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Mini Review

Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Identification of Pathogenic Bacteria

Halil Dündar*

Department of Pediatric Metabolic Disorders, Faculty of Medicine, Gazi University, Ankara, Turkey

***Corresponding Author:** Halil Dündar, Department of Pediatric Metabolic Disorders, Faculty of Medicine, Gazi University, Ankara, Turkey.

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Abstract

Recently matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has emerged as a rapid, sensitive, and cost effective tool for microbial identification and diagnosis. It offers an alternative to genotypic or phenotypic methods for the fast identification of microorganisms. Although techniques based on genomics such as 16 S rRNA sequencing are highly sensitive to identify bacteria at species level, they are costly and time consuming. MALDI-TOF MS is appealing because of its rapidity, cost effectiveness and strong potential in identification of pathogenic as well as food spoilage bacteria. It is a high-throughput identification method enabling accurate mass determination of whole cell proteins from bacteria. MALDI TOF fingerprint approach relies on mass spectral patterns of bacteria and allow for the identification of an unknown microorganism at the genus, species and even strain level by comparing the spectral profile with a library of mass spectra of reference strains.

Keywords: *Mass Spectrometry; MALDI-TOF-MS; 16S rRNA Sequencing; Pathogenic Bacteria; Identification*

Introduction

The accurate and urgent identification of bacterial pathogens is crucial in many infectious diseases as it provides a clear diagnosis, and allowing for the most effective treatment [1,2]. The conventional methods of identifying bacteria are based on phenotypic tests such as: Gram staining, catalase and oxidative tests, motility, colony morphology, which are complemented by secondary phenotypic tests using commercial kits. However, these phenotypic tests can be imprecise in assigning bacteria to its species [3]. Alternatively, molecular methods such as polymerase chain reaction (PCR) , real-time PCR, and loop-mediated isothermal amplification (LAMP) are available for the fast identification of microorganisms. Polymerase chain reaction (PCR) enables rapid identification of slow growing or uncultivable bacteria by targeting conserved genes such as those coding for ribosomal RNA [4] and RNA polymerase (rpoB) [5,6]. However, conventional PCR might lead to false-positive results due to the opening of tubes during post detection procedures [7,8]. Real-time PCR and LAMP are attractive because they are highly sensitive and specific, time efficient, and generate fewer false-positive results [8]. However, these molecular biology-based techniques are not suitable for routine identification since they are costly and require high level of technical expertise [3].

MALDI-TOF MS has become a suitable tool for the classifcation of complex biological samples, such as bacteria, fungi, and tissue samples of animals and plants since it allows investigation of intact lipids and proteins [9]. In MALDI-TOF MS analysis, the ribosomal proteins of microorganisms are ionized and separated from each other on the basis of m/z ratio, which is measured by time of flight (TOF) of the ions, corresponding to the time taken to reach the detector. Based on the TOF information, a peptide mass fingerprint with peaks spesific to genera and species is generated [9]. The unknown microorganism is identified by comparing its spectral profile from MALDI-

TOF MS against a library of mass spectra of reference strains deposited in the database [11]. Mass spectral patterns are mostly composed of highly abundant proteins including many ribosomal proteins, which are assumed to be characteristic for each bacterial species [12]. Since ribosomal proteins account for up to 21% of the cell's overall protein content and because of their constitutive expression as part of the cellular translation machinery, these proteins act stable biomarkers for fingerprinting [13]. Although the amino acid sequences of ribosomal proteins are highly conserved, slight amino acid sequence variations can occur even at the (sub)species and possibly strain level [14]. For example, *Bacillus cereus* group-specific biomarkers at 4,334, 5,171 and 5,886 Da are due to ribosomal proteins. Although *B. cereus* group members *B. anthracis*, *B. cereus* and *B. thuringiensis* display group-spesific peaks of 3,683, 4,334, 5,171, 5,886, and 7,368 Da, the mass peak of 5,413 is a species-spesific signal in the spectra of *B. anthracis*.

Direct colony smear on MALDI-TOF plates and ethanol/formic acid extraction are two methods described for the preparation of bacteria for identification by MALDI-TOF-MS. Although direct smear method is fast, the results are sometimes inconsistent compared to the chemical extraction [15-17]. Even standard ethanol-formic acid extraction procedure alone might not allow the membrane proteins to be ionized by matrix because of the thickness and hydrophobicity of the cell wall, as is the case of Nocardia, of which cell wall contains a large amount of mycolic acids like the wall of mycobacteria [18]. Boiling of colonies in distilled water for 30 minutes or plunging of them three times into liquid nitorgen at -196°C for 5 seconds were shown to improve the quality of the spectra generated [18]. In an effort to compare three sample preparation methods (direct transfer, the direct transfer-formic acid method with on-target formic acid treatment, and ethanol-formic acid extraction) for the identification of Gram-positive cocci with MALDI-TOF MS, it was found that direct transfer-formic acid demonstrated high reliability [19].

Studies have demonstrated that the identification of bacteria by MALDI-TOF MS is as accurately as [15,20] or higher than 16 S rDNA sequence analysis [21]. Although 16S rRNA sequencing couldn't differentiate among strains of *B. cepacia* complex, MALDI-TOF MS identified this strain with a high score, showing its higher discriminatory ability [21]. In addition, it is possible to obtain the identification within minutes starting from whole cells or cell lysates [22]. However, the first high initial cost of purchasing the instrument and the requirement of a database are the major limitaitions of MALDI-TOF-MS. The database for emerging pathogens might not be as available as those for commonly studied species [23] because of the absence or the availability of only a small number of isolates of a given species in the reference database [18]. An extended database was shown to improve identification of *Nocardia* species, where correct identification to the species level increased from 44% to 88% with a score of ≥ 2. Hence, MALDI-TOF MS is recommended for rapid and accurate identification of the most commonly encountered isolates [18].

MALDI-TOF-MS has proved its efficacy for the identification of various bacterial species including *Bacillus anthracis* [13,24,25], anaerobic bacteria such as *Actinomyces* sp., *Anaerococcus* sp., *Bacteroides* sp., *Clostridium* sp., *Fusobacterium* sp. [17], *Listeria* sp. [26], *Enterococcus* sp. *Staphylococcus* sp., *Streptococcus* sp. [19], *Nocardia* sp. [18], *Clostridium tertium* [27], *Mycobacterium* sp. [28-30]. In addition to its use for the identification of clinically important bacteria in microbiological diagnostic, it has proved to be a reliable technique for the surveillance and early detection of antibiotic resistant pathogens to control their spread and rapidly initiate adequate infection control measures. MALDI-TOF-MS technique has been employed to as a typing tool for methicillin-resistant *Staphylococcus aureus* [31-34], vancomycin-resistant enterococci [35], multidrug-resistant *Corynebacterium striatum* [36].

Conclusion and Future Prospects

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) has been proposed as a promising tool for identification and classification of microorganisms due to its rapid performance and cost-effectiveness. MALDI-TOF MS identification of microorganisms is in most cases comparable to that yielded 16S rRNA sequence analysis and superior to that of 16S rRNA sequence analysis in particular cases.

Besides routine diagnostics, it is a valuable technique to discriminate antibiotic resistant strains of the same species.

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