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Research Article

Utility of MALDI TOF AUTOF MS 1000 System for Identification and Authentication of Bacterial Strains using Standard ATCC Cultures

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Abstract

Precise bacterial identification depends on the reliability and efficiency of the technique being utilized. Scientists have incessantly adapted every advancement for better, quicker, and meticulous bacterial identification. MALDI-TOF MS is a microbial identification technique based on the soft ionization principle coupled with mass spectrometer. It became popular in this decade for its speed, uniformity, cost-effectiveness, ease of sample preparation, and accuracy. MALDI-TOF MS involves using a matrix to mix and bind the sample, which absorbs laser energy, causing fast heating, vaporization, and the ionization of the analytes; then the ions are distinguished based on the time they take to get to the detector, based on the principle that all ions with the same charge are given equal kinetic energy. Due to many diverse and extensive features of this exclusive detection method, this study principally aims to authenticate 38 bacterial strains using MALDI-TOF Autof MS1000 system. These strains included gram positive bacteria namely *Enterococcus faecalis, Staphylococcus aureus, Listeria monocytogenes, Clostridioides difficile* and gram-negative bacteria namely, *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Hemophilus influenzae.* The bacterial strains were accurately identified with a score value of more than 9.2 for all the strains, indicating high confidence in identification up to species level.

Keywords: Matrix Assisted Laser Desorption/Ionization (MALDI) Mass Spectrometry (MS); Autobio MALDI TOF MS 1000; Rapid Bacterial Identification; Accurate Bacterial Detection; Peptide Mass Fingerprint (PMF)

Abbreviations

API 20E: Analytical Profile Index 20 E; kDa: Kilodalton; m/z: Mass to Charge Ratio; MALDI: Matrix Assisted Laser Desorption/Ionization; MRSA: Methicillin-Resistant *Staphylococcus aureus*; MS: Mass Spectrometry; MSSA: Methicillin-Sensitive *Staphylococcus aureus;* PCR: Polymerase Chain Reaction; PMF: Peptide Mass Fingerprint; TOF: Time of Flight

Introduction

One of the crucial challenges in microbiology is accurate bacterial identification. It is essential for the scientists involved in a wide range of applied research and industry, from clinical microbiology to food production. Bacterial identification demands the understanding of their physiological, biochemical, morphological, and genetic characteristics [1]. Conventional detection techniques demonstrate many combats such as excess time consumption for identification that consists of several derided, labor-intensive, material-consuming, non-automated steps, and difficulty in pathogen quantification [2]. The first technique created to examine the human microbiota was the bacterial culture method, which uses an artificial medium that enables the growth, separation, and identification of bacteria [3]. The API 20E (Analytical Profile Index 20 E) testing device, which consisted of a plastic component with 20 cupules that contained pH-based substrates and allowed the identification of over 100 different substances, had been utilized and considered "gold standard" by researchers for many years up to 1992. The major disadvantage associated was due to excessive time consumption. Henceforth, other automated methods to reduce turnaround time were developed [4].

In the past few decades, advancements in molecular biology have evolved the approach of bacterial identification by relying on nucleic acid analysis such as PCR sequencing. These techniques are extremely sensitive. However, due to several PCR inhibitors in complex samples and negligible sample contamination, it can provide a false-positive signal. Additionally, the inability of PCR and PCR-based techniques to distinguish between live, transient, and dead bacteria restricted its pervasive application [5]. This led to the development of accurate and rapid techniques for bacterial identification. Ideally, an identification method should be simple, reliable, highly specific, uniform in analysis, and cost-effective as much as possible. The bacterial chemotaxonomic technique known as matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been acknowledged to be qualifying all these conditions [1].

Matrix Assisted Laser Desorption/Ionization (MALDI) mass spectrometry (MS) was conceptualized by researchers in 1985 and later in 1988, MALDI was coupled with mass analyzer time of flight (TOF) [6]. In 1990, a very groundbreaking application of MS in microbiology unveiled that intact bacterial cells could be characterized using MALDI coupled to a time of flight (TOF) analyzer. General purpose sample preparation for almost all microorganisms is the significant advantage of MALDI-TOF MS over conventional methods [7]. A detailed comparative analysis is depicted in Table 1.

Parameters	**Detection Techniques**	
	Culture Method	**Autof Maldi-TOF MS 1000**
Time Required for Sample Preparation for bacterial identification	72 to 120 hrs	5min*
Total Time Required for Analysis (Enrichment, biochemical, staining etc.)	72 to 120 hrs	2 sec per culture
Technique Complication (Manual, Chemical, Mechanical)	Very High	Very low
Likelihood of errors during the whole procedure	Very high	Very low
Laborious & Tedious Method	Very high	Very low
Failure Rate of the Bacterial Identification	It is presumptive identification, and one is expected to know the possible class of the microbe	Very low and confirmed identification upto species level

Table 1: Comparison between Culture method and MALDI TOF MS Detection Method of bacterial identification.

*: After pure culture is available.

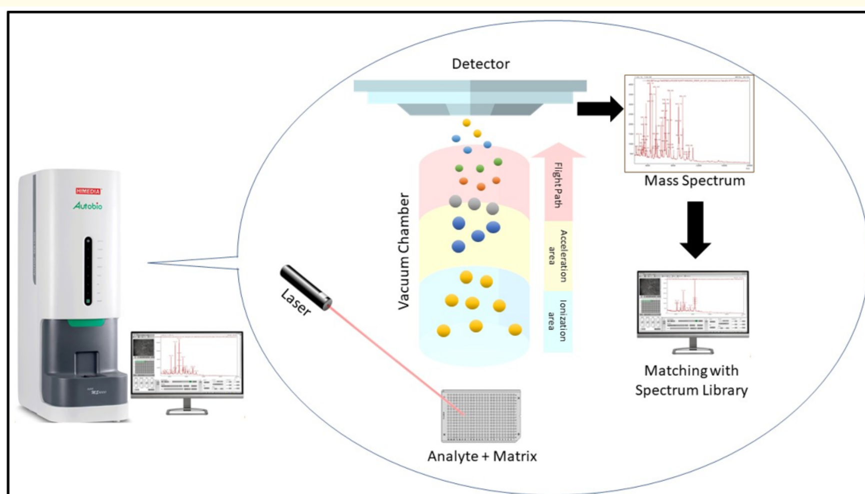

Figure 1: Working Principle of MALDI-TOF.

The principle of MALDI-TOF MS (Figure 1) is based on a soft ionization technique that preserves the integrity of the sample, as the energy from the laser is spent on volatilizing the matrix rather than sizeable fragmentation [8,9]. The preparation of samples requires the coating or mixing of a solution on an organic compound known as the matrix that absorbs energy and crystallizes while drying whereas co-crystallization occurs in the sample embedded in the matrix [7]. Matrix functions to reduce intermolecular interactions among sample molecules, protection of sample from laser decomposition, induces ionization by energy transmission to sample, and isolation among polymers [10]. The laser beam generates individual protonated ions of the analytes in the sample by desorption and ionization of the sample in the matrix. The protonated ions are then accelerated through a fixed potential and separated by their mass-charge ratio (m/z) of an ion which is measured by determining the time taken for an ion to cross the total flight tube length. On the basis of TOF-MA (Time of flight-Mass Analyzer) information, a characteristic spectrum known as the peptide mass fingerprint (PMF) is generated for the analytes in the sample. Microbial identification using MALDI TOF MS entails comparing the PMF of the unknown organism to the PMF in the database or comparing the mass of the biomarker in the unknown organism to the proteome reference database [11].

The present study contributes towards the identification and validation of 38 standard reference ATCC bacterial strains by using MALDI TOF MS.

Materials and Methods

Thirty-eight different ATCC bacterial strains were selected for the analysis, that included both gram-positive and negative bacteria (Table 2). The samples included drug-resistant bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis.*

For the MALDI-TOF analysis on Autof MS1000 system, bacterial samples were prepared by direct deposit method. Briefly, a single colony was spotted on the target slide to form a homogeneous smear and then treated by the ready-to-use matrix solution, with a-cyano-4-hydroxycinnamic acid (HCCA) as the main component. After drying at ambient temperature, the target slide was inserted into the hatch of the MALDI-TOF Autof MS1000 machine. Microbial identification was performed by comparing the spectra generated from the samples with the reference spectra in the database. For Autof MS 1000, the target slide is a metal reusable slide with ninety-six sample sites [12]. Spectra were acquired by the Autof Acquirer and then analyzed using the latest version (Autof Acquirer Version V2 -V2.0.157) of software on the Autof Analyzer. The calibration was done using Autobio calibrating agent. The manufacturer's criteria for interpretation of the results were used to identify the bacteria, based on the homology score values as follows: If identification scores ≥9 considered positive at the species level, scores of 6–9 considered positive at the genus. Level, and scores < 6 defined as not identified.

Gram Positive	Gram Negative
Enterococcus faecalis ATCC 49532	*Klebsiella pneumoniae* ATCC BAA- 1705 Strain ART 2008133
Clostridioides difficile ATCC 43593	*Escherichia coli* ATCC BAA- 2469 Strain 1001728
Staphylococcus aureus ATCC BAA- 1690 Strain HFH- 29744 (MRSA)	*Klebsiella pneumoniae* ATCC BAA- 2472 Strain 1100975
Streptococcus pneumonia ATCC 700675 Strain S. Africa 6B- 8	*Enterobacter cloacae* ATCC BAA- 2468 Strain 1000654
Listeria monocytogenes ATCC 700301	*Cronobacter sakazakii* ATCC 29544 Strain CDC 4562-70
Enterococcus faecalis ATCC BAA-2365	*Pseudomonas aeruginosa* ATCC BAA- 2110 Strain PGO 2338
Enterococcus faecalis ATCC 49532	*Acinetobacter baumannii* ATCC BAA- 1799
Enterococcus faecalis ATCC 51299	*Klebsiella oxytoca* ATCC 51983
Enterococcus faecalis ATCC 51575	*Enterobacter hormaechei* ATCC 49162
Enterococcus faecium ATCC BAA-2316	*Hemophilus influenzae* ATCC 33930
Enterococcus faecium ATCC BAA -2317	*Escherichia coli* ATCC BAA- 2340 Strain 1101362
Enterococcus faecium ATCC BAA- 2318	*Escherichia coli* ATCC BAA- 2471 Strain 1100101

Enterococcus faecium ATCC 700221	*Escherichia coli* ATCC BAA - 2523 Strain 1109131
Staphylococcus aureus subsp. *aureus* ATCC BAA-41 Strain NYBK 2464 (MRSA)	*Enterobacter cloacae* ATCC BAA- 2341 Strain 1101152
Staphylococcus aureus subsp. *aureus* ATCC BAA-42 (MRSA)	*Enterobacter cloacae* ATCC BAA - 2468 Strain 1000654
Staphylococcus aureus subsp. *aureus* ATCC BAA-44 (MRSA)	*Klebsiella pneumoniae* ATCC BAA- 1705 Strain ART 2008133
Staphylococcus aureus ATCC BAA- 1683 Strain HFH- 30364 (MRSA)	*Klebsiella Pneumoniae* ATCC BAA - 2473 Strain 1100770
Staphylococcus aureus ATCC BAA- 2313 Strain M10/ 0148 (MRSA)	*Klebsiella pneumoniae* subsp. *pneumoniae* ATCC BAA-2524 Strain 1103199
Staphylococcus aureus subsp. *aureus* ATCC BAA-2094 Strain B8-31 (MRSA)	
Staphylococcus aureus subsp. *aureus* ATCC 33592 (MRSA)	

Table 2: List of selected bacterial strains with its nature (Gram positive and Gram negative).

Results

All the bacterial species were identified correctly with a score value in the range of 9.252 to 9.736, which indicates highly reliable species identification and most probable sub-species identification (Figure 2). A score value in the range of 0-6, indicates that the identification is not reliable.

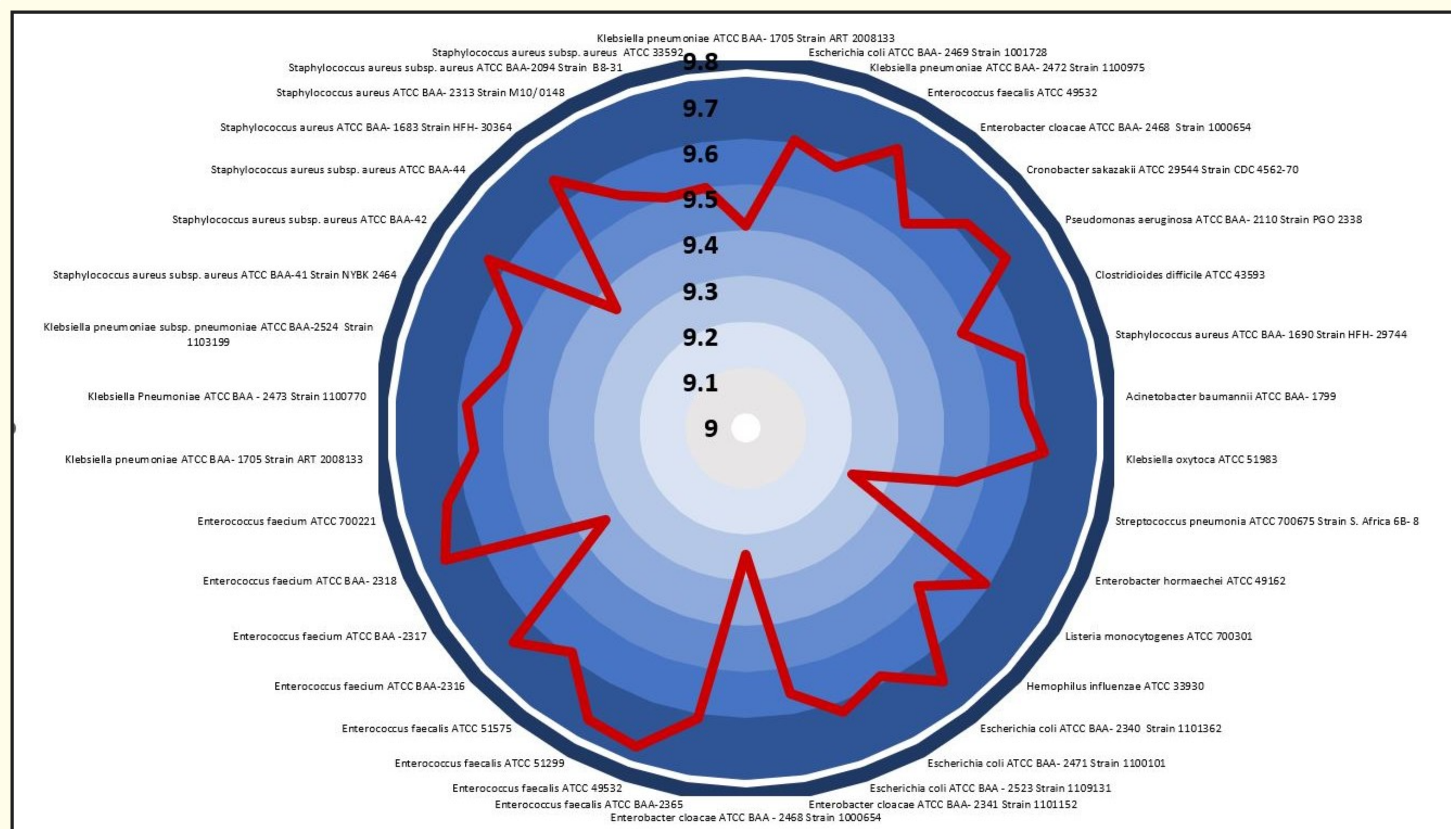

Figure 2: Score value of all 38 bacterial species obtained after using Autof Maldi-TOF MS 1000.

Gram positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Klebsiella pneumoniae* indicated score value in the range of 9.3-9.5, whereas all other strains exhibited score value of 9.5 and above. The spectrograms of a few of the strains that are based on m/z ratio are shown in Figure 3.

In this study, we correctly identified 20 Gram-positive bacteria and 18 Gram-negative bacteria with score value of above 9 with negligible failure rate.

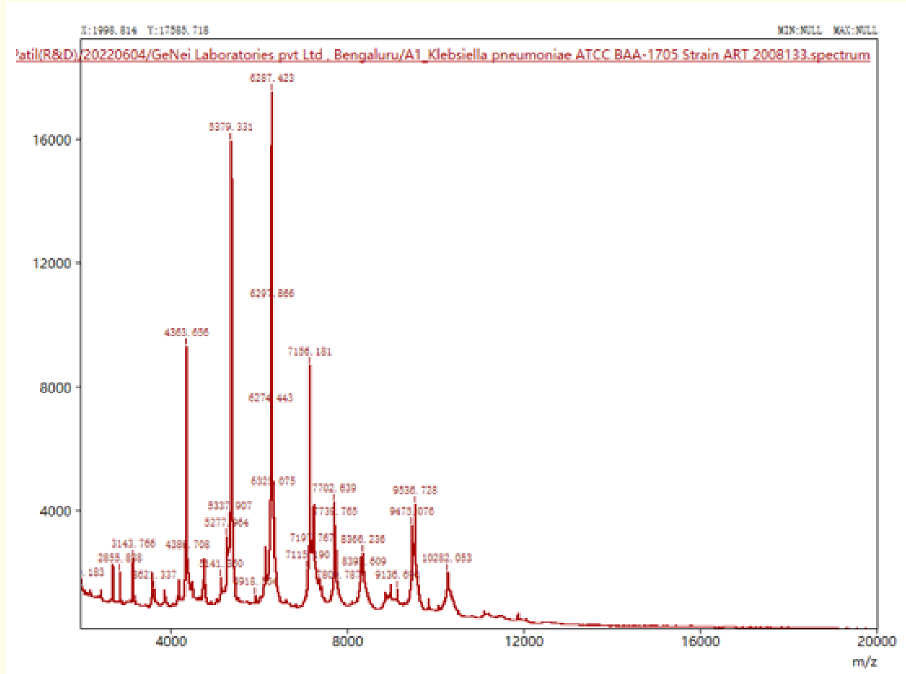

Figure 3a: Spectrograms of the few selected bacterial strains.

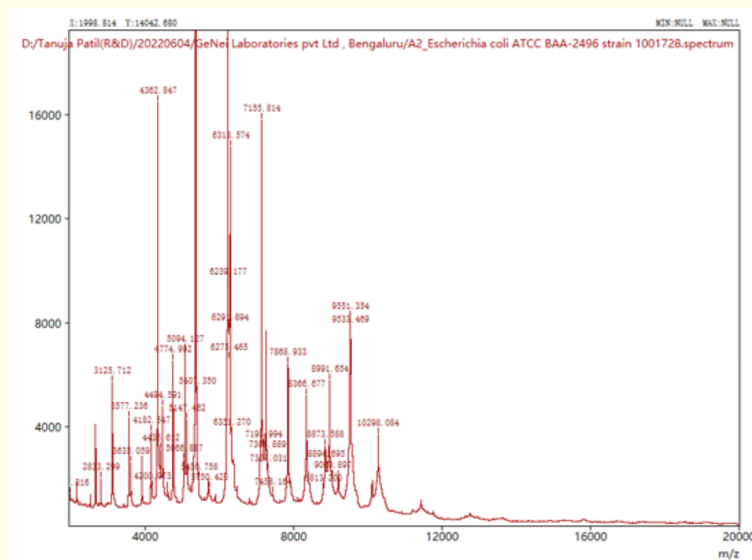

Figure 3b: Spectrograms of the few selected bacterial strains.

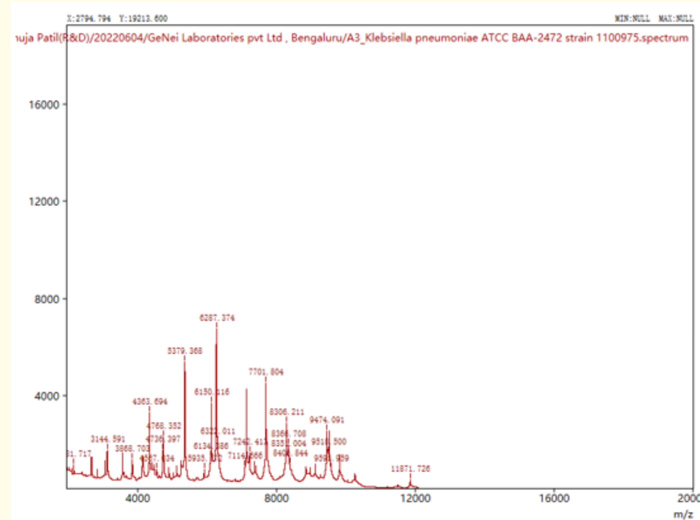

Figure 3c: Spectrograms of the few selected bacterial strains.

Discussion

Mass spectra of peptides from every individual strain, also known as peptide-mass fingerprint (PMF), varied and formed peaks at numerous m/z ratios in the range of 2 to 20 kDa. These variations were observed due to the presence of characteristic ribosomal proteins in the sample. Wang., *et al*. reported the highest accuracy of about 98.6% by Autof MALDI-TOF MS 100 in the comparative study of bacterial identification on various detection instruments. The study also reported that the Autof MALDI TOF MS 1000 correctly identified the highest number of strains at the genus level [12]. One of the factors on which the accuracy of identification by MALDI-TOF depends is the collection of fingerprint sequencing of protein for all potential strains in the database. In another study, the superiority of Autobio MALDI TOF Autof MS 1000 in the detection of bacterial strains, with increased specificity at species and sub-species level. In this study, fungal and yeast species were also identified with analogous accuracy [13]. Similarly, the identification of *Legionella qingyii* sp. nov. and other various ATCC and novel strains were reported using Autobio MALDI-TOF Autof MS 1000 at the genomic sequencing level [14].

In clinical studies, the process of identifying bacteria from a cultured sample involves assigning the genus of the bacteria (such as *Staphylococcus* or *Streptococcus*) by using a combination of methods, including observing their physical characteristics such as colony size and color, examining them under a microscope after Gram staining, and conducting rapid biochemical tests to check for catalase and/or oxidase activity [15]. The Gram stain is widely used to identify bacterial smears *in vitro*, as it can distinguish between Gram positive and Gram-negative bacteria based on their differing cell walls. The Gram staining technique has a significant drawback, as some Gram-positive bacteria tend to lose their color more easily than others, which can cause them to be mistakenly identified as Gram-negative. Several factors, including the composition of the growth medium and the age of the culture, can affect the tendency of Gram-positive bacteria to lose their color during the staining process [16].

The present study demonstrates the high utility, and rapidity of the Autobio's MALDI TOF Autof MS 1000 for identification of bacteria up to species level with high confidence. The wider database of the system is perhaps one of the reasons which ensures it. Moreover, the simplicity with which it is done is worth mentioning here as all these cultures were identified by the direct method without any protein extraction.

Conclusion

Autobio MALDI-TOF Autof MS1000 system was found to be an efficient system for bacterial identification. Simplicity in sample preparation and shorter time required for analysis have been the

attractive features of this method. The spectral graphs generated by the soft ionization of the samples coupled with comparison of the spectral data stored in the system database reliably authenticated the identity of the organisms. The score values of all the 38 ATCC bacterial strains, including both Gram positive and Gram-negative bacteria, varied in the range of 9.2 to 9.7, indication identification with very high reliability. With certain modifications, this system can be used for high throughput identification of large number of bacterial strains with high level of confidence. With a laboratory-friendly footprint, analytical versatility along with the option to upgrade from TOF to TOF/TOF capabilities, this platform may offer significant value in providing innovative analytical solutions for better and faster identification and authentication of bacterial strains in academic and clinical diagnostic settings.

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Conflict of Interest

All authors declare no conflict of interest.

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