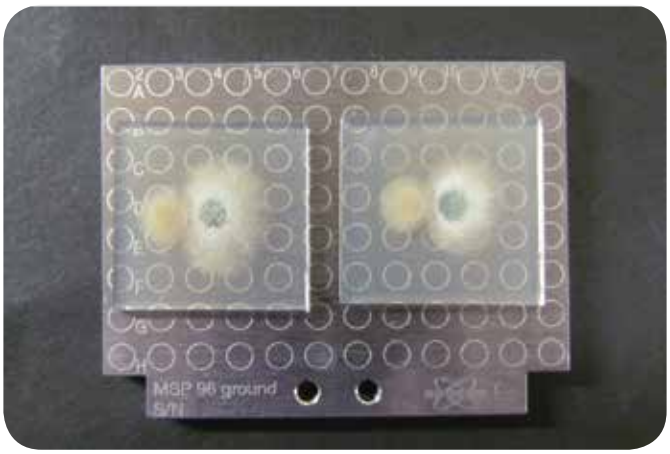

Application Note FTMS-48

Microbial Imaging Mass Spectrometry with Fourier Transform Ionization Mass Spectrometry

Abstract

In this application note we describe a straightforward approach for the preparation and molecular analysis of a bacterial-fungal interaction and the visualization of metabolic exchange factors using an FT-ICR high resolution mass spectrometer.

Introduction

Since the discovery of penicillin it has been widely recognized that microbes produce biologically active molecules, some of which have been found beneficial for treating infections (e.g. penicillin, vancomycin and daptomycin), enabling transplantations (e.g. rapamycin) and lowering cholesterol (e.g. lovastatin). Despite the importance of these molecules in agriculture, diagnostic and therapeutic areas, there are few methods that capture molecular information directly from microbial cultures.

In 2009, microbial imaging mass spectrometry was developed as a way to capture the molecular information from cultured microbes directly from agar surfaces[1]. Microbial imaging mass spectrometry is an extension of the field of histological imaging mass spectrometry[2] and has resulted in the capture of otherwise hidden molecular microbial phenotypes and metabolic exchange of compounds that promise new therapeutic and biochemical

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activities[1, 2 – 13]. Often however, these bioactive compounds can be small-molecules that are isobaric with intense matrix ions or other endogenous compounds. When imaging with MALDI-TOF systems it is often useful to supplement MALDI imaging analyses with other techniques such as TOF/TOF imaging or LC-MS in order to fully characterize molecular structures and/or molecular distributions. However these approaches are not without disadvantages. MALDI-TOF/TOF imaging provides high molecular specificity which lends itself quite well to verification of a suspected molecule but is limited for discovery experiments. On the other hand, LC-MS strategies provide access to a wide dynamic range of compounds but at the sacrifice of enriching specific molecules via extraction and the LC procedure itself, as many molecules are difficult to extract and/or chromatograph poorly, as well as missing the spatial information.

Although MALDI-TOF based imaging is incredibly useful for microbiology, Bruker's solariX high-performance mass spectrometer offers an enhanced strategy for MALDI imaging that is well suited to studying microbial metabolites and other small molecules[12]. It's high mass-resolution capabilities in MS-mode facilitates discovery studies by separating metabolite images from the interferences presented by ions that are very close in mass. At the same time, solariX's high measurement accuracy can often provide unambiguous molecular formula assignment for each of these resolved images. The same resolution and accuracy capabilities are also present in MS/MS mode if more detailed structural confirmation is needed. In this note we show the power of solariX for metabolic imaging of a Pseudomonas aeruginosa and Aspergillus fumigatus interaction, two organisms found in cystic fibrosis lungs.[3]

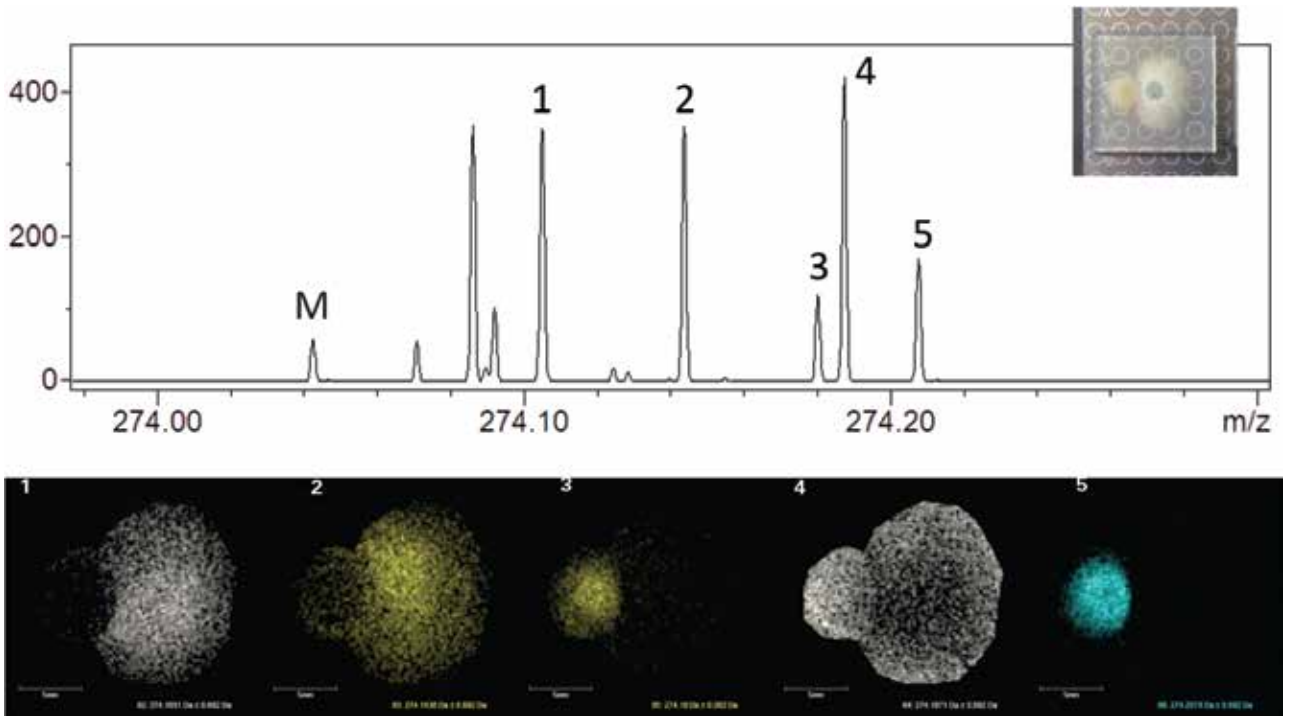
Methods

Detailed descriptions of the microbial sample preparation can be found in the references and several minor variations exist. Typically, 10-11 ml of 1-2% agar with necessary nutrients was poured into a Petri-dish to form a layer of agar ~1mm thick. Pseudomonas aeruginosa and Aspergillus fumigatus were subsequently added to the agar and incubated until colonies began to interact. During the incubation period it is important to seal the Petri-dish in order to minimize evaporation. When the microbes reached the desired phenotype the agar was cut and peeled from the Petri-dish and placed on top of an unpolished MALDI target plate. A digital photo was taken of the sample before matrix was applied. While the sample was wet, it was 'dusted' with crushed crystals of 50:50 DHB:HCCA (Sigma-Aldrich #50149-1G-F) from a manually-agitated 53 µm sieve. After the sample became visibly saturated with matrix, it was dried at 370C for 1-3 hours. As it dried the agar shrank from 1 mm to ~120 µm thick. Residual matrix was then removed using a gentle stream of compressed air. It is important to note that the plate can be analyzed immediately or stored in a desiccator. Although molecular decomposition during prolonged storage is possible, in our hands, samples stored in a desiccator for two weeks still produce strong ion signal with minimal degradation. A video of the sample preparation is available on youtube (http://youtu.be/RIQ4BtjSCvI) and the experimentalist wanting to perform these experiments should read ref 11.

MALDI imaging was performed using a 9.4T solariX equipped with dual ESI/MALDI ion source and Smartbeam II laser set to minimum focus. The m/z range acquired was 200 – 360 with a transient length of 700ms for a broad band resolving power of ~150,000. Single-scan spectra consisting of 500 accumulated laser shots were acquired at a rate of <1s/spectrum and calibrated using the DHB cluster ion at m/z 273.03936 as lock mass before saving. Data were post-processed using a proprietary data reduction algorithm which eliminates baseline data points but keeps intact full peak information. Fleximaging software was used to register the digital photo of the sample plate to the X-Y stage of the instrument so that measurement regions could be drawn around the colonies directly on the imported digital image of the sample. This enabled the direct correlation of the chemistry of microbes with the phenotypes of the microbes and surrounding agar. For the present study, high spatial resolution wasn't a requirement and so MALDI images were acquired at a spatial resolution of 300um.

Results and discussions

When two microbes interact, metabolic exchange factors are secreted as a means of chemical communication resulting in synergy, commensalism and antagonism. Key to identifying and characterizing compounds with high activity is to map relationships between compound distribution and any underlying microbial response. Figure 1 illustrates the performance benefits of microbial imaging with solariX. The example shows a narrow segment at m/z 274 from the microbial interaction in which at least 18 distinct ion images are resolved. Five of these ions present clear differentiation to either P. aeruginosa or A. fumigatus as seen from the images shown in the figure. When investigating the activity of known target ions or discovering new compounds considerable molecular specificity is gained by separating isobaric ion images. The observation that some of the individual images are clearly specific to an individual microbial colony would otherwise go undetected in lower resolution systems. It is important to note here that ion desorption in solariX is decoupled from the mass measurement, unlike traditional MALDI-TOF, and we therefore observe minimal degradation of resolution or measurement accuracy from the non-conductive agar substrate.



Higher molecular specificity is accompanied by higher measurement accuracy which helps identify the molecular entity being imaged by allowing assignment of a molecular formula to each detected peak. For many metabolites, a measurement accuracy of 1 ppm or less is more than sufficient to determine a single molecular formula for an ion. An example of this is illustrated in Figure 1 using the ion images denoted as 1-5. Each of these five peaks was analyzed by SmartFormula to identify combinations of C, H, N and O would sum to within 5 ppm of the measured m/z and the results are presented in Table 1. With exception of ion 5, m/z 274.2075, a search window as large as 5 ppm returns a single molecular formula for each ion image. Enlarging the search window to 10 ppm returned a single formula, listed in Table 1 as ion 5a, but on examination, the difference of 10 ppm observed between the calculated and measured m/z for 5a is out of line with differences for peaks 1-4. One must consider the possibility that peak 5 does not represent a monoisotopic ion but rather one that contains 1 or more heavy isotopes. Indeed, the peak labelled M was measured at m/z 274.04249, a difference of 0.80 ppm from the calculated mass of the A+1 peak of [DHB-H2O]2+H+. When we included heavy isotopes in the potential formulas peak 5 it matches very well to the A+2 peak of nitrohydroquinone (Row 5b, Table 1) a known molecular product of Pseudomonas. The difference between the calculated and measured m/z is consistent with peaks 1-4. Further, assignment to this compound is also supported by its image, with its distribution being localized to this colony.

peak	Meas. m/z	only formula in search win.	Calc m/z-meas m/z	Search window
1	274.1051	$C_{11}H_{12}N_7O_2$	-1.57 ppm	5 ppm
2	274.1436	$C_{16}H_{20}NO_3$	0.62 ppm	5 ppm
3	274.1800	$C_{17}H_{24}NO_2$	0.53 ppm	5 ppm
4	274.1875	$C_{11}H_{24}N_5O_3$	-0.34 ppm	5 ppm
5a	274.2075	$C_9H_{24}N_9O$	8.40 ppm	10 ppm
5b	274.2075	$^{12}C_{16}{}^{13}C_2H_{26}NO$	-0.36 ppm	5 ppm

Conclusion

Microbial imaging is a strategy that enables the chemical analysis of microbes grown on agar surfaces under controlled conditions. Using solariX to carry out MALDI-MS imaging, one can utilize its high resolving power to differentiate these species, especially in the lower mass range where the matrix signals and many of the relevant microbial metabolic exchange factors, virulence factors, and quorum sensors are found. In addition improving the specificity of images solariX can provide molecular formulas to ion images of interest. These capabilities can be invaluable tools for understanding the molecular discussions associated with microbial interactions.

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