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Review

Identification of bacteria using mass spectrometry techniques

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ABSTRACT

The possibility to rapidly identify bacteria is required in many different fields. Due to rapid progress in the development of mass spectrometry devices during the last few years, identification by means of mass spectrometry has become a very powerful and usable tool. These methods offer fast analysis of biomarker ions, providing reliable information on bacteria characterization even at the sub-species level. Therefore, these approaches have been successfully established as routine methods, together with classical biochemical tests and genome sequencing. This review focuses on common biomarkers and on different mass spectrometry techniques which have been used for bacteria identification throughout the third millennium.

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Abbreviations: 2-AA, 2-aminoacetophenone; A, adenine; BAMS, bioaerosol mass spectrometry; BHP, bacteriohopanepolyol; C, cytosine; CE, capillary electrophoresis; CI, chemical ionization; DART, direct analysis in real time; DESI, desorption electrospray ionization; DGDG, diglycosyldiacylglycerol; DMAN, 1,8-bis(dimethylamino) naphthalene; DNA, deoxyribonucleic acid; DPA, dipicolinic acid; dTTP, 2'-deoxythymidine-5'- triphosphate; dUTP, 2'-deoxyuridine-5'- triphosphate; EI, electron ionization; ESI, electrospray ionization; FAB, fast atom bombardment; FAME, fatty acid methyl ester; G^-, Gram-negative; G, guanin; G^+, Gram-positive; GC/MS, gas chromatography/mass spectrometry; HPLC, high-performance liquid chromatography; LC, liquid chromatography; LDI, laser desorption/ionization; LOS, lipooligosaccharides; LPS, lipopolysaccharides; MAB, metastable atom bombardment; MALDI, matrix-assisted laser desorption/ionization; MALDI-RE, matrix-assisted laser desorption/ionization re-sequencing; MRM, multiple reaction monitoring; MRSA, methicillin-resistent *Staphyloccocus aureus*; MS, mass spectrometry; NIH, National Institute of Health's; PCA, principle component analysis; PCR, polymerase chain reaction; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PSD, post-source decay; Py, pyrolysis; RNA, ribonucleic acid; SASP, small acid soluble proteins; SIFT, selected ion flow tube; SPA, Selective proteotypic-peptide analysis; SPME, solid phase micro-extraction; T, thymine; TOF, time-of-flight; U, uracil; UV, ultra-violet; VNTR, variable-tandem repeats; VOC, volatile organic compound.

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1. Introduction and short historical overview

The detection or identification of bacteria is required in many different spheres of human activity. Rapid and correct pathogen identification is crucial in medicine as well as in other fields such as food analysis, veterinary science, ecology, agriculture, etc. Various methods for identifying microorganisms, such as microscopic observation, biochemical testing, immunochemical assays and molecular biological methods, have been developed. Each of these methods has some particular advantages and disadvantages. Biochemical testing is a non-targeted form of analysis; however, a pure microbial culture is required. Immunochemical assays can be performed in mixtures of microbial cultures, but the affinity and specificity of the antibody is the limiting factor. Molecular biology can also be performed in a mixture of microbial cultures, and even non-cultivable microorganisms may be identified. On the other hand, these benefits are reflected in the higher cost of the analysis.

Mass spectrometry (MS) is very attractive for work in microbiology because of its speed. Methods based on intact cell MALDI-TOF experiments provide comprehensive information within 5 min; the other advantage is that just one single colony is required for analysis. However, it was not always like this.

Despite the enormous application of mass spectrometry after the Second World War, the first experiments to identify individual microorganisms using this technique were performed in the 1970s in conjunction with the mission to Mars and analysis of the Martian soil [1,2]. In these studies, pyrolysis-gas–liquid chromatograph coupled with mass spectrometer was used for the analysis of soil and *Micrococcus luteus* and *Bacillus subtilis* whole cells. The first bacterial Py-MS mass fingerprint was measured by Meuzelaar and Kistemaker using Curie-point pyrolysis-MS in 1973 [3], however, the measured m/z range between 10 and 50 Da was not sufficient. The real breakthrough was performed by Anhalt and Fenselau [4], who measured the pyrolysis products of ubiquinone, phospholipids, and other volatile compounds from in source heated bacteria. Lower temperature used for pyrolysis enabled recording of the mass spectra up to 700 Da. Subsequent experiments performed by pyrolysis-gas chromatography/mass spectrometry allowed for example the characterization of microorganisms [5] or Gram-type differentiation [6,7]. However, the soft ionization techniques that emerged in the 1980s have proven to be more effective. Fast atom bombardment (FAB) ionization was used by Heller et al. [8] to measure lipid biomarkers from the whole bacteria lysate. This method appeared promising; thus, correlation functions and a library-matching system for bacteria identification were created [9]. Nevertheless, insufficient sensitivity and an insufficient dynamic range and confidence level have disqualified FAB from practical use.

In comparison with FAB, electrospray ionization (ESI) offers high sensitivity and is very useful for the ionization of macromolecules such as protein biomarkers. Early experiments were carried out with bacterial lysates [10]; however, Goodacre et al. [11] developed a method for the intact cell characterization of bacteria. The major disadvantage of this approach is the clogging of the electrospray needle and the high complexity of obtained spectra, which often cannot be resolved easily. Despite this, ESI MS is used for the identification of bacteria, spores [12], and viral particles [13].

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry entered the field of microorganism identification in 1996 [14–16] and took the leading position in just a few years. This success is based on MALDI's short analysis time, high sensitivity, and intact cell measurements, as well as the possibility of automation; therefore, MALDI mass spectrometry has become the method of choice. The other advantage of MALDI is its compatibility with different analyzers and its wide m/z range. On the other hand, the process of MALDI ionization brings some limitations and problems, such as suppression effects, "sweet spots", and selective ionization [17].

In the last few years, DESI-desorption electrospray ionization [18,19], SIFT-selected ion flow tube [20], and BAMS-bioaerosol mass spectrometry [21] have appeared and become more widespread. Although their use is more limited than that of MALDI, they are able to provide useful information about lipids and/or various metabolites (e.g., volatile organic compounds, ammonia, etc.).

In this review, we summarize both common and rare biomarkers used in bacteria identification. We summarize developments and innovations in mass spectrometry methods applied to bacteria identification over the last decade, and compare current commercial systems designed for clinical use. The review focuses on mass spectrometry; thus, it will deal only with those biomarkers measurable by current mass spectrometry techniques.

2. Biomarkers

Biomarker is a word used in many different contexts in medicine, cell biology, microbiology etc. The official National Institute of Health's (NIH) definition of a biomarker is: "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" [22]. Nevertheless, this definition is inadequate for the needs of microbiology. Hence, other definitions have arisen, such as: "A biomarker is a molecule that allows for the detection and isolation of a particular cell type" or "A biomarker can be any kind of molecule indicating the existence, past or present, of living organisms". These fit better. In any case, the specificity of a particular molecule (or molecules) is a key characteristic. From the perspective of bacteria identification, the ideal biomarker is present in all bacteria, but it is specific for each of them and measurable.

If we take a look at molecules present in a bacterial cell, we find biopolymers (nucleic acids, polysaccharides), small organic molecules (lipids, saccharides, metabolites), and inorganic molecules. Nucleic acid is the molecule which almost meets the requirements of an ideal biomarker. The genome is fundamental and encapsulates unique information about each organism in existence. But there is only one copy of DNA per cell, which makes it difficult to measure by any technique without previous amplification. Mass spectrometry is no exception. Fortunately, PCR products are measurable using soft ionization techniques such as MALDI or

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