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Metabolomics workflow construction for *Aspergillusniger* detection using advanced gas chromatography

Sílvia M Rocha
Universidade de Aveiro, Portugal

Opportunistic infections by *Aspergillus niger* have increased in the last years, either in paediatric patients as adults, presenting a high mortality rate, therefore strongly suggesting the need for prevention or earlier diagnosis and treatment.Microbial metabolomics has been breaking new ground as very useful tool in several areas, including those related to microbial detection, since microorganisms produce several volatile metabolites that can be used as unique chemical fingerprints of each species, and possibly of strains. This richness of information holds the promise for diagnosing infections in situ (e.g. from body fluids, food products, environmental samples, among others), circumventing the laborious recovering of microbes or their genetic material. Microbial metabolomics studies have been mainly focused on the study of the volatile fraction by using 1D-GC. Nevertheless, the use of comprehensive two-dimensional gas chromatography (GC×GC) has revealed that sensitivity and limits of detection are improved compared to 1D-GC. Several challenges should be overcome, since microbial culturing in representative conditions, alongside the technical difficulties to identify and/or quantify trace metabolites within complex matrixes, as well as the inherent problems related to data processing are partially responsible for the paucity of information on the full volatile metabolome of common microbial pathogens. Thus, this talk aims to discuss new developments towards the establishment of a comprehensive platform for *A. niger* detection management, contributing to in-depth explore its exometabolome, which was studied upon different growth conditions, using a methodology based on headspace-solid phase microextraction combined with GC×GC-ToFMS, an advanced gas chromatographic based methodology with high resolution and high throughput potentialities. Partial Least Squares-Discriminant Analysis (PLS-DA) and cross validation were performed to assess both the predictive power and classification models robustness.In addition, PLS-DA-Variable Importance in Projection was applied to highlight the metabolites playing major roles in species distinction; decreasing the initial dataset to only 16 metabolites (*A. niger* Biomarker pattern). The data pre-processing time was substantially reduced, and an improvement of quality-of-fit value was achieved. This study goes a step further on exploring the potentialities of metabolomics for constructing *A. niger* omics pipeline that can be proposed as a high throughput tool towards its future detection based on a molecular biomarkers pattern.

smrocha@ua.pt

Fibrinolytic activity in bovine bile lipid: Application of chromatography, mass spectrometry and *In-vivo* wound healing assay

Namrata Singh
Indian Institute of Chemical Biology, India

An ether extract of nine different bacterial metabolites combined with two step (ether followed by ethanol) extract of bovine bile lipid is used as an immune stimulatory drug. While characterizing the drug, we observed fibrinolytic activity in the extract through fibrinogen plate assay and fibrin zymography.Background literature emphasized major role of fibrinolytic enzymes in activating immune systems. This increased our curiosity to understand the role of these enzymes in this drug in human physiology. This fibrinolytic enzyme/s has no similarity with plasmin in terms of cross reactivity in *immunoblot* assay and hydrolysis of the specific substrate S-2251. In RP-HPLC analysis, the lipid extract was fractionated into several components. Interestingly, fibrinolytic activity was confined to all the fractions. To purify the enzyme, it was extracted from the lipid by aqueous buffer extraction and applied to CNBr activated fibrinogen substrate affinity column. Purified enzyme was tested for activation of complement system and wound healing through C3 binding and *in-vivo* wound healing assay respectively. The enzyme will be identified by mass-spectrometric analysis. Also, we propose to finger-print protein components present in bile lipid by MS analysis to have a better insight of the functionality of the lipid component of the drug.

namratasingh819@gmail.com