact of nanoparticle size on the morphological switch of dimorphic fungi has not been investigated yet. To examine the size-dependent activity of AgNPs on the morphological switch of *Candida albicans,* citrate-coated nanoparticles of 7, 21 and 50 nm average size were synthesized by chemical reduction method. The effect on the hyphae formation and the biofilm production of *C. albicans* was detected on fungal monocultures, and on co-cultures using human keratinocytes. The yeast-to-hyphae conversion was completely inhibited by 24-hour exposure to small sized nanoparticles (AgNP-I). When fungal cells were treated with medium sized AgNPs (AgNP-II) and with large particles (AgNP-III), both yeast and elongated cellforms of *C. albicans* were observed. Biofilm formation was not detected upon AgNP treatments after 72 hour incubation. Moreover, AgNP-I reduced the number of elongated *C. albicans* cells co-cultured with HaCaT cells to a greater extent than AgNP-II, while AgNP-III did not affect the hyphae formation of the *C. albicans* compared to the control.

In summary, the smallest AgNPs were the most effective in inhibiting biofilm production of *C. albicans* in monoculture and in co-culture with keratinocytes, respectively. The medium sized AgNPs were also efficient, but the biggest AgNPs did not influence the morphological switch of *C. albicans* co-cultured with keratinocytes. These results suggest a size-dependent impact of AgNPs on the morphological conversion of opportunistic pathogenic fungi.

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Minimising the risks related to resistance evolution to therapeutic antimicrobial peptides

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Antimicrobial peptides (AMPs) are key effectors of the innate immune system and promising therapeutic agents. Yet, knowledge on how to design resistance-proof AMPs with minimal cross-resistance to human host-defense peptides remains limited. Here, we systematically assessed resistance evolution of *Escherichia coli* against a large set of different AMPs by applying metagenomics, chemical-genetics, and adaptive laboratory evolution. Although generalizations about AMP resistance are common in the literature, in general, we found that *E. coli's* capacity to evolve resistance to AMPs with different physicochemical properties and cellular targets varies considerably. The modes of action of the AMPs also influence the cross-resistance to human host AMPs. A special group of AMPs emerged with lower potential to induce resistance by horizontal gene transfer or genomic mutations. Our work can inform future efforts on how to minimize cross-resistance between therapeutic and human host AMPs.

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Biocontrol agents from field-to-lab and lab-to-field: genus *Trichoderma* in the spotlight

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The filamentous fungal genus *Trichoderma* is a popular subject of basic and applied mycology research, which is due to the fact, that *Trichoderma* species play important roles in various agricultural environments. Several members of the genus have the potential to biologically control plant pathogenic fungi and nematodes by antagonistic action based on efficient competition, antibiosis and/or mycoparasitism. The ability of certain *Trichoderma* species to promote plant growth and induce systemic

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resistance in plants can also be exploited within the frames of environmentally friendly agricultural practices.

The first step of the development of biocontrol means based on *Trichoderma* is generally the isolation of *Trichoderma* strains from agricultural soil samples derived from the fields of the crop plants to be protected. Isolated strains are screened in the laboratory for their antagonistic abilities towards the target pathogens, and properly characterized to select potential biocontrol agents (BCAs). The species-level identification of the selected BCA candidate *Trichoderma* strains by sequence-based molecular tools is a crucial step of the process, as besides the positive implications of the genus in biocontrol, certain *Trichoderma* species may be harmful as the causal agents of green mould infections affecting cultivated mushrooms, while others are able to cause opportunistic infections in humans. An exact and reliable identification of BCA candidates enables to avoid the development of potentially harmful members of the genus to biocontrol products, thus lowering the risks of *Trichoderma* biocontrol. Strain improvement by mutagenesis, protoplast fusion or genetic transformation can be performed to enhance the beneficial properties of the selected *Trichoderma* strains, however, the improvement by genetic manipulation may restrict the applicability of the resulting BCAs in several countries with strict GMO regulations. Subsequent steps are optimization of fermentation for the selected BCA and selection of the proper formulation and delivery. If the beneficial effects of the BCA candidate can be confirmed by greenhouse and field trials, registration and commercialization can follow. The development of proper, specific monitoring tools for the applied *Trichoderma* strains may provide important information about their population dynamics and performance in various agricultural systems.

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Molecular investigation of salt tolerance development in eukaryotic green algae

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Enhancement of algal salt accumulation was the general aim of the study. Metabolic and genomic characterization of salt tolerance as well as the identification of novel efficient salt metabolizing eukaryotic green algae strains were the major tasks of this project. We have also tested the possibilities of experimental evolution of salt tolerance in selected green algal species. As a first step the biomass potential of a series of algal species in our collection was assessed under low salt conditions. Also, new algae isolates were recovered and purified from specific environments with high NaCl conditions (special soils and ponds). Biomass potential and initial salt tolerance were determined for the novel isolates as well.

Algae are thriving in complex communities in nature, they are in permanent interactions (mutualistic, neutral and parasitic) with a number of other microorganism, mostly with bacteria. Thus, we were interested in the effect of selected bacterial partners on the algal salt accumulation performance. We have tested the effects of natural bacterial partners and also designed fully synthetic algal-bacterial consortia. We have continuously monitored the algal and bacterial living cell number, morphology and photosynthetic efficiency. The salt accumulation performance of the algae was determined in selected stages of the adaptation process, salt tolerance of axenic algae was compared to algae associated with bacterial partners. Significant increase in salt tolerance was observed for certain algae strains (*Chlamydomonas reinhardtii* cc124 and *Chlorella* sp. MACC360). We suggest, that salt adaptation may occur through various ways as indicated by the highly different morphological changes of the applied algae strains. In order to get a better understanding of the underlying molecular changes, genome-level experiments were initiated, the whole transcriptome of the ancestor and adapted algae strains are under investigation either under axenic and bacterial associated conditions. The possible mutations (SNPs, deletions and insertions) will also be investigated beside the gene expression profiles.

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Examination of the effects of zinc oxide nanoparticles on plants using proteomics

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Zinc oxide nanoparticles (ZnO NP) have broad range of radiation absorption, high chemical, mechanical, thermal and photostability and high electrochemical coupling coefficient. These rare properties make ZnO NP useful in *e.g.*, biomedicine, agriculture. NP can be synthetized in a wide variety of 3D structures and sizes, which have an impact on their toxicity. Their effects on plant species are mostly dose-dependent, lower concentrations are proven to be beneficial, while higher concentrations are toxic. In this study the involvement of nitrosative stress in ZnO toxicity was examined.

We studied the effect of 6 nm ZnO NP on two plant species, Indian mustard (*Brassica juncea* L. Czern. cv. Negro Caballo) and rapeseed (*Brassica napus* L. cv. GK Gabriella). Plants were grown for five days on sterile ½ MS media which contained control (0), 25 mg/l or 100 mg/l ZnO NP-s. We examined plant biomass production, cell viability, enzyme activities involved in superoxide radical homeostasis and protein tyrosine nitration.

25 mg/l ZnO NP concentration was beneficial to plants, indicated by increased tolerance index and biomass production. Cell viability in the root meristem decreased slightly, with changes in enzyme activities and protein tyrosine nitration pattern. On the other hand, 100 mg/l ZnO NP-s had toxic effect on both plant species, since it reduced biomass production, tolerance index and cell viability. Protein tyrosine nitration and enzyme activities were modified compared to control. *Brassica juncea* was more tolerant to ZnO NP toxicity.

Our data suggests, that ZnO NP toxicity is in strong correlation with protein tyrosine nitration, implying the importance of future studies in this research field.

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Developing a novel protein-tagging, immundetection and purification system

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Over the past years our laboratory has produced several monoclonal antibodies (mAb) that specifically recognize the p54 protein, the polyubiquitin receptor subunit of the 26S proteasome. We found that anti-p54 mAb1 recognizes the C-terminal region of the protein with unusually high specificity. Preliminary results suggested that the epitope of the antibody is relatively short, hence, it can be used to generate an epitope-tagging system for protein labelling, detection or purification. Therefore, I started to map the epitope of anti-p54 mAb1 and found that an 8-mer sequence localized in the C-terminus of the p54 protein serves as the epitope of anti-p54 mAb1. I aimed to compare the anti-p54 mAb1 with a commercially available immunoglobulin (anti-Flag M_2) that is widely used (however very expensive) in immunological applications due to its high specificity towards an artificial 8-mer epitope (tag) fused to the protein of interest. I did the comparison on whole cell lysates of *Drosophila* embryos or Chinese Hamster Ovary and human cells, respectively, representing different proteome complexities, using the same concentration of the two antibodies in Western-blot experiments. I found that anti-p54 mAb1 performed significantly better under the same conditions and did not produce any background at exposure when anti-Flag M_2 already generated non-specific bands. After that, I started to examine proteins, which were tagged with the anti-mAb1's 8-mer epitope on their N- or C-termini. When the 8-mer epitope was fused to either the N- or the C-terminus of the model proteins it always behaved as an immuno tag and the chimeric proteins were recognized by the anti-p54 mAb1 in Western-blot experiments. During this study I also generated plasmid vector systems in which any gene of interest can be fused to the 8-mer epitope (N- or C-terminally) and expressed in bacteria or Eukaryotic cells in a constitutive manner or under the regulation of inducible promoters. This would allow us to tag any proteins of interest with this 8-mer epitope for immuno-detection, staining or purification from bacteria to tissue culture cells.

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Nitric oxide signaling during nickel stress responses in plants

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Excess heavy metals may result in branched, shallow root systems due to primary root (PR) shortening and concomitant lateral root induction (LR). Although, nickel (Ni) can be a relevant environmental contaminant, its relationship with these special root morphological responses is largely unknown. Also, the putative involvement of nitric oxide (NO) signalling in plant nickel stress requires further research. Therefore, in the third year of my HAS János Bolyai Research Scholarship (2016-2019), I am currently studying the nickel stress-induced root system alterations with special attention to NO signalling. In connection with this research program, my goal was to disseminate the scientific topic and the experimental results.

Wide concentration-range (0, 25, 50, 75, 100 μM) nickel chloride treatments were applied to *Arabidopsis thaliana* L. (Col-0) and *Brassica juncea* L. Czern. (cv. Negro Caballo) and 50 μM Ni proved to induce altered root morphology (shorter PR and increased number of LRs compared to control). Moreover, the greater Ni tolerance of *B. juncea* was associated with smaller Ni-induced changes in its nitroproteome, as well as in the amount of S-nitrosoglutathione enzyme and in NO, hydrogen sulphide and reactive oxygen species levels.

These results were presented in the frame of a course (title: *Reactive nitrogen- and oxygen species in plants*) which is attended by both Biology Bsc. students and Biology Msc. students. Moreover, a poster (title: *Nitrosative stress in nickel-exposed Arabidopsis and Brassica*) will be presented at Straub Days organized by HAS Biological Research Centre in Szeged. I dealt with the topic in two seminar lectures. Additionally, two Bsc. theses were prepared (Kitti Sándor, Bsc.: Essentiality and toxicity of nickel in plants and Olivér Gaál, Bsc.: Nickel phytoremediation). Collectively, the dissemination of my research topic and experimental results has been realized by integrating them in university education and in research training, by presenting them to scientific community and by promoting them to a wider social circle (Researchers' Night 2018, Facebook page).

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Investigation of nanoparticle-endothelial cell interactions in a microfluidic chip model of the blood-brain barrier

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The blood-brain barrier is a dynamic interface composed of brain microvascular endothelial cells that restrict the brain penetration of most neurotherapeutics. Thus, central nervous system diseases are especially challenging to treat pharmaceutically.

Our research group has been developing a novel and versatile microdevice, which enables the co-culture of two or three cell types, the flow of the culture medium, visualization of the cells by microscopy, monitoring of the transcellular electrical resistance and the measurement of monolayer permeability. Our integrated lab-on-a-chip device is suitable for modeling the blood-brain barrier and mimicking the cerebral blood flow. The aim of this study was to optimize this chip device developed in our laboratory for drug delivery experiments and to test the effect of fluid flow on nanoparticle-endothelial cell interactions.

hCMEC/D3 human cerebral microvascular endothelial cell monolayers were cultured in the device under static and dynamic flow conditions. The growth of cells and the integrity of the layer was monitored by phase contrast microscopy and

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transendothelial electrical resistance (TEER) measurements. Liposomes loaded with rhodamine B were prepared and tested for permeability across hCMEC/D3 monolayers. Cellular uptake of liposome-loaded rhodamine B in endothelial cells was also visualized by fluorescence microscopy.

We have successfully optimized our chip device for drug delivery experiments and showed that dynamic fluid flow plays an important role in the permeability and cellular uptake of the nanoparticle cargo. Our results indicate that the utilization of microfluidic chip devices could be an important step in drug delivery research.

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The role of autophagy in the regulation of axon degeneration during aging

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Structural integrity of neuronal processes is maintained by finely tuned protein degradation programmes. Macroautophagy is a compartmentalized form of both bulk degradation of cytoplasmic components and selective elimination of proteins and organelles. Autophagy is indispensable for the maintenance of normal cellular homeostasis and is especially active in the nervous system. During selective autophagy, selective autophagy receptors such as p62/Ref(2)P recognize several ubiquitin chain types on proteins followed by binding of the receptors to LC3/Atg8a that mediates the degradation of the whole complex. Loss of core autophagy genes in the mouse nervous system results in shortened lifespan, motor and behavioural problems due to apoptotic cell death and brain atrophy. Axon terminal dystrophy is also observed in these mice. We hypothesized that in the *Drosophila* wing nerve, late-onset axon degeneration could be induced by lack of proper autophagic processes. We knocked out or specifically downregulated in neurons core components of the autophagic machinery and examined aged animals. We found that while in young autophagy-deficient flies no change in axon structure could be observed, aged animals, earliest at 30 but mostly at 60 days of age developed axon fragmentation and loss. Currently no evidence exists concerning the death mechanisms that instruct axon destruction in lack of autophagy. To shine a light on this, we tested the role of caspases, mitochondrial oxidative stress and the injury-induced Wallerian degeneration pathway that have been implicated in diverse axon disintegration events. We found no role for caspases but curiously, overexpression of both Sod2, a mitochondrial superoxide dismutase and Wld^S, a potent inhibitor of Wallerian axon degeneration rescued axon fragmentation in *Syntaxin-17*-depleted animals. We validated our results using genetic mosaic analysis with autophagy gene null mutants and in a different axon model in the fly brain. In summary, we established a fly model for genetic dissection of late onset axon degeneration in autophagy deficiency and pinned down the downstream mechanisms mediating axon degeneration.

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Investigation of immune activation and immune regulation in rodent models and human patient-derived blood

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The immune system is highly plastic owing to the complexity of cellular fates to execute the versatile polarization of lymphoid and myeloid cells. The activation and polarization evolve from the interactions in the network of immune cells with the surrounding soluble or anchored signals. The evolutionary advantage of this versatile polarization is the elimination of

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pathogenic invaders and cancerous cells. However, due to genetic and environmental factors the polarization of leukocytes may be influenced and disturbed to render the immune system overwhelming or ineffective against pathologies.

To achieve high resolution measurement of cellular features, single cell mass cytometry has been used in our laboratory combining advantages of the single cell resolution of traditional fluorescence-based flow cytometry with the multiplexicity of inductively coupled plasma-mass cytometry. Instead of fluorophores detection for mass cytometry is based on stable heavy-metal isotope labeled antibodies. Thus, the autofluorescence and spectral overlapping are eliminated. This unique feature enables researchers to multiplex up to 45 different antibodies in one single tube. PBMCs of human systemic autoimmune diseases (RA, SLE, SSc), rat model of colitis and non-small cell lung cancer patients have been investigated by different antibody panels using mass cytometry. Plasma proteome profiling is also carried-out by Luminex and Legendplex technologies. Large data sets, disease associated patterns of cellular and plasma protein markers have been analyzed.

The combination of the analysis of cytokine production with the complex view of the heterogeneity of PBMCs at single cell resolution could potentially reveal novel pathways to identify therapeutic targets.

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Analysis of genes encoding possible spore coat-like proteins in opportunistic human pathogenic fungi

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Members of the order Mucorales can be agents of life-threatening, opportunistic human infections, known as mucormycosis. Because of the devastating outcome of this disease, which has been observed despite the current treatment options, it is urgent to identify the possible virulence factors. It was previously shown that the spore coat CotH proteins played an important role in the pathogenesis of Mucorales, hence arise the main objective of our study to determinate the possible role of CotH5 protein in the pathogenesis of *M. circinelloides*.

In this experimental setup, a linearized deletion cassette was used along with the Cas9 enzyme and the gRNA complex for direct transfer without *in vitro* RNP formation and the use of plasmids to disrupt the *cotH5* gene in a homology directed repair mechanism. This approach resulted in stable transformants, in which gene disruption occurred by the integration of the selection marker at the expected position. The mutation was proven by PCR analysis and the lack of the appropriate transcripts was proven by qRT-PCR. Phenotypic characterization of mutant was performed and the possible role of the protein in the process of phagocytoses was also analyzed. We identified the CotH5 protein as a possible spore coat-like protein based on its amino acid sequence. Construction of strains, in which the *cotH5* gene is disrupted or overexpressed can provide a good tool to investigate its relevance in the pathogenicity and other biological mechanisms. Based on the results we think that the CotH5 protein may have a role in the sporulation of the fungus.

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Examining the efficiency of exercises aiming the development of oral health literacy and critical thinking

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In our previous research, we examined the brushing habits of secondary school and university students and experienced that the ratio was high of those in both sample, who has problems with the evaluation and application of information. The development of oral health literacy may be connected to the development of critical thinking since the evaluation as a cognitive operation is a common feature of these two areas. The aim of our present research is to examine the efficiency of exercises aiming the development of oral health literacy and critical thinking in classrooms.

We tried two exercises in our research: one of them was an interview with false information in which they had to find the mistakes ("Interview with a dentist") and another was an article with questions ("Which brushing method is the best?"). After that, the students filled out a questionnaire about their motivation and the efficiency of the exercises.

According to 83.34% of the students (n=54, 35.2% of them female, 64.8% of them male) they could solve the tasks individually, and 90.74% of them could interpret the read text critically. Only 22.22% of the answering students agreed completely that the tasks demanded from them to apply their previous studies of health literacy taught in Biology classes. The 83.33% of the participants like reading in topics related to their health, and 74.07% likes critical thinking. Most of the students could not tell why plaque is dangerous, and they could not evaluate the significance of fluorides properly.

The results highlighted that students require to be concerned with the development of oral health literacy and critical thinking in classrooms. We set ourselves the target to develop more exercises in continuing our research.

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Development and characterization of microbial lipase systems for industrial applications

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Lipases catalyze lipolytic reactions in which they release free fatty acids, diacylglycerols, monoacylglycerols and glycerol. Under certain conditions, lipases can synthesize lipid compounds through the catalysis of esterification processes. As a result of the above reactions, functional lipids, *i.e.* polyunsaturated fatty acids and alkyl esters, potentially applicable in different industries can be produced. Therefore, there is a need for identification of new lipases, and analysis of the background of reactions in specific conditions relevant for their practical application. With experiments from our laboratory a number of producer strains and enzymes have been characterized from the zygomycetes fungal group. Using these biocatalysts, and other enzymes from microbial sources, our aim was to develop and characterize lipase systems utilizable for future production of bioactive lipids. In line with this, immobilization and regiospecificity studies, as well as assays on lipases' hydrolytic potential against oils from various vegetable and animal sources were also planned.

For the investigations we selected the *Mortierella echinosphaera* and *Mucor corticolus* lipases that have been purified by our group prior to this assay. In addition, commercial forms of *Aspergillus niger*, *Rhizopus oryzae*, *Rhizopus niveus* and *Rhizomucor miehei* lipases were also included to the studies. In summary, the lipases of *M. echinosphaera*, *M. corticolus* and *R. oryzae* were successfully immobilized onto the polypropylene Accurel MP1000 carrier. The immobilized enzymes have shown great efficiency in catalysis of lipolytic reactions, and they exhibited improved biochemical properties, *e.g.*, storage and temperature stability, compared to the free enzymes. Moreover, the enzyme-support complexes proved to be reusable in several reaction cycles as well. Concerning regiospecificity, the studied enzymes exhibited different properties that affect (with potential) their hydrolytic behavior against oils substances. Finally, in a pilot experiment, a comparative analysis has also been initiated in which we examine the hydrolytic activity of selected lipases on vegetable and menhaden fish oils using a simple and fast chromogenic

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test. Results so far showed high variability in the oil degradation potential of the catalysts tested.

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Interaction of neutrophil granulocytes with *Curvularia lunata*

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Emerging fungal pathogens, like *Curvularia lunata* represent a continuously increasing problem, because of the growing population with underlying conditions, the difficulties of diagnosis and the high antifungal resistance of certain fungal agents. This filamentous fungus is known as a trans-kingdom pathogen, which is able to cause infection both in plants and humans. The mycoses in humans can manifest as fungal keratitis, sinusitis, cutaneous lesions or invasive infections, depending on the immune status of the host. The aim of this study was to investigate the neutrophil response to hyphal form of *Curvularia lunata* and expose the fungal defence mechanisms.

In the present study, *C. lunata* SZMC 23759 and *A. fumigatus* SZMC 23245, both isolated from human eye infection were examined. Activation of neutrophils was measured in the presence and the absence of the supernatant of germinating conidia and after serum treatment. Effector mechanisms of the immune cells focused on oxidative burst and NET formation was studied. Melanin production and extracellular acidification by *C. lunata* was also analysed as a potential defence action of the fungus.

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Cell division of microalgae investigated by protein derivatives

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Cell division is the most important process of living organisms. Bacterial cell division has been studied intensively for decades, but many fundamental questions remain unanswered. Cyanobacteria, which belong to microalgae, are biotechnologically important, photosynthetic Gram negative bacteria, the ancestors of plant plastids. Understanding the mechanisms of the bacterial cell division can lead to the development of new antibiotics, to biotechnology, and furthermore to the understanding of plant plastids development, division. In prokaryotes, and in the eukaryotic cell organelle plastids and mitochondria a tubulin homologue GTPase protein, the FtsZ initiates the cell division by the polymerization into a ring-like structure (Z-ring) at midcell. The Z-ring subsequently functions as a scaffold for recruitment of downstream factors that promotes the formation of the division septum. The positioning and timing of the Z-ring is crucial for the cells. It is believed that bacteria generally divide by binary fission producing two identical daughter cells, although in some cases asymmetrical division was also observed. Following the development of the division ring is possible by fluorescent microscopic techniques, when the FtsZ protein is fused with *e.g.*, green fluorescent protein (GFP). According to published information, the FtsZ-GFP chimeric gene cannot be completely segregated from the endogenous *ftsZ* gene, because the FtsZ-GFP chimera cannot form complete division rings without wild-type FtsZ (Buske PJ et al. 2015; Ma X et al. 1996). This might indicate that the presence of GFP can disturb the ring formation. At the same time the presence of chimera can cause alteration in the cell size influencing the proliferation time (Quin S et al. 1998). We have created new FtsZ-GFP chimeras in *Synechococcus* sp. PCC7942 cells with different linker between GFP and FtsZ to avoid possible interferences with the potential interactors. The reporter molecule made

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the FtsZ ring be visualized. The constructs allow us to examine the FtsZ protein interactions and to study the influence of the environmental factors on cell division.

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Evolution of long-distance resource transport in complex multicellular fungi.

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With the evolution of complex multicellular life, organisms had to circumvent the limitation of diffusion, leading to the evolution of special animal and plant cells or tissues, which facilitate long-distance nutrient transport. Fungi can produce rhizomorphs, hyphal strands and cords (here Fungal Long-distance Transport Structure, FLTS) that enable the transportation of nutrients and water, have fundamental role in plant pathogenicity (*e.g.*, *Armillaria* spp.), the functioning of common mycorrhizal networks of plant communities and exploring and degrading wood material (*e.g.*, white rot fungi). Despite of the essential biological functions and the immense importance of FLTS, we have limited knowledge of its taxonomic distribution, macro-evolution and the genetic background of its development.

Therefore, we summarised more than 5.300 literature records on FLTS and performed macro-evolutionary analyses using a published mega-phylogeny (Varga et al. 2019, Nat. Ecol. Evol.) containing 5.284 species of mushroom-forming fungi (Agaricomycetes). We found that species in the phyla Ascomycota and Basidiomycota can develop FLTS, but the most complex morphologies can be found predominantly in Agaricomycetes, a class of Basidiomycota. In addition, FLTS is typical in most of the orders of Agaricomycetes, but it is most abundant in the Agaricales and Boletales. Furthermore, ancestral character state reconstructions showed that FLTS is possibly an ancient character state and first occurred in the most recent common ancestor of Phallomycetidae and Agaricales. To reveal genes responsible for FLTS development we want to compare transcriptomic data of species from two closely related FLTS orders, the Agaricales (*Armillaria ostoyae*) and the Boletales (*Serpula lacrymans*). Transcriptomic data are available for *A. ostoyae*, but the rhizomorph and fruiting body development of *S. lacrymans* is yet to be investigated. After examining the strain S7 on 11 different nutrient agars and in 12 different conditions, we found that culturing the strain on a complex media containing $NaNO_3$, KH_2PO_4, $MgSO_4$ and $FeSO_4$ in dark at 22 °C for one month resulted 1-2 mm wide rhizomorphs. Furthermore, using the same media, but exposing the culture to a sequence of temperature conditions in dark (22 °C, 4 °C, 18 °C) could induce fruiting body development.

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Drug delivery by nanoparticles

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The major obstacle of the pharmaceutical treatment of central nervous system disorders is the blood-brain barrier, which restricts the penetration of therapeutics to the brain. Nanocarriers hold great promise for drug delivery across the blood-brain barrier, but specific targeting is needed for the succesful brain delivery of nanoparticles, which is currently unsolved. Several clinically used drugs are known to be successfully cross the brain endothelial cells via the nutrient transporter solute carriers (SLC), however this pathway is not fully exploited for drug delivery. Ligands of SLC transporters at the blood-brain barrier are promising targeting vectors to increase the brain penetration of nanoparticles. The aims of this study were (i) to examine the mRNA expression levels of SLC transporters of different cell types, (ii) to compare the uptake of targeted nanoparticles in

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primary pericytes, astroglia cells and neuroblastoma cells, (iii) to verify the uptake mechanisms in different cell types.

High mRNA expression levels for hexose and neutral amino acid transporting solute carriers (SLCs) were found in isolated astroglia cells and pericytes so we prepared, characterized and tested nanoparticles from non-ionic surfactants (niosomes) functionalized with alanine, ligands of SLC-transporters and glutathione, a reference ligand as blood-brain barrier targeting molecules.

The presence of targeting ligands on niosomes, especially the dual-labeling, increased the uptake of the cargo molecule in pericytes, astrocytes and neuroblastoma cells. This cellular uptake was temperature dependent and could be decreased with a metabolic inhibitor and endocytosis blockers filipin and cytochalasin D. Our data indicate that dual-labeling of nanoparticles with glutathione and alanine, a ligand of SLC transporters can be effective for blood-brain barrier targeting.

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Role of the neurovascular unit and brain environment in the formation of central nervous system metastases

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Brain metastases are devastating complications of cancer and an unmet challenge for clinicians. Therapeutic resistance of brain tumours largely depends on unique aspects linked to the cerebral environment and the neurovascular unit. Among cells of the neurovascular unit, endothelial cells and astrocytes are the most active in immediately responding to and continuously associating with invading tumour cells.

Here we used advanced microscopy techniques – including two-photon microscopy in living animals, confocal and super-resolution (stimulated emission depletion/STED) fluorescence imaging and transmission electron microscopy – to understand the mechanisms of interactions of triple negative breast cancer cells with cerebral endothelial cells and astrocytes. We followed reaction of brain resident cells to the tumour cells during the extravasation step and the metastatic growth phase in the brain parenchyma.

We observed that mammary carcinoma cells induce up-regulation of N-cadherin in cerebral endothelial cells; however, this mechanism is dispensable for the trans-endothelial migration. Breast cancer cells are able to migrate via the transcellular pathway through the endothelium, leaving the tight junctions intact. Metastatic cells breach the end-feet layer of astrocytes that cover the outer surface of cerebral vessels and accumulate along the capillaries attached to the vascular basement membrane. This process, called vascular co-option, is the main mechanism of tumour vascularization in the brain.

In conclusion, we identified new mechanisms involved in the reaction of brain resident cells to invading breast cancer cells. Our results contribute to a better understanding of the complex cross talk between metastatic cells and brain resident cells, which is essential for the identification of future therapeutic targets.

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Response of keratinocytes to pathogenic and skin commensalist *Candida* species

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Invasive *Candida* infections are prevalent worldwide and threaten especially those with compromised immunity. Besides these severe diseases, cutaneous infections caused by *Candida* species may also develop. *C. parapsilosis* regularly colonizes the human skin as a commensalist and only seldom leads to pathological conditions there. On the contrary, *C. albicans* is not a member of the normal flora of the human skin but it is the commonest agent of cutaneous candidiasis. Keratinocytes are able to mediate

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immunological activities, yet little is known about their response to related commensalist and pathogenic yeasts.

Therefore, our goal was to examine the interaction of *C. albican*s and the less harmful *C. parapsilosis* with human skin keratinocytes.

We infected HaCaT and HPK-KER cell lines with *C. albicans* and *C. parapsilosis* strains. Damage of skin epithelial cells was monitored through lactate dehydrogenase activity in supernatants of infected keratinocytes. Cytokine release from infected skin cells was determined by enzyme-linked immunosorbent assay from cell culture supernatants. We used imaging flow cytometry to reveal association of keratinocytes with green fluorescent protein expressing strains of the two *Candida* species and also to test if pHrodo™ Red stained yeasts are ingested by keratinocytes. Recently, we have initiated a survey on the occurrence of *Candida* species on healthy skin surfaces of Hungarian volunteers.

C. albicans caused remarkable damage to keratinocytes as opposed to *C. parapsilosis*. Pre-treatment of HaCaT cells with live or heat killed *Candida parapsilosis* did not reduce cellular damage triggered by *C. albicans*. IL-8 production was induced by *C. albicans* in both cell lines and also IL-6 secretion in HPV-KER cells. Release of these cytokines was hardly affected by *C. parapsilosis*. To a low extent, cells of both *Candida* species were able to adhere to keratinocytes and we observed occasional phagocytosis of both yeasts by skin cells. To date, only two *C. parapsilosis* and no *C. albicans* strains were isolated from skin surfaces.

This project contributes to a better understanding of skin immunity to pathogenic and commensalist yeasts. It was supported by the UNKP 18-3-IV-SZTE-55 New National Excellence Program of the Ministry of Human Capacities.

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