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Mass spectrometry imaging: Towards mapping the elemental and molecular composition of the rhizosphere

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A B S T R A C T

This short review provides perspective regarding the use of mass spectrometry imaging (MSI) to study the rhizosphere. It also serves to complement the multi-omic-focused review by White et al. (in press). MSI is capable of elucidating chemical distributions within samples of interest in situ, and thus can provide spatial context to MS omics data in complementary experimental endeavors. Most MSI-based studies of plant-microbe interactions have focused on the phyllosphere and on the "associated rhizosphere" (our term for material that is not removed during harvesting). Sample preparation for these in situ analyses tends to be a limiting factor. These studies, however, have provided valuable insights into the spatial arrangement of proteins, peptides, lipids, and other metabolites within these systems. We intend this short review to be a primer on the fundamentals of MSI and its role in plant-microbe analysis. Lastly, we offer a perspective on the future of MSI and its use in understanding the molecular transformations beyond what we call the associated rhizosphere, one which extends to the rest of rhizosphere and into the bulk soil.

1. Imaging the rhizosphere by mass spectrometry

The rhizosphere is a highly dynamic environment where a diversity of correlated (bio)chemical and physical processes occur as a consequence of root growth, water transport, and microbial respiration, for example. The ability to map the chemical agents of such complex niches can be of tremendous value in elucidating many of these processes. Mass spectrometry imaging (MSI) is a method capable of revealing elemental and molecular distributions within samples of interest in situ. A striking advantage of MSI over other imaging techniques is that the creation of ion images can hint at the function and genesis of detected compounds (Watrous et al., 2013; Anderton et al. 2016). Specifically, MSI is able to visualize interactions between associated and coexisting species – that is, MSI can ascribe molecular structure to its origin – thus it could be a powerful tool for imaging the rhizosphere as one highly complex system. This was demonstrated recently when MSI was used to investigate the chemical interface in the invasion of rice (Klein et al., 2015) and *Arabidopsis* leaves (Ryffel et al., 2016) by bacterium. Elsewhere, MSI was used to decipher the array of metabolites involved in nitrogen fixation by *Medicago* root nodules (Ye et al., 2013).

MSI applications have been a staple in the human health and biomedical fields, but these techniques are now emerging as standard methodologies within the plant and microbial scientific community. A range of reports utilizing MSI to access the spatial distribution of elements (da Silva and Arruda, 2013), low molecular weight metabolites (Hemalatha and Pradeep, 2013), lipids (Velickovic et al., 2014a), and polysaccharides (Velickovic et al., 2014b) within plant tissues has shed light on factors that dictate plant growth, development, reproduction, and pathology, as well factors related to plant responses to biotic and abiotic stresses. In microbiology applications, MSI has demonstrated great potential in understanding molecular interactions and signaling across microbial communities (Watrous et al., 2013). However, the literature on measuring the rhizosphere with MSI is relatively limited. Perhaps this is due in part to the major challenge of preserving and preparing rhizosphere samples for analysis with most MSI methods. Especially, how does one preserve this complex system in its native state? Soil, after all, is loosely associated and thus an "unfriendly" medium.

2. A brief history and overview of MSI

The concept of MSI arguably started with J.J. Thompson's first mass spectrometric measurements (Thomson, 1913). But MSI remained limited in scope and breadth, in part, until the invention of better vacuum technology and soft ionization techniques. Early examples of MSI in plant and microbial applications focused primarily on the analysis of elements or on very small molecules (often molecular fragments), using methods like secondary ion mass spectrometry (SIMS)

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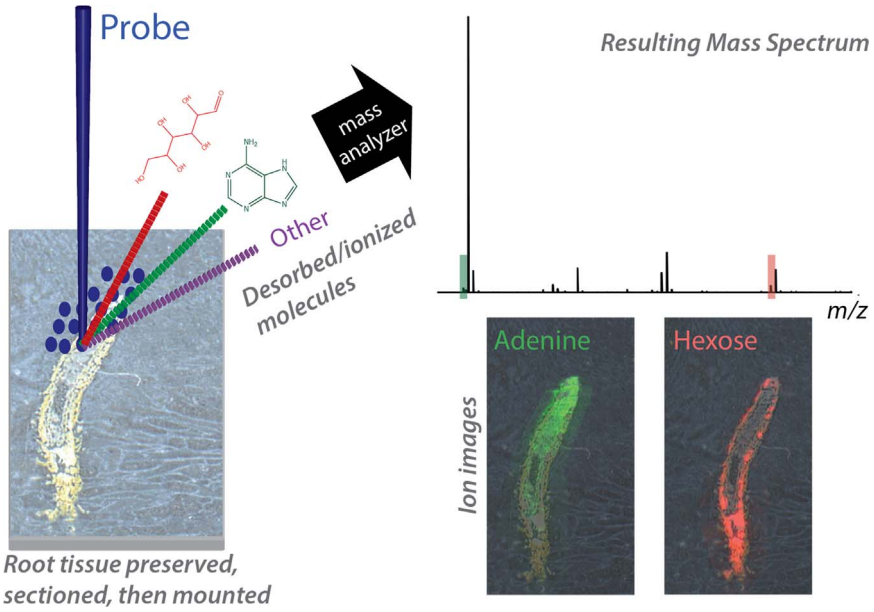

Fig. 1. Example of microprobe mode MSI analysis of a plant root tip. A probe is rastered over a sample (left), and at every raster spot (blue dots) a mass spectrum is acquired (top right). Ion images can be reconstructed to indicate the distribution of a species over the area probed (bottom right). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The latter half of the 1980s provided a major paradigm shift in mass spectrometry applications when matrix-assisted laser desorption/ionization (MALDI) (Karas et al., 1985) and electrospray ionization (ESI) (Fenn et al., 1989) demonstrated the ability to analyze intact organic compounds, especially large biomolecules. A decade later, the Caprioli group (Caprioli et al., 1997) used advances of MALDI, along with a robotic sample holder, to create the first MS image of intact biomolecules on biological tissue. After that, methodologies, instrumentation, and software for MSI developed rapidly.

The basic steps of a MSI workflow are straightforward. Preserved samples are commonly sectioned (e.g., frozen tissue) or excised (e.g., agar supported material). Then they are mounted on a substrate, which often needs to be conductive if ionization is occurring at or near the surface (method dependent). Mounted samples are then serially probed by an ionization source where localized mass analysis is achieved (Fig. 1). After a series of mass spectra is generated from predefined areas ("pixels" or "voxels"), one can reconstruct images that map the intensity of ions of interest. Note, the described method is the so-called "microprobe mode," which is most commonly employed. In an alternative approach called "microscope mode" MSI, the desorption/ionization beam is typically defocused to probe a larger area of the sample. By using ion optical elements that retain the spatial organization of the ion cloud, an image can be formed when ions reach a position-sensitive detector (Jungmann and Heeren, 2012). In both modes, created ion images can be merged mutually, with each ion image presented by a different color scale, or they can be superimposed onto a picture of the sample that was analyzed. Both image visualization methods invite profound insights into spatial biological questions.

Numerous combinations of ionization sources and mass analyzers can be employed in MSI applications, each with their own advantages and limitations. Essentially, there are three different types of desorption/ionization methods utilized in MSI (Fig. 2). SIMS, which offers the highest lateral resolution (< 100 nm), uses a primary ion beam that bombards the sample producing secondary ions from surface molecules (Fig. 2A). The primary ion beam can either be pulsed or can constantly bombard the sample. A distinction exists in SIMS between the so-called "static mode" and the "dynamic mode" regime once the primary ion dose exceeds 10^{13} ions/cm^2, and these different modes of operation can provide varying levels of chemical and spatial information. Note, the

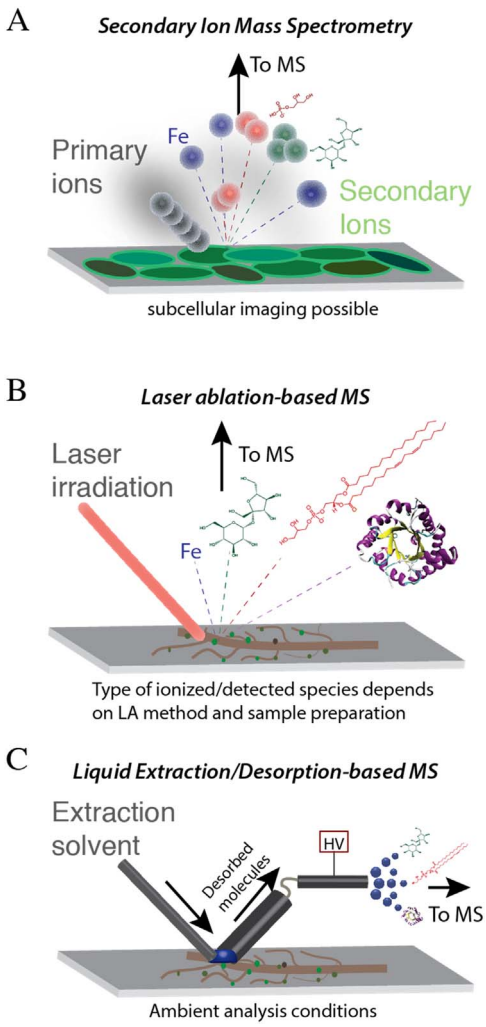

Fig. 2. Schemes of the three different types of desorption/ionization methods utilized in MSI.