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Analysis of fungal fatty acids and prostaglandin-like compounds

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Candida parapsilosis is the third most frequently isolated *Candida* species in candidiases, especially in special patients groups such as low birth weight neonates, where *C. parapsilosis* even outmarks *C. albicans*. A number of biochemical parameters could influence the virulence of these fungal species, whose research is important to understand the mechanisms of whole infection process. According the previous results of our research group based on the transcriptome analysis of the *in vitro* host-pathogen interactions process, the prostaglandin- and the fatty acid pathways of *C. parapsilosis* could play an important role in the pathogenesis. Therefore, we aimed to develop a reliable measurement method for the analysis of metabolites, which are originated from the pathogenicity-linked biochemical pathways and are involved through the interactions as the final chemical effectors including prostaglandins and fatty acids.

In the first part of our work, we developed a liquid chromatographic method using fluorescence detector to analyse prostaglandins from the ferment broths of the wild type and UGA3 (a putative transcription factor, that can play a crucial role in fungal prostaglandin biosynthesis) mutant *C. parapsilosis* strains. After the optimization of sample preparation on solid phase, the evaporated extracts were derivatized to create fluorescence-active derivatives of prostaglandins. In this reaction the fluorescence molecule (Br-DMEQ) was linked to the carboxyl group of prostaglandins in the presence of aprotic solvent and catalyzer. The amounts and the ratios of the reaction components were optimized for the amount of the reaction products leading to the opportunity of the more sensitive measurement. The separation after the testing of several stationary- and mobile phases was carried out on the Phenomenex XB-C18 column with water/acetonitrile supplemented both with 0.1% acetic acid resulting the determination of seven prostaglandins. For the enlargement of the number of the detectable prostaglandins a mass spectrometric analysis was also developed in negative ESI ionization mode, which was able to determine 18 prostaglandin components. Furthermore, the fatty acid content of the wild type and a fatty acid desaturase deficient strain (OLE2) were analyzed with GC-FID technique developed for the analysis of 37 both of saturated and desaturated fatty acid methyl esters.

In the second part of our study, we dealed with the development of a rapid, high-throughput analytical method for the monitoring of the economically important fungal products, fatty acids, from the biomass of the *Mortierella* species to collect information about the effect of abiotic parameters of cultivation media to the production and the composition. In the method, the carboxyl groups of the extracted fatty acids were also tagged with Br-DMEQ and a short HPLC run was applied on core-shell chromatographic column to separate eleven fatty acids, which were in the scope of the study.

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Application of various imaging techniques for plant stress diagnostics

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Plant growth is affected by various factors. The resistance of the plant to withstand various biotic and abiotic stress factors plays a vital role for its growth and development. Our objectives were to combine various non-invasive imaging techniques (Digital imaging, Near- infrared (NIR) imaging, thermal imaging and chlorophyll fluorescence imaging) for studying stress responses induced by drought, chemical treatment and fungal pathogens in wheat seedlings (*Triticum* spp). Severe drought stress was induced by growing drought tolerant and drought-sensitive wheat genotypes subjected to decreased soil water content (10% field capacity as compared to 60% in the well watered control), while chemical stress induced by silica nanoparticles (10-20 nm particle size, 1000 mg/L) was studied in hydroponically grown wheat seedlings. Two week old near-isogenic wheat lines possessing various tan spot resistance genes were infected with pyrenophora tritici-repentis (PTR) fungal pathogen and characterized. OJIP fluorescence induction kinetics showed characteristic differences between cultivars in response to drought stress as there was an increase in variable fluorescence in response to SiO_2 NPs treatment. Gas exchange measurements showed lower net photosynthetic CO_2 uptake during drought stress, while CO_2 uptake was enhanced in response to SiO_2 NPs treatment. Thermal imaging indicated stomatal closure based on lower transpiration rate under drought stress, while increased evaporative cooling through the stomata was seen in response to SiO_2 NPs. Increasing drought stress activates photosynthetic electron transport rates in water stressed drought sensitive cv. However, we observed higher quantum yields of PSI and PSII photochemistry in SiO_2 NPs treated wheat seedlings. Chlorophyll fluorescence imaging has proven to be a promising tool for characterization and early detection of tan spot disease in wheat *in vivo*. NIR images were able to detect the loss of water content in the area of tan spot infection on various wheat cultivars.

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