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Seeking novel antimicrobials derived from environmental bacteria

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Antibiotic resistance is an increasing health problem worldwide. The development of novel antibiotics has slowed down thereby narrowing the available antimicrobial armada. It is noteworthy however, that our natural environment still harbors numerous unexploited antimicrobial compounds, produced by yet uncultured microorganisms. We combine innovative microbiological and analytical chemistry techniques to screen environmental microorganisms for their ability to produce novel antimicrobial compounds with drug potential.

We collected about 300 soil samples from 15 countries. The samples were screened for antimicrobial producing bacteria by agar overlay assay. Preliminary identification of the positive isolates was determined by Sanger sequencing of the 16SrRNA genes. Crude extracts (CE) were prepared by cultivation in broth followed by solvent extraction. The CEs were tested against a panel of opportunistic pathogens. CEs were thereafter purified with High Performance Liquid chromatography (HPLC) to obtain biologically active fraction(s) which were analyzed with Mass Spectrometry (MS) to determine the novelty of the active compound. The whole genomes of few isolates suspected to be producing new compounds were sequenced using Illumina technology.

Thirty antimicrobial producing strains were isolated and identified, the CEs from most of which showed broad spectrum inhibitory activity. MS analysis of the active fractions derived from *Paenibacillus* and *Brevibacillus* strains shows production of antimicrobial peptides belonging to the class of polymyxins and bogorols, respectively. BLAST search using antiSMASH of the two genome sequences confirmed the presence of Biosynthetic Gene Clusters (BGCs) coding for the production of both compound classes in their respective hosts. Currently, we have two antimicrobial peptides suspected to be novel.

Our ongoing experiment include chemical synthesis of the suspected antimicrobial peptides to verify their activity and expression of their (BGCs) in heterologous host. We also plan to continue analytical screening of the crude extracts from the remaining isolates.

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Building a bioinformatics resource for evolutionary studies on *Armillaria* pathogenesis

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Armillaria sp. are spread worldwide, and they are known cause severe root rot diseases in a variety of woody plants. As they possess a great threat to the forests and garden plants, better understanding of the pathogenic and virulence factors is crucial to develop proper control strategies and tackle the infection caused by them. The main objective of our study was to elucidate the role of secretory proteins, lncRNAs (long non-coding RNAs) and microbiome which could be involved in *A. ostoyae* pathogenicity. We also performed *A. ostoyae-Trichoderma atroviride* interaction assay to study the *T. atroviride* bioeffector strategies.

To study the *A. ostoyae* secretory genes, lncRNAs and microbiomes, we infected 2-year-old Norway spruce seedlings in natural environment and isolated the mycelium that was growing in the cambium of the spruce seedlings and sequenced them. We performed gene differential expression analysis study and identified the secretory genes and lncRNAs which were expressed in mycelium that was either killing the plant or causing symptoms like wilting. Our results indicate that there were 2214 differentially expressed genes whose log2FoldChange (logFC) was above |1| and p-value < 0.05. Genes related to processes such as lignocellulose, hemicellulose degrading was upregulated in the mycelium that was infecting the woods. We filtered out differentially expressing secretory genes (279 secretory genes) in *A. ostoyae* mycelium and then used it for comparison with other fungi. Machine learning based feature identification identified some of the orthogroups that could contribute to *A. ostoyae* infection strategies. We also identified 131 differentially expressing lncRNAs in *A. ostoyae* during the infection and associated

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the genes that were *cis-trans* regulated by those differentially expressed lncRNAs. The results of *Armillaria-Trichoderma in-vitro* interaction assay and double RNA-seq study indicate that *Trichoderma* employ different CAZymes, Peptidases and metabolite related genes such as non-ribosomal peptide synthetase (NRPS), Polyketide synthase (PKS), PKS-like and NRPS-PKS hybrid to tackle *A. ostoyae. A. ostoyae* in turn turned on a self-defense system by expressing genes related to alpha-cuprenes. In our study, we identified plant cell degrading enzymes and other secretory proteins are crucial to establishing infection in host plants and we also identified that many of the genes related to pectin degradation, phenolics degradation and secondary metabolite production were regulated by lncRNAs. *A. ostoyae-Trichoderma atroviride* interaction assay showed that *T. atroviride* slowly killed *A. ostoyae* by negatively impacting its genes related to cell cycle control and DNA repair. Our study provides a detailed insights on the molecular repertoires that are crucial for *A. ostoyae* virulence and pathogenicity; it also sheds light on *A. ostoyae* genes that are involved in mycoparasitic interaction.

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Characterization of azole resistant *Candida auris* strains generated by laboratory microevolution method

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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 clinical manifestations.

Fluconazole, posaconazole and voriconazole resistant strains were generated from two (0381, 0387) independent triazole susceptible isolates using the *in vitro* microevolution method and the effects of the acquired resistance were further examined. Collected data implies that acquisition of triazole resistance effects the fitness of the fungal cells under host modeling conditions, as 0381 POS^{evo} 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 0381 originated strains showed slightly increased fungal burden (especially in the brain), while 0387 derived strains had a significant decrease in virulence in BALB/c mice. Generated strains were sequenced and compared to the susceptible isolate. 0381 FLU^{evo} and VOR^{evo} strains harbored an SNP in *TAC1b* that corresponded with increased *CDR1* 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 triazole resistance in *C. auris* and may indirectly influence its virulence.

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Cardiovascular effects of cannabinoids in different rat models

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In previous years, the presence of cannabinoid receptors (CB1, CB2) and their various ligands have been explored in the cardiovascular system. Cannabinoids, due to the distribution of their receptors, have a positive effect on vascular contraction

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and play an important role in the regulation of metabolic processes as well. The aim of our work was to examine the effects of anandamide (AEA) and rimonabant (RIMO) on various physiological processes affecting the cardiovascular homeostasis. AEA is a well-known agonist of cannabinoid receptors, while RIMO is a synthetic cannabinoid, considered as an inverse agonist of CB1 receptors. We investigated the impacts of the cannabinoid receptor modulators in two different experimental designs. Twenty-month-old female and male rats were used in the RIMO study, whereas sham-operated and ovariectomized female Wistar rats were involved in the AEA study. Both substances were administered *intraperitoneally* for 2 weeks, at a daily dose of 1.0 mg/kg. At the end of the experiments, rats were sacrificed, and their hearts were removed; the biochemical parameters were measured from heart tissues in both studies. Our results proved that two-week-long RIMO treatment significantly reduced the cardiac reactive oxygen species via improving antioxidant defense mechanisms including heme oxygenase (HO) system, superoxide dismutase and glutathione content. At the same time, it successfully decreased age-related inflammation, since it significantly reduced the concentration of tumor necrosis factor-alpha, the activity of myeloperoxidase enzyme and the expression of nuclear factor kappa B. Regarding the hormone-depleted female rats, it can be concluded that estrogen deficiency caused a significant decrease in both nitric oxide synthase and HO parameters, as well as regarding the transient receptor potential vanilloid 1 (TRPV1) and calcitonin gene related peptide (CGRP) values. However, two weeks of AEA treatment upregulated the cardioprotective pathways and favorably restored these adverse changes. Our findings prove that low-dose RIMO was beneficial against age-associated inflammatory and oxidative damages in rat heart, while AEA may be an effective therapeutic strategy in estrogen-deficient cardiovascular conditions.

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Examinations of kynurenic acid (KYNA) analogues with basic research methods of ischemic stroke

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Ischaemic stroke stands on the second step of the mortality list of WHO and this disorder causes the most cases of acquired disability. Despite, there are numerous research groups investigating many ways of healing pathways, possibilities are restricted now. Our research group examine a molecule as known as kynurenic acid which is the one known endogen N-methyl-D-aspartate receptor (NMDAR) antagonist in the human system. This molecule has neuroprotective effects in central nervous system (CNS), but its penetration ability is poor through the blood brain barrier (BBB). Colleagues in Pharmaceutical Chemistry Institute synthetised and synthetising constantly kynurenic acid analogues with the aim of create a molecule which as neuroprotective as KYNA, but it has a higher permeability through the BBB.

Firstly, we examined the analogues in an *in vitro* electrophysiological method using male Wistar rats. Acute brain slices were prepared with vibratome (300 μm thick, coronal) and were measured in Haas-type chamber. Artificial cerebrospinal fluid was streamed around the slices and the analogue wash into the chamber during the process. If we experienced consequent repeatable effects at a given analogue in normal 'physiological' circumstances, the second level of examination was conducted in ischaemic conditions. During oxygen-glucose deprivation (OGD) we found a molecule as known as SZR104 can prolong the normal operation of hippocampal pyramidal cells with 4 min and 9 sec.

In the meantime, The Neurovascular Research Group (Biological Research Centre, Szeged) could prove that the molecule of SZR104 has an excellent penetration ability through the blood brain barrier. Finally, based on the results above, we started an *in vivo* tissue distribution investigation in which we administered SZR104 in three concentrations (0,15 mmol/kg, 0,03 mmol/kg, 0,003 mmol/kg) to the animals in physiological saline solution through their tail-vein. After the treatment we collected liquor, plasma, and brain tissue from the animals at four time (30, 60, 90 min and 24 h). Based on our preliminary mass spectrometry (cooperation with Institute of Pharmaceutical Analysis, University of Szeged) experiments the analogue SZR104 can penetrate through the blood brain barrier highly also in a living animal.

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Evaluation of the biostimulant effect of two microalgae species from the Chlorophyta division

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Microalgae are ubiquitous, produce a plethora of bioactive compounds and achieve huge biomass within a short time in relatively smaller spaces than plants. Thus, they are suitable substitutes to plants in several industries such as fuel, pharmaceuticals and organic fertilizer production. The biostimulant effect of processed microalgae (various methods are used for extraction) have been reported. However, limited studies report on microalgae-plant-biostimulation in line with sustainable agriculture.

Therefore, the objective of our work was to test the biostimulant action of the following selected microalgae; *Chlamydomonas reinhardtii* cc124, *Chlorella sp.* MACC-360 and *Chlorella sp.* MACC-38. Our aim was to investigate if they stimulated growth in *Medicago truncatula* and *Solanum lycopersicum*. Our experiments were designed to eradicate chemicals, reduce energy consumption and simplify extraction and application procedures. Thus, the plants were treated with wet microalgae biomass. Either live cells alone or live cells plus the spent media were applied to plants via soil drench method. Also, the cells were crushed in liquid nitrogen then suspended in water to prepare the water extract which was sprayed on leaves. Physiological, biochemical and photosynthetic parameters were then analyzed. Transcription studies were also conducted for the tomato experiments and microscopy was done to check emanation of extracellular material from algae. We observed that the tested microalgae had strain-specific biostimulant effect on tested plants. All strains mostly increased leaf chlorophylls and carotenoids, thus, consequently affected photosynthesis. *Chlorella sp.* MACC-360 had a more profound effect compared to the other strains especially on flowering; this strongly correlated with the profuse extracellular polysaccharides (EPS) production. Transcriptomic studies revealed differential expression of genes participating in responses to abiotic stresses especially cold and drought stress, in microalgae-treated-tomato plants. Overall, our results imply the feasibility of using microalgae in agriculture as an alternative to chemical fertilizers.

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Purification of fungal metabolites by chromatographic methods

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There might be several purposes for purifying microbial metabolites. In some cases, the purification from fermentation environment is the easiest and most inexpensive way to produce large quantities of a well-known compound for further experiments or for using as standard compound in quantitative and qualitative measurements. In other cases, purification of a non-described metabolites is also required to determine its exact structure. Several preparative chromatographic techniques are available to address these challenges including flash chromatography or liquid-liquid chromatography, which is becoming increasingly common in the purification of natural compounds.

The aim of our work was the development of purification processes for ochratoxin A (OTA), a toxic secondary metabolite of *Aspergillus albertensis* and for two undescribed intermediate metabolites in nicotinic utilisation pathway of *A. nidulans*. OTA separation was carried out by centrifugal partition chromatography after a three-step liquid-liquid extraction. To reach the most suitable biphasic solvent system, "shake flask" method was used, and the samples originated from the upper and lower phases, were analysed by HPLC-UV technique. Based on the test results, the instrumental optimization was implemented with hexane-isopropanol-water 30-24-40 (V/V/%) system. The run was carried out with 8 ml/min flow rate in descendent mode at 2000 rpm rotational speed. Finally, the purity of OTA was more than 99% according to HPLC run on 333 nm and 280 nm. The two metabolites of *A. nidulans* were purified by flash chromatography on silica and strong anion exchange stationary phase. Method development was started on thin layer chromatography (TLC) and the spots on TLC plate were scraped off, dissolved in methanol, and measured by HR-MS. The flash chromatographic runs were carried out on 25 g silica loaded column with solid loading type. Eluents were ethyl acetate with 1% NH_3 (A) and methanol with 4% NH_3 (B). Metabolite with molecular weight of 131 g/mol was separated with an isocratic elution at 20% B, and metabolite with molecular weight of 147 g/mol

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was separated with isocratic elution at 40% B. The collected fractions were analysed by HR-MS and the purest fractions were analysed by NMR techniques for structural determination.

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Short-term effects of TRPML activation on autophagic flux

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Autophagy is a cellular degradation process that is conserved in all eukaryotic cells and induced by different stimuli such as nutrient deprivation or oxidative stress. Autophagy activation results in sequestering intracellular cargo in vesicles called autophagosomes which then fuse with lysosomes for degradation and recycling. Two crucial steps are required for autophagic cargo degradation: autophagosome-lysosome fusion and lysosomal degradation. Defects in any of these two steps block autophagic flux. Notably, Ca^{2+} signalling from the endolysosomal cation channel TRPML1/MCOLN1 is required for retrograde transport of lysosomes towards the perinuclear region during autophagy. While TRPML involvement in autophagosome biogenesis, its lysosomal fusion and passive regulation of lysosomal pH is well-established, much less is known about autophagic consequences following short-term TRPML Ca^{2+} efflux.

Therefore, the object of our work was to study the role of calcium in lysosome regulation. Our specific aim was to understand how short-term TRPML Ca^{2+} efflux controls autophagosome-lysosome fusion. Our experiments were based on TRPML1 activation by ML-SA1 and HEK293 cells transiently expressing dominant-negative (TRPML1 D471-472K-pHcRed) TRPML1 mutant. We firstly found that intracellular Ca^{2+} chelation by BAPTA-AM led to LC3-II accumulation and reversal of acidic lysosomal pH. To monitor TRPML Ca^{2+} mobilization during ML-SA1 (a TRPML agonist) treatment, we generated a lysosome-targeted genetically encoded Ca^{2+} sensor (GCaMP6m-TRPML1). TRPML Ca^{2+} efflux by short ML-SA1 treatment (10-15 min) correlated with its acidification and hydrolase activity, even in pH neutralized lysosomes. Moreover, ML-SA1 potentiated autophagosome-lysosome fusion and degradation of autophagic cargo. Interestingly, lysosomal migration was found unaffected during this short ML-SA1 treatment. We also saw that localization of the small GTPase Rab7 to autophagosomes increased following TRPML activation, which may indicate its mechanistic role in fusion. In line with this, overexpression of dominant-negative Rab7 prevented AP-LY fusion. Further studies relating to how TRPML Ca^{2+} efflux regulates migration-independent autophagic fusion is underway.

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Direct organogenesis in *Arabidopsis thaliana*: investigation the interplay between polyamines and plant hormones

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De novo shoot meristem formation is one possibility for vegetative propagation during which plants can be regenerated from different tissues or explants. In *Arabidopsis* a very efficient direct regeneration system was developed which based on the capability of the lateral root primordias (LRPs) to convert into shoot meristems (SMs) in definite stages of their development. To the formation of LRPs to SMs a well-balanced cytokinin (CK) and auxin ratio is essential, but these hormones also may interact with other plant hormones and polyamines (PAs). PAs has a common precursor with ethylene and NO, respectively. Furthermore, catabolism of PAs results in generation of reactive oxygen species (ROS). Besides amine oxidases, NADPH oxidases are also major ROS generators in plants. Nevertheless, during the last years, convergent action of PAOs and NADPH-oxidases was identified in several processes, including developmental and stress responses of the plants.

Our aim was to investigate the role of PA metabolism and its possible interplay with NO, ET, and ROS during LRP- SM

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conversion of *Arabidopsis* plants. Among PAOs, the expression of Arabidopsis *POLYAMINE OXIDASE 5* (*AtPAO5*) increased during the process and its ectopic overexpression enhanced the LRP-to-shoot conversion efficiency. *pao5-2* mutants with elevated thermospemine (t-spm) level or exogenous treatment with t-spm in wild type plants (Col) showed a decrease in the expression of hemoglobin genes (*AtGLB1, AtGLB2*) and a decrease in the regeneration efficiency. Treatment with exogenous ethylene activator (1-Aminocyclopropane-1-carboxylic acid) caused an increase in NO level through to the enhance of relative expression of *AtNIA1* and the decrease of *AtGLB1* and *AtGLB2*. ACC treatment also decreased the mRNA level of *AtPAO5* and the efficiency of the conversion of LRP to SM. In wild type *Arabidopsis* plants relative expression of *AtRBOHs* did not change. However, in *atpao5-2 plants*, or under exogenous t-spm treatment the relative expression of *AtRBOHD* was significantly higher. Accordingly, *rbohd* mutant showed increased regeneration efficiency compared to the wild-type plants. Taken together, poliamine metabolism involves in the direct formation of SM and they cross talk with ethylene and NO in this process.

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Investigation of a possible relationship between trepanations and other pathological lesions on osteoarchaeological remains

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Trepanation are divided into three groups in the Eastern European osteological nomenclature: surgical, cultical and symbolic trepanations. Classical surgical interventions are conducted on living subjects, and all the three layers of the cranial vault are removed.

My examinations were conducted in skeletal series with skulls bearing surgical trepanations that derive from the Conquest Period and the early Árpádian Age (9^{th}-11^{th} c. CE). I examined all cases applying a unified protocol for comparability, and I also made efforts to verify the dating and the trepanation diagnosis of each possible case. This type of work creating a cadastre of 10-11^{th} century trepanations is unprecedented in the Hungarian osteological practice. In 51 cases, the earlier diagnosis of trepanation was verified. I examined all the available remains of each affected individuals, and also investigated 5 more individuals of the same sex and similar age-at-death in each series for comparison. Instead of the usual nosology-based palaeopathological diagnostical process, I observed types of lesions in the endocranial and ectocranial surfaces and made statistical comparison between the trephined and non-trephined samples. The work hypothesis was that trepanations were conducted for real therapeutic reasons. If pathological features are more frequent among individuals with trepanations than their peers, it indirectly implies that trepanation might have been applied as a therapeutic measure in most cases and was not a ritual intervention as often hypothesized elsewhere.

Beside the comparative work, we also put forward a change in nomenclature; we wish to introduce the word 'cranioglyph' instead of 'symbolic trepanation', since the latter tends to be confused with other interventions in the Western practice.

In the future, we wish to carry on the comparative approach to postcranial elements and activity-related changes to further elucidate the indication of these ancient surgical techniques.

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Investigation of the interaction of keratinocytes and *Candida* species

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Our skin provides immunological protection against several pathogens. Skin epithelial cells respond to microbial stimuli in various ways. In this project, we aimed to investigate how human keratinocytes interact with different *Candida* species, as

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common colonizers of the skin. Human skin keratinocyte cell lines (HaCaT, HPV-KER) were applied and challenged with *C. albicans* (SC5314 and WO-1 strains) and *C. parapsilosis* (GA1 and CLIB214 strains) strains. We aimed to determine the extent to which *C. albicans* and *C. parapsilosis* damage human keratinocytes, their attachment to host cells, the keratinocytes' ability to internalize these fungi and to examine cytokine production in response to stimuli. Furthermore, we explored these parameters mentioned before, following *C. albicans* infection which pre-incubated with the skin commensal *C. parapsilosis* strains (primed immunity).

Our results suggest that *C. albicans* causes significantly more damage to human keratinocytes than *C. parapsilosis* and the HPV-KER cell line was more susceptible to the infection. In both HaCaT and HPV-KER cells, the production of IL-6 and IL-8 increased primarily after *C. albicans* infection. Based on the adhesion studies, there was a low degree of association in case of *C. parapsilosis* GA1 and CLIB214 compared to *C. albicans* SC5314 and WO-1. The efficiency of phagocytosis was found to be much lower in *C. parapsilosis* strains compared to *C. albicans* strains in both cell lines. During the pre-incubation experiments, host cells were pre-treated with *C. parapsilosis* cell, then *C. parapsilosis* cells were removed and host cells were stimulated with *C. albicans* cells. In these experiments uninfected conditions were used as controls for subsequent comparisons. LDH assay showed no difference in the extent of damage compared to pre-incubation free infections. There was no change in the extent of adhesion compared to pre-incubation free infections. When the efficacy of phagocytosis was tested following pre-incubation infection, only the HPV-KER cell line and only *C. albicans* SC5314 strain showed lower levels of phagocytosis. Both cell lines showed elevated levels of IL-6 and IL-8 cytokine/chemokine secretion at higher MOI values of fungal infection and predominantly after live *C. parapsilosis* treatment.

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Microfluidic platforms designed for morphological and photosynthetic investigations of *Chlamydomonas reinhardtii* on a single-cell level

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Green algae are of outstanding ecological and increasing biotechnological importance. *Chlamydomonas reinhardtii* is a well-known model organism but, the evaluation of its life cycle processes and photosynthesis on a single-cell level is largely unresolved. To facilitate the study of the relationship between morphology and photochemistry, we established microfluidics in combination with chlorophyll *a* fluorescence induction measurements.

Microfluidics devices available for *C. reinhardtii* enabled only steady-state fluorescence measurements so far. Devices for *C. reinhardtii* are available almost exclusively for studying a micropopulation of cells and rarely single-cell analysis. To fill this gap, we designed, constructed, and employed two types of microfluidic platforms for single-cell investigations: The traps of the "Tulip" device are suitable for capturing and immobilizing single cells, enabling the assessment of their photosynthesis for several hours without binding to a solid support surface. Using this "Tulip" platform, we performed high-quality non-photochemical quenching measurements and confirmed our earlier results on bulk cultures that non-photochemical quenching is higher in ascorbate-deficient mutants (*Crvtc2-1*) than in the wild-type. We have successfully measured the light adaptation kinetics in alga cultures at two different light intensities. At 151 and 383 μmol photons $m^{-2}s^{-1}$, NPQ was higher in an ascorbate-deficient mutants (*Crvtc2-1*) and they regenerated more easily than the wild type (CC-4533). We obtained very similar results in acetate-containing growth medium and in carbon-limited minimal medium. The traps of the "Pot" device were designed for capturing single cells and allowing the growth of the daughter cells within the traps. Using our most performant "Pot" device, we could demonstrate that the F_V/F_M parameter, an indicator of photosynthetic efficiency, varies considerably during the cell cycle. With this platform, we demonstrated that photosynthetic efficiency changes during the cell cycle, which is in line with earlier photosynthetic activity measurements and transcriptomics data. Our microfluidic devices, therefore, represent versatile platforms for the simultaneous morphological and photosynthetic investigations of *C. reinhardtii* on a single-cell level.

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Characterization and functional analysis of zinc trafficking in the human fungal pathogen *Candida parapsilosis*

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Zinc is essential for cellular life and its uptake by fungal pathogens is crucial for maintaining both viability and pathogenicity. We aimed to assess the role of zinc uptake and homeostasis in the human fungal pathogen *C. parapsilosis* and to examine their role in virulence. Our *in-silico* predictions suggested the presence of at least six potential zinc transporters in *C. parapsilosis*: orthologues of *ZRC1*, *ZRT3* and *ZRT101*, but an orthologue of *PRA1* zincophore was not found. In addition, we detected a species-specific gene expansion of the novel zinc transporter *ZRT2,* as we identified three orthologue genes in the genome of *C. parapsilosis*. Based on predictions, we created homozygous mutant strains of the potential zinc transporters and characterized them.

We have identified and characterized for the first time five zinc transporters in *C. parapsilosis.* Like *C. albicans*, growth of *C. parapsilosis* in an acidic environment is mediated exclusively by the membrane zinc transporter CpZrt21. However, in this species, two additional Zrt2 orthologues are also present that may be under the same regulation as *CpZRT21* in the setting of zinc limited conditions. We revealed that *C. parapsilosis* forms zincosomes under high zinc supplemented environmental conditions, although, in contrast with *C. albicans,* in a Zrc1-independent way. Instead identified a *C. parapsilosis* vacuolar-localised zinc transporter, CpZrc1, which is essential for zinc detoxification and also protects *C. parapsilosis* cells against killing by murine macrophages. Based on our findings we can hypothesize that, once *C. parapsilosis* cells are phagocytosed by macrophages, zinc ions accumulate in the phagolysosome which may contribute towards fungal killing.

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Single-cell image analysis and machine learning methods using the cellular microenvironment

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Modern automated microscopes produce thousands of images routinely. They help to understand the complex phenotypic composition of cellular systems which is essential to answer major questions of cell biology. However, analyze these images manually is almost impossible. Due to their efficiency, machine learning based software have become widely used tools in this field. However, an important limitation of such methods is that their decision is based solely on the local properties of the cell of interest. In our approach, we use various features from the surrounding environment. We tested, that such additional information can improve single-cell-level phenotypic image analysis in 2D, and we are curious what results we could get in pictures of 3D cell cultures and tissues.

The relevance of 3D cell cultures is in that, that although 2D cell-based study is the most used method in the beginning of drug testing, its limitations have been increasingly recognized. In vitro 3D cultures could fill the gap between conventional 2D in vitro testing and animal models. In our laboratory, we use different kind of plates with gravity-based approach for spheroid (a type of 3D culture) generation. With current microscopy technologies, it is difficult to visualize individual cells in the deeper layers of 3D samples mainly because of limited light penetration and scattering. To overcome this problem several optical clearing methods exist. We work with many of them (for example: sucrose, ScaleA2, Clear) to be able to acquire good-quality images with a light-sheet microscope. We created a software tool (3D-Cell-Annotator) that can segment the single cells of the spheroids. With that we can collect training data for machine learning. Our main aim is to test the effect of microenvironmental features in 3D.

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Protective effects of H_2S donor, Lawesson's reagent in TNBS-induced rat colitis

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Inflammatory bowel diseases (IBD) are chronic, immune-mediated disorders, which affect the gastrointestinal (GI) tract with periodic inflammation and ulceration. It has been showed that oxidative stress contributes to epithelial and vascular injuries. The formation and releasing of neutrophil extracellular traps (NETs) is called NETosis. It is increasingly clear that NETosis is an important process of the immune response during inflammation, however its mechanism is still not precisely known. It is presumed that hydrogen sulphide (H_2S) as an important gasotransmitter has a wide range of regulatory functions and plays a role in many physiological and pathological processes.

Therefore, the aim of our study was to investigate the protective effects of H_2S and to determine whether H2S has an impact on the heme oxygenase (HO) and NETosis markers in 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced rat colitis. To model IBD, TNBS was administered intracolonically (i.c.) to Wistar male rats. Then animals were treated per os with various doses of Lawesson's reagent as a H_2S donor 2 times/day for 3 days. To clarify the role of HO in the H_2S-mediated protection an HO inhibitor, SnPP was also administered in a co-treatment with H_2S donor. Colon samples were collected after 72 hours of TNBS treatment. Our results showed that the most effective daily dose of H_2S donor (18.75 μM/kg/day) significantly attenuated TNBS-provoked pro-inflammatory factors such as myeloperoxidase (MPO) activity, tumor necrosis factor-α (TNF-α) content, the expression of nuclear factor-kappa B (NF-κB) and high mobility group binding 1 (HMGB1), while increased the expression of ubiquitin C-terminal hydroxylase L1 (UCHL-1), a potential anti-inflammatory mediator. Furthermore, H_2S significantly elevated the colonic HO enzyme activity, however the protective effect of H2S was abolished by the co-treatment with HO inhibitor. Finally, our measurements showed that treatment with H2S donor significantly inhibited the expression of the NETosis markers: peptidylarginine deiminase 4 (PADI4), citrullinated histone H3 (CitH3) and MPO. Taken together, our results showed that H_2S may exert anti-inflammatory effects through modulation of the antioxidant HO enzyme and inhibtion of NETs formation, suggesting new therapeutic targets against IBD.

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Nitrate assimilation by green microalgae

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Green microalgae *Chlamydomonas* sp. MACC-216 and *Chlorella* sp. MACC-360 were investigated for their growth and nitrate removal capacity in the modified tris-acetate-phosphate (TAP-M) medium supplemented with various concentrations of nitrate (5 mM, 10 mM, and 15 mM). Both microalgae removed 100 % nitrate from the TAP-M medium containing 5 mM nitrate, but this nitrate removal percentage decreased when the concentration of nitrate was increased in the medium. Furthermore, increased accumulation of lipids was observed with increasing concentration of nitrate only in *Chlamydomonas* sp. MACC-216. Both microalgae were also cultivated in synthetic wastewater (SWW) where both microalgae could grow well and showed significant nitrate removal, however, *Chlamydomonas* sp. MACC-216 performed better than *Chlorella* sp. MACC-360.

Chlamydomonas sp. MACC-216 was further selected to assess nitrate removal under various combinations of light colours (red, blue, blue + red, and white) and intensities (50 μmol m^{-2} s^{-1}, 100 μmol m^{-2} s^{-1}, and 250 μmol m^{-2} s^{-1}). *Chlamydomonas* sp. MACC-216 removed the highest nitrate under blue + red light when grown in TAP-M. Also, nitrate removal was found to be directly proportional to light intensity as nitrate removal increased when the light intensity was increased from 50 μmol m^{-2} s^{-1} to 250 μmol m^{-2} s^{-1}. *Chlamydomonas* sp. MACC-216 was further grown under red, blue, blue + red, and white light colour with a constant 250 μmol m^{-2} s^{-1} light intensity in SWW. Even in SWW, *Chlamydomonas* sp. MACC-216 removed the highest nitrate under blue + red light. Moreover, the highest nitrate reductase activity was also observed under blue + red light. Expression of seven nitrate assimilation pathway-related genes (*Nrt1*, *Nrt2.1*, *Nrt2.2*, *Nia*, *Mcp*, *Nii*, and *GS2)* was analysed and for all the genes except *Nii,* the highest expression was observed under blue + red light. Based on the results, blue + red light

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together with 250 μmol m^{-2} s^{-1} light intensity represents an optimal light condition for efficient nitrate removal by applied microalgae from wastewater.

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Analysis of β-galactosidase activities in Mucoromycota fungi

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β-Galactosidases catalyze the hydrolysis of lactose to glucose and galactose. Through transgalactosylation reactions, the β-galactosidases can synthesize lactose and lactulose derived molecules, i.e., galacto-oligosaccharides (GOS) and OsLu, which are non-digestible sugars and can affect positively the growth of beneficial gut microorganisms. There are β-galactosidase-active microorganisms, however, little attention has been paid on the production and activity in Mucoromycota. These fungi are members of the former Zygomycota and have different applications as producers of industrial enzymes such as cellulases and lipases. In fact, for the functional food production, there is a continuous need to develop new microbial galactosidase systems for which the Mucoromycota fungi may be potentially suitable.

In screening studies, 99 strains belonging to the order Mucorales and Mortierellales were subjected to β-galactosidase production test by using a chromogenic method. A total of 66 β-galactosidase active strains were identified mostly within the genera *Lichtheimia*, *Mortierella*, *Rhizomucor* and *Umbelopsis*. Strains with high activity were selected for fermentation in submerged and solid-state systems containing lactose and/or wheat bran substrates as inducers for the enzyme production. Wheat bran was found as a good substrate for high yield β-galactosidase production, and the *Lichtheimia ramosa* and *Rhizomucor pusillus* were excellent sources of the activity. In real-time PCR assays, the relative transcript levels of the β-galactosidase coding genes were also examined in wheat bran surroundings, in which the relative transcription levels and activities measured agreed during the course of the cultivation. Furthermore, crude forms of the *L. ramosa* and *R. pusillus* β-galactosidases were able to synthesize oligosaccharides on lactose and skim milk substances. The produced oligosaccharide enriched cocktails exhibited a growth promoting effect towards commercial probiotics *in vitro*. Through ion exchange and size exclusion chromatographies, β-galactosidase-active proteins with molecular mass of about 80-90 kDa, and temperature and pH optimum of 50 °C and pH 6.0, respectively, were purified from the *L. ramosa* and *R. pusillus*. Finally, a plasmid free CRISPR-Cas9 system for *L. ramosa* has been optimized and an uracil auxotrophic mutant strain was developed for future β-galactosidase gene manipulation experiments.

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Investigation of tumor heterogeneity: a novel, laser microdissection-coupled lipidomic method

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Lipid metabolic reprogramming is a newly recognized hallmark of malignancy. Most normal cells build up their membranes from dietary lipids. In contrast, cancer cells re-activate the de novo lipogenesis, which is also promoted by oncogenic signalling. Thus, the lipid metabolic network is flexible and tuned to fulfil the requirements of cancer cell.

Tumors resemble complex organs, consisting of tumor cells and highly heterogeneous host-derived stroma. Because the communication between tumor and stromal cells influences the aggressiveness and metastatic potential of tumors, the exploration of tumor heterogeneity is of great interest but also a great challenge. Currently available data on lipid metabolic reprogramming derive from measurements conducted in 2D cell cultures (unable to mimic metabolic zonation) or they examine relatively large tumor pieces (low spatial resolution). Mass spectrometry imaging techniques are capable of high spatial resolution but do not provide quantitative data and have limited lipidome coverage.

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Here, we present the development of a novel laser dissection-coupled microlipidomic method including preparation of parallel native and hematoxylin-eosin-stained cryosections from spheroids and tumor samples, cross-referencing of stained and autofluorescence images, laser dissection of marked regions (100-150 cells), microextraction and mass spectrometry analysis. By using this workflow, we could identify a radial gradient in the lipidomic profile of 3D spheroids, whereas the reproducibility of matching areas from parallel sections was excellent. We could also dissect different tumor regions (cancer and stromal cells) from mouse allografts and human tumors. In summary, the novel approach ensures high throughput, semi-automatic analysis of the lipidome, provides quantitative data with broad-range coverage and good spatial resolution, thereby enabling an unbiased, hypothesis-generating research.

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