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Emerging mass spectrometry techniques for the direct analysis of microbial colonies

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One of the emerging areas in microbiology is detecting specialized metabolites produced by microbial colonies and communities with mass spectrometry. In this review/perspective, we illustrate the emerging mass spectrometry methodologies that enable the interrogation of specialized metabolites directly from microbial colonies. Mass spectrometry techniques such as imaging mass spectrometry and real-time mass spectrometry allow two and three-dimensional visualization of the distribution of metabolites, often with minimal sample pretreatment. The speed in which molecules are captured using these methods requires the development of new molecular visualization tools such as molecular networking. Together, these tools are beginning to provide unprecedented insight into the chemical world that microbes experience.

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Introduction

Historically, advances in microbiology closely follow the development of technologies. The invention of microscope in early 1660s by Anton van Leeuwenhoek (1632–1723) for example enabled the visualization of a hidden living world [1]. Similarly, modern techniques such as fluorescence microscopy revolutionized our understanding of membrane fluidity, calcium sensing, vesicle formation, distributions of proteins and many other important processes in microbiology [2–6]. Although mass spectrometry has been used to analyze microbiological samples for decades, it never gained a solid footing in the arsenal of routine tools used by microbiologists [7–19]. However, there is a resurgence in the adaptation of mass spectrometry toward the analysis of microbial samples due, in part, to the increased sensitivity, the increase in need to understand the mechanistic roles of microbial behavior at the chemical level, new instrumentation, ease of use of modern instruments and novel microbial and mass spectrometry compatible workflows that are being developed.

This year we are celebrating the 100-year anniversary of mass spectrometry. With the invention of cathode rays by Sir Joseph John Thomson (1856–1940), measuring the mass of atoms and molecules became possible [20]. Over the past few decades, as softer ionization methods have become available, entirely new ways were opened up to peek into the fascinating world of microbes. Liquid chromatography coupled with mass spectrometry (LC–MS) has been used to understanding the intricate complexities of microbial metabolism, protein–protein interactions and post-translational modifications [21–24,25••,26–32].

However, LC–MS is not the only way to investigate microbial systems. Direct analysis of microbes can be accomplished without a separation step. The ability to directly analyze microbial colonies has led to strain identification workflows that are now approved for clinical use. Furthermore, recent mass spectrometry advances enabled the mapping of microbial molecules spatially, the observation of molecules produced by living microbial colonies and of microbial molecules at the single cell level.

Clinical use of microbial mass spectrometry

In the 1970s, the lab of Catherine Fenselau published their pioneering research using mass spectrometry to identify cultured bacteria from patients' specimens. It was realized that direct mass spectrometry of microbes gives rise to fingerprint type signatures that can be correlated to a database of known species and a microbe's fingerprints as obtained with a mass spectrometer. These methods have now advanced to the clinic [33,34]. This pioneering work set the stage of the development of clinical microbiology identification tools such as Biotyper and VITEX-MS. The Bruker *in vitro* diagnostics (IVD) matrix-assisted laser desorption/ionization (MALDI) Biotyper is a MALDI-time of flight (ToF)-mass spectrometry based platform for the identification of bacterial and yeast. Biotyper uses the unique molecular fingerprint such as the most abundant proteins, to identify the microbes [33,35,36]. The VITEK MS from bioMérieux

is a similar platform as the Biotyper from Bruker [37]. The FDA clearance in 2013 for both of these microbial tests marks an exciting transition for clinical diagnostic mass spectrometry. It is likely that these and other mass spectrometry techniques will be widely introduced into clinic laboratories in a foreseeable future since they are faster, less labor intensive and cheaper than their counterparts to determine the phylogenetic branch that microorganisms belong to [38].

Direct analysis of microbial colonies in recent years has gone beyond microbial identification. Imaging mass spectrometry (IMS) and real-time mass spectrometry are two new and exciting areas (Table 1). These developments are also leading to the development of new data visualization tools, such as molecular networking. Due to the volume of data that is generated, it has given rise to the need for a molecular Genbank to compare and contrast the data.

Table 1

Overview of novel mass spectrometry techniques used in microbiology.

Techniques			Representative Results
Microbial Imaging Mass Spectrometry	Static SIMS	Dynamic SIMS	Study intact membrane lipids and proteins at single cell level [55,56].
	MALDI IMS		Capture the metabolic transformation in polymicrobial community [75,76].
	NanoDESI /NanoDESI IMS		Monitoring of intact molecular species under ambient at single colony directly from Petri dish [91].
Microbial Real-Time Mass Spectrometry			High-throughput identification and characterization molecules secreted from bacteria colonies [80,90,97,98].