ADAM ZĄBEK

ABSTRACT

During the recent decades, it can be noticed a significant increase in the infection diseases caused by various opportunistic fungal strains, especially in immunocompromised patients. Moreover, more often it can be observed the formation of dangerous and highly resistant to antifungal drugs biofilms. The metabolomics tools are one of the most innovative research approach which are useful in understanding of the resistance mechanisms as well as the analyze of the composition microbial metabolites. In this doctoral thesis I focused on a comparison of metabolic profiles of drug resistant and drug susceptible strains of *Candida* species. The drug resistant and drug susceptible strains of *Candida albicans* and *Candida glabrata* species were investigated using a ^{1}H NMR-based metabolomics approach. An analysis of the metabolic fingerprints of cell extracts based on a PCA model revealed a clear separation between the tested strains. Additionally, the OPLS-DA model was built to explore the characteristic metabolites in the drug resistant strains. According to the results that were simultaneously obtained from the multivariate and univariate analyses, the drug resistant strains exhibited higher concentrations of acetate, formate and tartrate, while in the lower level were the following metabolites: histidine, glycine, trehalose, *myo*-inositol, succinate, pyruvate, glutamate, 4-aminobutyrate, alanine and lactate. These metabolites were strictly related to resistance properties of *Candida* species. The changes in metabolite concentrations allowed us to tentatively describe the biochemical pathways that are altered in drug resistant and drug susceptible *Candida* species.

In the present study also shows an innovative approach in taxonomy based on targeted metabolomics analysis with using spectroscopy ^{1}H NMR. The taxonomical classification among fungi kingdom in the last decades was evolved. In this study we report that targeted metabolomics study with using ^{1}H NMR spectroscopy combined with chemometrics tools could be used for classification fungi. Each tested species, *Aspergillus fumigatus*, *Fusarium oxysporum* and *Geotrichum candidum* belonged to three various classes revealed specific metabolic profile of primary endo-metabolites. The species of *A. fumigatus* is represented by the highest concentration of glycerol, glucitol and Unk_5. The positively correlated metabolites in *F. oxysporum* species include propylene glycol, ethanol, 4-aminobutyrate, succinate, xylose, Unk_1 and Unk_4. In *G. candidum*, 3-methyl-2-oxovalerate, glutamate, pyruvate, glutamine and citrate exhibit positive correlation. Additionally, a detailed analysis

of metabolic changes among *A. fumigatus*, *F. oxysporum* and *G. candidum* shows that *A. fumigatus* seems to be the most pathogenic fungi. The obtained results showed that targeted metabolomics analysis could be utilized in the future as a taxonomical tool.

The *Aspergillus fumigatus* – related infections become worldwide problem of healthcare systems due to increasing drug resistance of this microorganism and increasing number of immunocompromised nosocomial patients. These infections are related with *Aspergillus* ability to form settled communities referred to as the biofilms. The small compounds known as *Quorum Sensing* molecules allow microorganism to coordinate virtually all processes taking place during biofilm formation. In the study presented, the HRMAS ^{1}H NMR metabolomic approach was applied to define composition of extra and intracellular metabolites produced by biofilm and planktonic (aka free-swimming) cultures of pathogen and to evaluate impact of different *Quorum Sensing* molecules on biofilm formation.

The scanning Electron Microscopy was used to confirm *Aspergillus* ability to form biofilm *in vitro*, while multivariate and univariate data analysis was applied to analyze data obtained. The *Aspergillus fumigatus* strain was able to form strong biofilm structures of biofilm *in vitro*. The statistical analysis revealed significant changes of metabolite production depending on Aspergillus culture type (biofilm or plankton), time and addition of various QS molecules. The data obtained, if developed, might be used in future NMR diagnostics as markers of *Aspergillus* biofilm-related infection and lead to shorten time between identification and treatment introduction.

The increase in the incidence of fungal infections caused by drug resistant species of fungi leads researchers to search for new effective drugs, which can resist the invasion of pathogens. One of the directions of therapy is the analysis of natural products, among which is a very rich collection of various substances with antifungal properties. In this study, grapefruit essential oil (GEO) extracted from *Citrus paradisi* was demonstrated to be a potential antifungal agent against *Aspergillus fumigatus*. To determine the major differences in fungal metabolism that occurred with GEO treatment, a metabolomics study was performed. The results obtained provide new insight into our understanding of the mechanisms of activity of GEO. This complex essential oil affects *A. fumigatus* cells holistically, thereby causing cell wall disintegration and inactivation of the synthesis of cell wall/membrane components, protein degradation, activation of anti-oxidative stress mechanisms as well as ineffective oxygen respiration.

The results suggest that the metabolomics approach is a very good tool for increasing knowledge of the mechanisms of action of various compounds antibacterial and antifungal. It

allows you to provide basic information about the metabolic changing as an important starting point for further analysis of the areas of omics sciences, i.e. genomics, transcriptomics and proteomics.