Challenges and Applications – MS Imaging Aplications

Analysis of fungal co-culture metabolites using both classic and imprinting Mass Spectrometry Imaging

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Introduction: Fungi are able to produce a wide range of bioactive metabolites, which enhanced when challenged in co-culture[1]. For a better evaluation of these metabolites, the Mass Spectrometry Imaging (MSI) can be used to provide complementary information through the metabolite spatial localization [2]. However, some adaptations are required on available methodologies in MSI for applications in microrganisms[2], particularly on sample preparation. The imprinting method has been shown to be a robust method when applied to preparation of samples analyzed by DESI-MSI[3], but it has never been tested for MALDI-MSI for microrganisms, in our knowledge.

Methods: Herein we applied both Classic and Imprinting MALDI-MSI to analyze metabolites produced by *Aspergillus terreus* (ATCC® 20542TM) and *Pleurotus pulmonarius* fungi in mono and co-culture. The fungi were inoculated, after 8 days, on PDA medium with MALDI glass slides to classic method and they were transferred to filter paper by manual pressure to the imprinting method. After, the samples were dehydrated and submitted to HCCA matrix application by sublimation. The chemical images were acquired by MALDI-MSI and the metabolites were identified by LC-ESI-MS/MS.

Results: Twelve ions were detected by MALDI-MSI, using classic (*m/z* 210, 277, 307, 321, 329, 346, 351, 462 and 484) and imprinting (*m/z* 313, 379 and 404) methods. The ions were more abundant in co-culture, and they were highlighted in the interaction zone between fungi, especially the ions *m/z* 329, 351 and 484. Probably these ions may be related to metabolites involved in communication between microrganisms, because these fungi formed a mutualistic interaction [4]. All ions were investigated by LC-ESI-MS/MS, and only two of them were identified. The ion *m/z* 379 was assigned as rubrophen ($C_{22}H_{20}O_6$) and the ion *m/z* 346 as adenosine monophosphate ($C_{10}H_{14}N_5O_7P$). The metabolites identified correspond to primary metabolites, but most bioactive compounds refer to secondary metabolites. And, these metabolites are rarely reported in MS/MS databases, which explain the difficulty in the identification of these compounds.

Conclusions: Despite of the challenges encountered on the sample preparation and metabolite identification, using both classic and imprinting MALDI-MSI for bioprospection of fungi

metabolites is a promising approach in the biotechnological applications. Moreover, the efforts toward entering data into MS/MS microorganism databases are important to easily identify compounds of fungi origin.

References:
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