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Application of MALDI-TOF MS for identification of environmental bacteria: A review

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ABSTRACT

Bacteria play a variety of roles in the environment. They maintain the balance in the ecosystem and provide different ecosystem services such as in biogeochemical cycling of nutrients, biodegradation of toxic pollutants, and others. Therefore, isolation and identification of different environmental bacteria are important to most environmental research. Due to the high cost and time associated with the conventional molecular techniques, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has gained considerable attention for routine identification of bacteria. This review aims to provide an overview of the application of MALDI-TOF MS in various environmental studies through bibliometric analysis and literature review. The bibliometric analysis helped to understand the time-variable application of MALDI-TOF MS in various environmental studies. The categorical literature review covers various environmental studies comprising areas like ecology, food microbiology, environmental biotechnology, agriculture, and plant sciences, which show the application of the technique for identification and characterization of pollutant-degrading, plant-associated, disease-causing, soil-beneficial, and other environmental bacteria. Further research should focus on bridging the gap between the phylogenetic identity of bacteria and their specific environmental functions or metabolic traits that can help in rapid advancements in environmental research, thereby, improving time and cost savings.

1. Introduction

Microorganisms are living organisms with a size of a few millimeters to nanometers and are present in all environmental matrices i.e., water, air, and soil. They almost always exist as a network of biologically diverse groups, interacting with each other and fighting for the limited nutrients (Braga et al., 2016). Bacteria, fungi, plants, algae, protozoa, and viruses get involved with each other during these biological interactions. They are present almost everywhere, thanks to their diverse metabolic processes and ability to adapt and survive in extreme environments. It has been estimated that a few grams of soil may contain tens of thousands of different species of bacteria (Mauchline and Malone, 2017). Similarly, the water matrix may contain various types of microorganisms including beneficial as well as pathogenic bacteria (Costello and Chaudhary, 2017; Zancarini et al., 2017). Moreover, it has been noted that the biological composition of the air matrix of an indoor environment can be determined by the occupants and can be correlated to the solid surfaces found in the environment (Hanson et al., 2016; Weikl et al., 2016).

Microorganisms in the environment provide a variety of ecosystem services. They play a significant role in the biological decay of organic matter in the soil, biogeochemical cycling of various elements, provide nutrients for plant growth, detoxify, or biodegrade various environmental pollutants, inhibit the growth of other harmful bacteria/fungi, participate in the fermentation of various food products, and contribute to countless other natural processes. On the other hand, some of the microorganisms (such as pathogenic bacteria) can be harmful to humans as they may cause infections and diseases that are sometimes life-threatening. In addition, the growth of some bacteria in food results in the spoilage of food items and products making them unfit for consumption. In conclusion, the role of bacteria in the environment and ecosystem cannot be ignored and their isolation, identification, and characterization are important to fulfill various human needs.

The techniques used for bacterial identification are based on the use of culture media and morphological, physiological, and biochemical based tests. Generally, these traditional identification methods take at least 3–5 days and require expert skills (Popovic et al., 2017). Other than these methods, various molecular (nucleic acid-based) methods have

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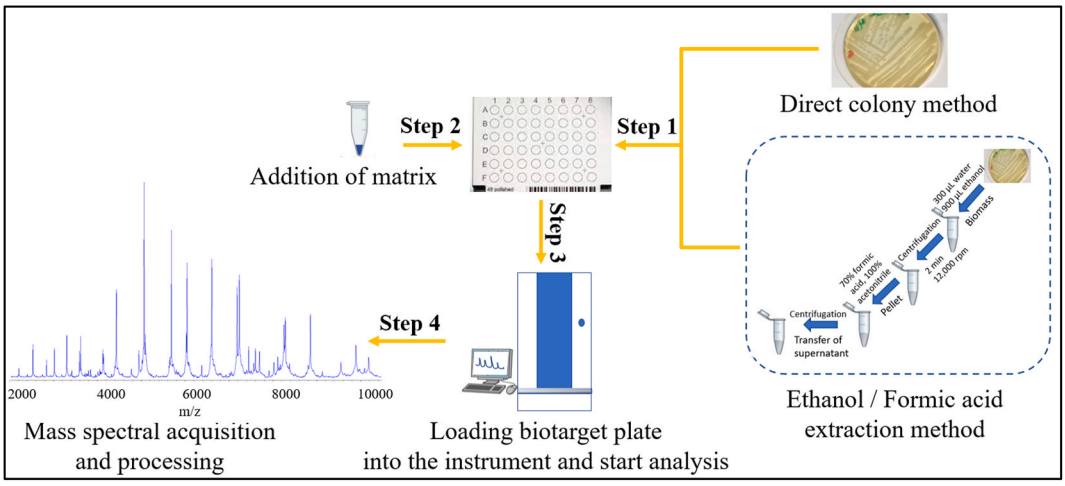


Fig. 1. Workflow for bacterial identification using MALDI-TOF MS.

also been developed such as DNA-DNA hybridization, 16s rRNA, G+C ratio, real-time polymerase chain reaction (RT-PCR), and fluorescent in-situ hybridization (Okafor, 2011). Some of these techniques like 16s rRNA and RT-PCR do not require the cultivation of bacteria making it possible to know the biological composition without going through culturing on microbiological media. However, these techniques are costly, time-consuming, and require high expertise (Braga et al., 2013). Therefore, accurate and rapid identification methods are required for bacterial identification to cope with pathogenic environmental bacteria and to support the isolation and identification of novel environmental bacteria.

MALDI-TOF-MS stands for Matrix-Assisted Laser Desorption Ionization – Time of Flight – Mass Spectrometry has been invented a long time before, but spectral fingerprints were obtained from whole bacterial cells for the first time in 1996 (Holland et al., 1996). During the same year, the spectral fingerprints of different Bacillus species such as *Bacillus anthracis, Bacillus melitensis,* were acquired by employing the MALDI-TOF technique (Krishnamurthy et al., 1996). Since then, much attention was directed towards the identification of not only bacteria but also yeast and mold using MALDI–TOF (Kostrzewa et al., 2013). However, MALDI-TOF was started to be routinely utilized as a first-line identification method in Microbiology labs during the last 12–15 years. The technology offers many advantages over conventional microbiological and molecular techniques, which include reliability and rapidness as it takes only a few minutes for identification of microbes (Fenselau, 2012), simplicity, cost-effectiveness, and non-requirement of highly skilled people (Seng et al., 2009). Moreover, the technology has the ability to identify many different types of microorganisms and thus possesses the potential to replace other identification methods in Microbiology labs (Biswas and Rolain, 2013).

Although the MALDI-TOF MS has found its routine application in clinical Microbiology labs, its utilization for the identification of environmental bacteria is still underestimated. There are several review articles published related to MALDI-TOF based identification of bacteria such as Tsuchida et al. (2020), Hou et al. (2019), Jang and Kim, (2018), and Singhal et al. (2015). However, both Tsuchida et al. (2020) and Hou et al. (2019) are entirely focused on clinical samples, while, the reviews by Jang and Kim, (2018) and Singhal et al. (2015) adopted a broader perspective and have discussed briefly its application in both clinical and environmental samples. In comparison, the more focused and comprehensive discussion of MALDI-TOF MS for its application in environmental microbiology was given by Santos et al., 2016. Nevertheless, in that review article, in addition to the bacterial identification by MALDI-TOF, other areas of environmental microbiology such as protein profiling, metabolomics, and others were also discussed. Therefore, the current review article aims to comprehensively explain the application of MALDI-TOF MS for bacterial identification in various environmental fields. Using bibliometric analysis, the trends of its application in different fields with time are explained for the first time. In addition, the application of MALDI-TOF MS for bacterial identification from water, soil, plants, air, food, and other environmental surfaces is also presented in detail.

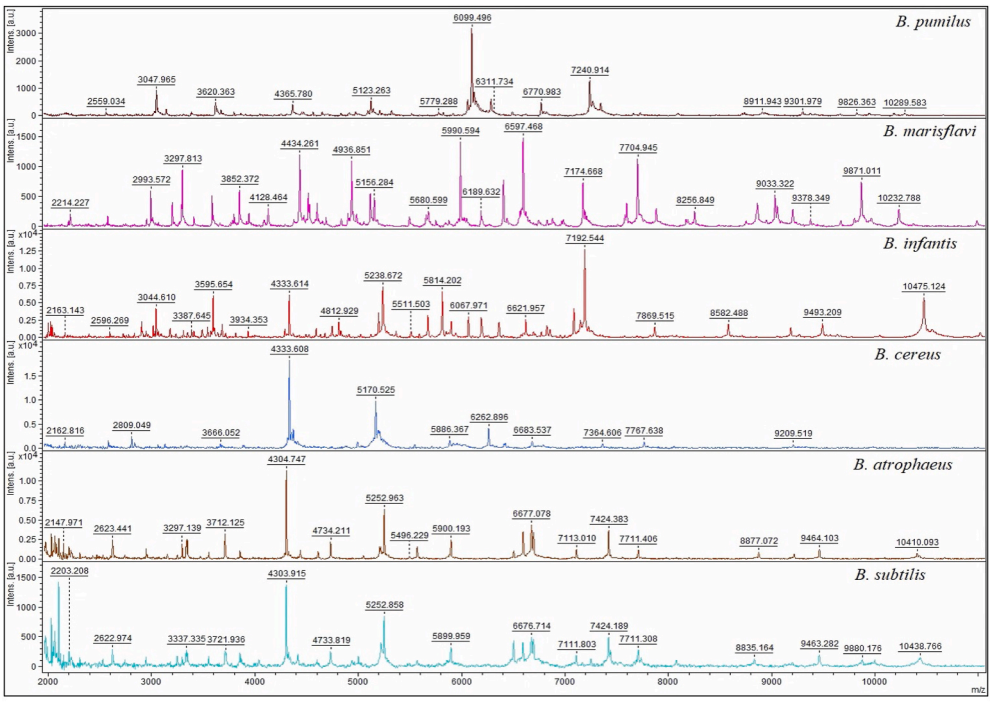
2. MALDI-TOF MS, the technique

MALDI-TOF MS relies on the detection of mass to charge ratio abbreviated as m/z of ribosomal proteins of the bacteria, which helps to provide a unique mass spectrum of the bacteria within a short period of time (Carbonnelle et al., 2011). The technique does not consider biomarker ion peaks identification in a spectrum. Instead, it relies on the characteristic mass profile obtained by a set of ion peaks, which represents a "fingerprint" of the bacteria (Dieckmann and Malorny, 2011). The identification is provided by comparing the spectrum with the spectra of reference strains based on the closest match (Popovic et al., 2017).

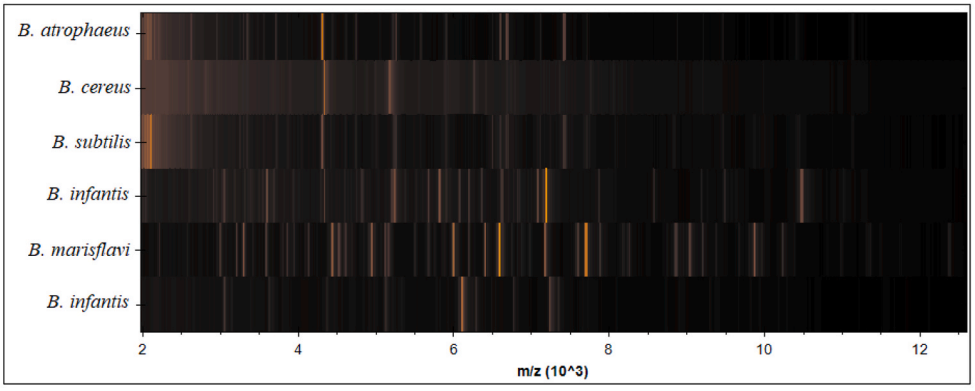
The technique can be used to analyze different types of organic molecules such as nucleic acids, organic molecule solutions, proteins, and completely microbial cells. However, the proteins and whole microorganisms are most extensively utilized for Microbiology applications (Dekker and Branda, 2011). The most reliable biomarkers for the identification of bacteria are the proteins specifically the ribosomal proteins. The features of ribosomal proteins favor efficient ionization, which includes their abundance and medium hydrophobicity (De Carolis et al., 2014). Thus, using this technique, an adequate amount of stable mass signals for the ribosomal protein peptides typically 2000 to 20,000 Da can be obtained. The mass signals are used to produce profile spectra, which comprise a series of peaks that are conserved at the genus, species, and even subspecies level (Benagli et al., 2012).

2.1. Sample preparation

There are mainly two methods for sample preparation i.e., the Direct Transfer method and Protein extraction methods (Fig. 1). The former method is easy and fast which is based on using bacterial cells directly from the pure culture plates using sterile inoculating loops or after harvest action by centrifuge in the case of liquid media. This method is mostly utilized for identification i.e., for almost 90–95% of the samples. The protein extraction method constitutes the extraction of proteins from bacterial cells using solvents of different types (Sedo et al., 2011). It is strongly recommended when a higher quality of spectra is needed.



(a)



(b)

Fig. 2. Comparison of bacterial protein profiles in MALDI-TOF MS using MALDI Biotyper software (a) Stack view, (b) Gel-view.

There are different types of solvents that can be used for the extraction of proteins. However, the combination of ethanol and formic acid is being widely utilized and is generally recommended by the manufacturers of MALDI-TOF MS systems (Bruker Daltonics, Microflex LT Biotyper operating system) (Step 1, Fig. 1). The bacterial cells directly from plates or extracted proteins are used to deposit on the MALDI biotarget plate (Step 2, Fig. 1), which is then overlaid with the suitable matrix solution (1:1). There are various organic compounds that have been used as matrices for MALDI-TOF MS such as α-cyano-4-hydroxycinnamic acid (CHCA), 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid), and 2, 5-dihydroxy benzoic acid (DHB). However, the most commonly used matrix is CHCA. After drying the mixture of matrix and bacterial cells/proteins at room temperature, the biotarget plate is then loaded onto the MALDI-TOF instrument for which the spectrum is obtained for comparison with the database (Step 3 and 4, Fig. 1). The most common manufacturers for the MALDI-TOF MS system are Bruker Daltonics and BioMerieux (Vitek MS).

2.2. Microorganism identification

Various commercial databases available can be used for comparing the acquired mass spectra of the unknown bacteria with the mass spectra of the known bacteria such as the MALDI Biotyper library (MBL). MBL contains a database of species-specific fingerprints of a wide variety of bacteria and yeasts. The identification is done by matching the unknown spectra obtained with the known spectra in a database using Biotyper software (Clark et al., 2013). Varieties of algorithms are developed to calculate the similarity level. Alternatively, there are various search engines and fingerprint libraries have been developed to search for databases of bacterial protein profiles (Bright et al., 2002). In addition, there are bioinformatics-based approaches like Swiss-Prot/TrEMBL or NCBInr data, however with albeit a lower number of protein entries for environmental bacteria of partially sequenced genomes (Mazzeo et al., 2006).

The identification accuracy via MALDI depends upon the database. In general, its accuracy of identification up to the species level is above 90% (Martiny et al., 2012). Therefore, the database needs to be updated to enhance the identification of the MALDI instrument (De Carolis et al.,

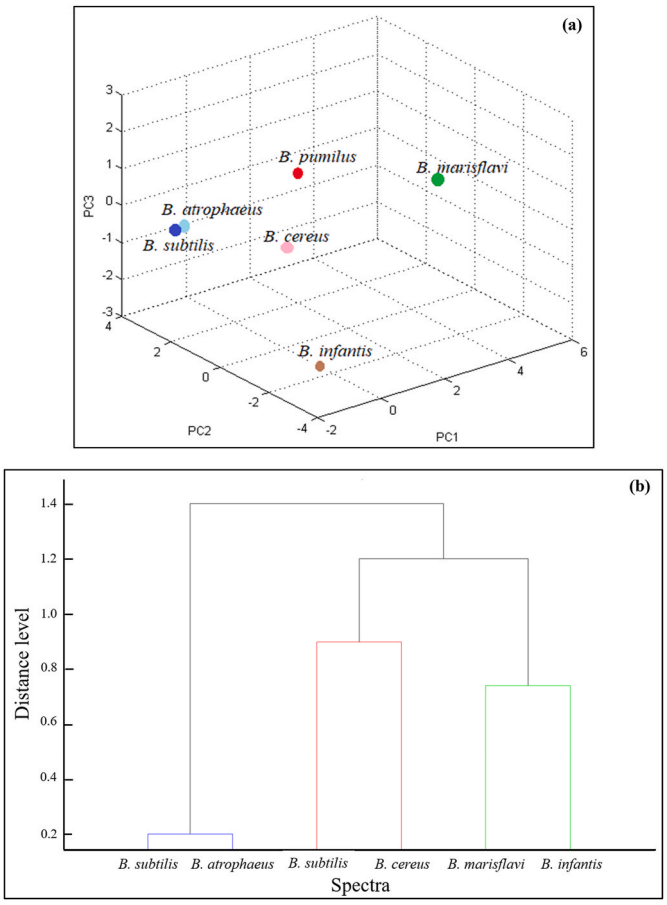


Fig. 3. Chemometrics or Statistical methods for comparison of protein profiles (a) PCA; (b) Dendrogram.

2014). The reproducibility of MS Spectra via MALDI also depends upon the sample culture, its preparation, and storage as well as the matrix type used (Fenselau, 2012).

2.3. Microorganism typing

The MALDI-TOF technique has been recently applied for the purpose of bacterial subtyping and classification. Although the instrument has been widely used for genus and species level identification, its utility to be utilized up to subspecies level is not yet fully explored (Sandrin et al., 2012). The subspecies level identification is quite challenging, as the strains (within the same species) tend to be very similar in terms of genotype and phenotype (Sandrin et al., 2012). However, subspecies level typing using MALDI-TOF MS has been reported for bacteria like *Yersinia enterocolitica* (Rizzardi et al., 2013). *Methicillin-resistant S. aureus lineages* (Wolters et al., 2011), *S. enterica* (Dieckmann and Malorny, 2011), and *Streptococcus agalactiae* (Lartigue et al., 2009).

There are different approaches to identification at strain level using MALDI-TOF MS as depicted in Fig. 2. The mass spectra are loaded for different *Bacillus* strains i.e., *B. marisflavi, B. pumilus, B. infantis* (Abu--Dieyeh et al., 2019) and *B. subtilis, B. cereus, B. atrophaeus* (Alsayegh et al., 2021) in a stack view (Fig. 2a). One way of differentiating similar species is differentiating them based on the presence/absence of one or more discriminating peaks (Sandrin et al., 2012; Ashfaq et al., 2019, 2020) through visual comparison of profiles. Similarly, the mass spectra can also be compared using gel-view as depicted in Fig. 2b. The gel-view makes the comparison easier as the presence/absence of peaks can be easily seen. The significant differences in the profiles of different species of Bacillus can be easily identified using Fig. 2 such as the presence of peak around 4300 and 5200 m/z can be considered biomarker peak for genus Bacillus. In addition, species-specific biomarker peaks like 7704, 8256, and 9033 m/z are only present in *B. marisflavi*, while peaks like 3044, 3595, and 5800 m/z are only present in *B. infantis*.

There are also statistical/chemometric methods that can be used to differentiate the species such as principal component analysis (PCA) (Fig. 3a), dendrograms (Fig. 3b), and composite correlation matrix. The PCA diagram in Fig. 3a shows 95% of the variation within the mass spectral profiles of the 6 strains through PC1, PC2, and PC3. From Fig. 3, it is evident that the strains of *B. atrophaeus* and *B. subtilis* are similar to each other as they are lying close to each other on the 3D diagram of PCA as well as on dendrogram, which can also be noted from their respective protein profiles presented in Fig. 2. Hence, the PCA helps to categorize the mass spectra of strains into groups (clusters) such that the species following in the same group are similar to each other than the ones in different groups. While the dendrograms show the hierarchical relationships between the strains and the composite correlation matrix provides information about intra- and inter-specific percentages of similarity between species (Abdel Samad et al., 2020).

Table 1
Categorization of environmental studies based on the core research objective.

#	Main Research Objective	Assigned category
1	Identification and characterization of new bacterial strain, Studying biodiversity of different environments such as polluted sites	Ecology
2	Comparison of MALDI-TOF MS with other established methods such as molecular techniques (16s rRNA, Multi-locus sequencing technique (MLST), and Vitek 2)	Methodology Validation
3	Identification of bacteria present in water, soil, and at different surfaces to investigate the presence of known indicator organisms (coliform bacteria), or to track the source of contamination	Environmental Monitoring
4	Identification, and characterization of human pathogens in the environment such as in water, indoor air, etc.	Environment and Health
5	Characterization of bacteria in water at different treatment stages, evaluating the efficiencies of treatment techniques for bacterial removal	Water Treatment Technique
6	Isolation, identification, and characterization of pollutant-degrading bacteria	Environmental Biotechnology/ Bioremediation
7	Isolation, identification, and characterization of mineral forming bacteria	Biomineralization
8	Identification of plant-growth-promoting (PGP) bacteria or growth inhibitors of pathogenic fungi to protect crops or increase yield	Agriculture and Plant Sciences
9	Identification of plant-associated bacteria involve in phytoremediation	Phytoremediation
10	Identification of plant pathogens	Plant pathology
11	Evaluation of different techniques for disinfection of surfaces	Disinfection Technology
12	Identification of foodborne pathogens, fermentative bacteria, lactic acid bacteria and others	Food Microbiology

3. Bibliometric analysis

For bibliometric analysis, the main data source is Web of Science (WOS), PubMed, and Scopus. In this research, the WOS was employed as the source database to obtain articles as many as possible. Additionally, the manual search was also conducted thoroughly to avoid missing any articles. To investigate the application of MALDI-TOF MS usage for bacterial identification from environmental samples (water, soil, plants, and others), the data was obtained using three main terms i.e., "MALDI-TOF MS" "Bacteria", and "Identification". These three terms were chosen based on preliminary analysis with different terminologies as they

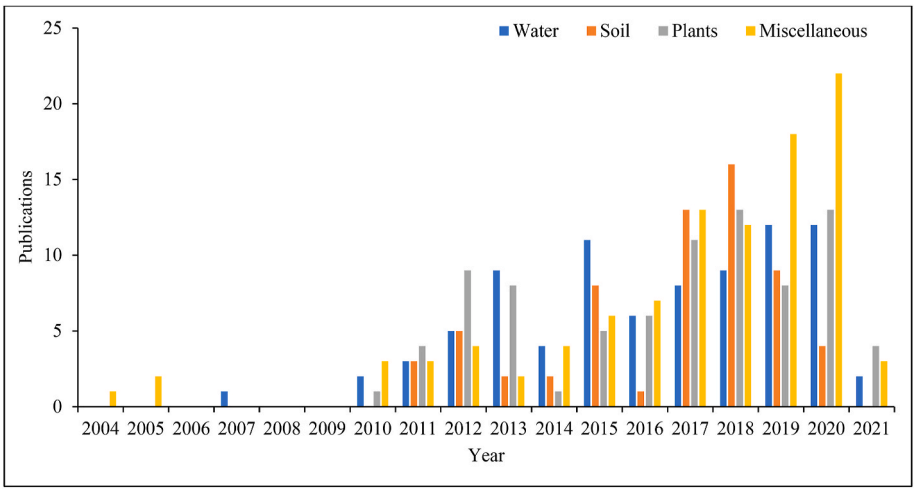


Fig. 4. Number of publications per year for different environmental compartments.

Table 2
Total number of articles published for environmental samples.

	Water	Soil	Plants	Miscellaneous	Total
Total no. of articles	85	64	65	104	318
% Percentage of total articles	26.75	20.1	20.45	32.7	100

provided the greatest number and most relevant results. The analysis was performed on 30th March 2021 and the obtained results were exported as text files.

Further analysis was performed using MS Excel to separate the articles/studies related to water, soil, plants, and other environmental compartments (air, food, and others). The application of MALDI-TOF MS for bacterial identification in environmental samples was studied through studying development trends (number of publications per year) by considering different sample types (water, soil, plants, air, food, and others), WOS categories (Microbiology, Environmental Sciences, etc.) and study areas (ecology, water treatment, food technology, bioremediation, etc.). In this paper, the study areas were defined based on the main core objective of the research article, which is summarized in Table 1. There were few studies, which could fall into more than one category; the study was then categorized based on the dominant one.

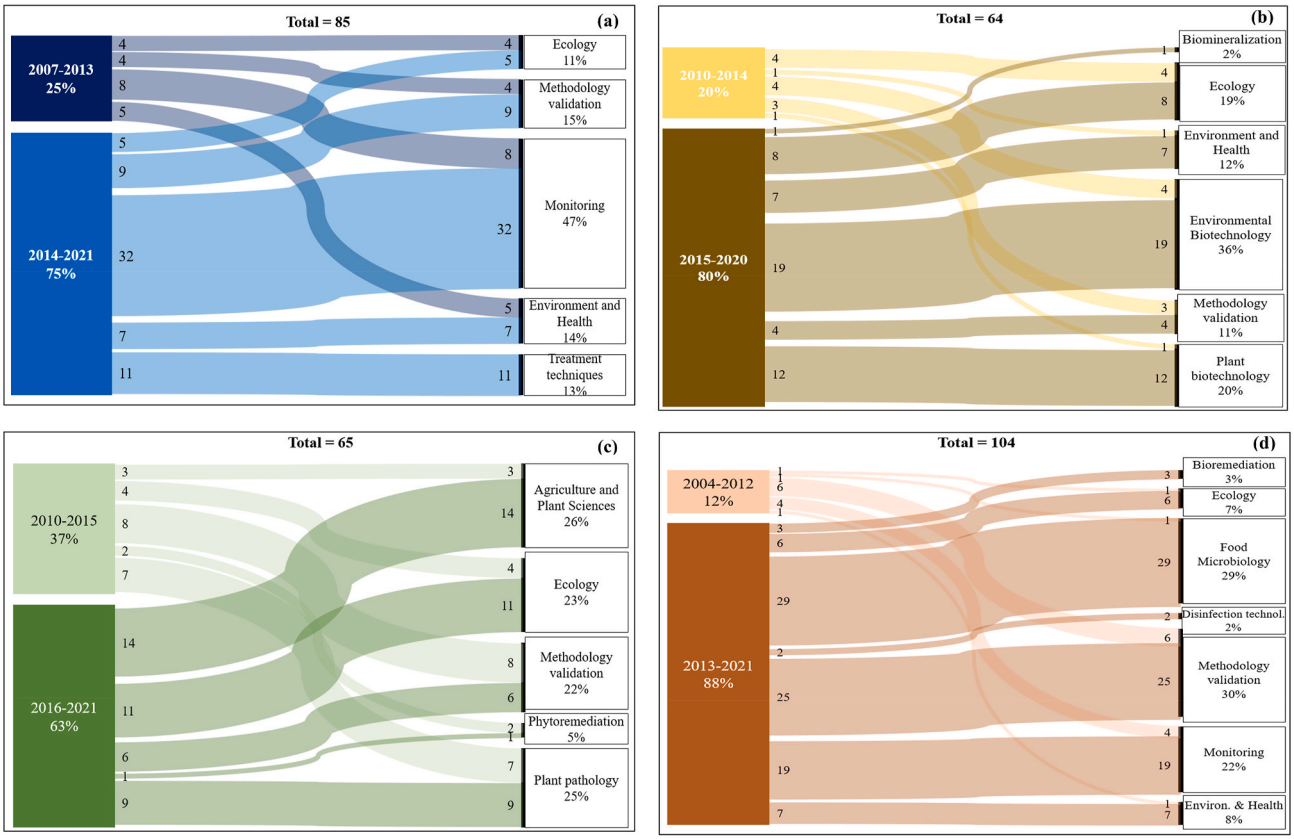
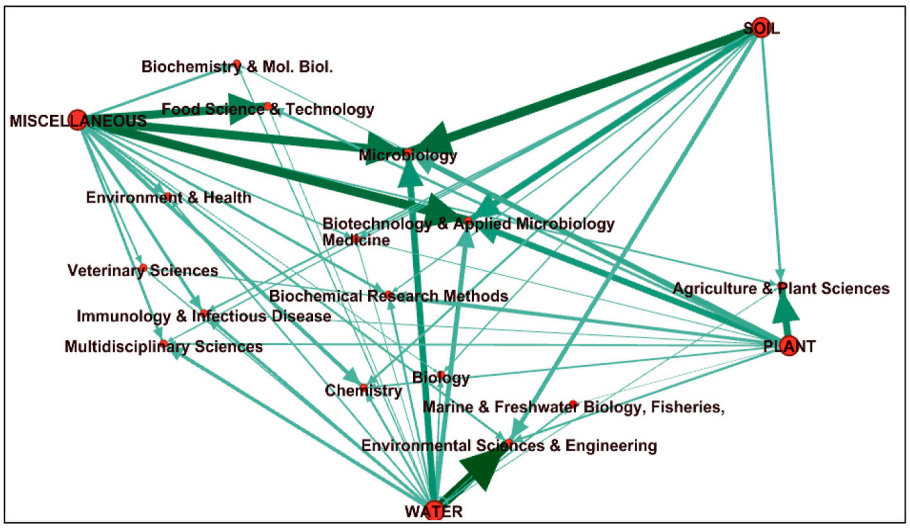
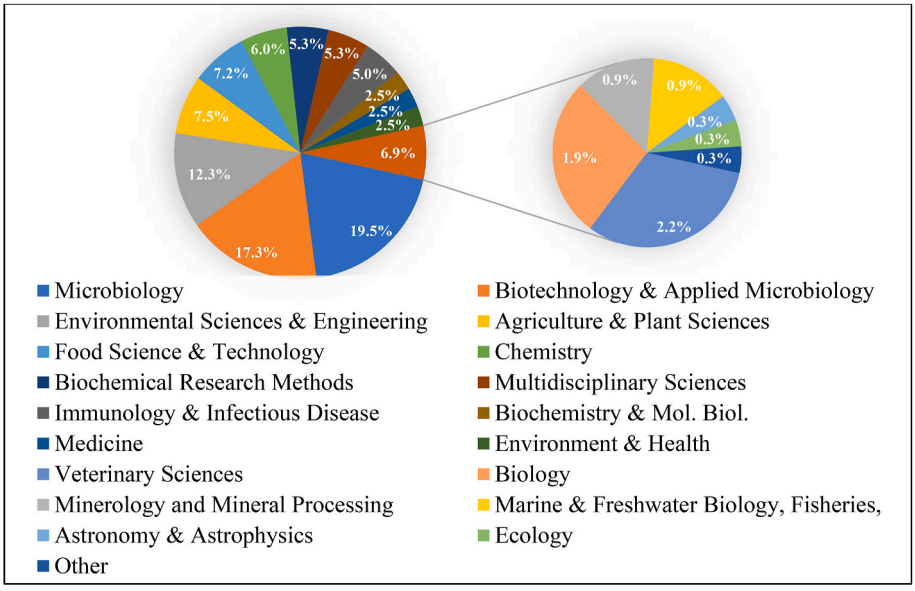


Fig. 5. Sankey diagram showing the evolution of MALDI-TOF MS technology for various types of studies in (a) Water; (b) Soil; (c) Plants; (d) Miscellaneous environmental samples.



(a)



(b)

Fig. 6. (a) Network of WOS categories as per different environmental compartments (b) Pie chart showing % of articles published in different WOS categories.

4. Application of MALDI-TOF MS for identification of environmental bacteria

4.1. Field development with time

The temporal trend of publications related to "MALDI-TOF MS", "Identification" and "Bacteria" is depicted in Fig. 4. It is evident from Fig. 4 that the technology has received greater attention for the identification of environmental bacteria during the last decade. Using the three strings in WOS, the total number of publications comprising original articles and research papers was 2533. During preliminary analysis, the review papers, and original articles pertaining to the application of MALDI-TOF MS in the medical industry and proteomics were omitted which yielded 318 articles. These articles were then further categorized based on the source of bacterial isolates and study types as summarized in Table 2. Table 2 shows that a total number of 85 articles were belonging to "Water", 64 to "Soil", and 65 to "Plants". While the remaining 104 articles, which did not belong to any of these environmental samples, were sub-categorized under "Miscellaneous" in which articles related to bacterial identification from food and surfaces were in abundance.

The results of Bibliometric analysis showed that MALDI-TOF MS was used for the identification of environmental bacteria in 2004 (Fig. 4). In 2004, Krader and Emerson (2004) tested MALDI-TOF MS using a linear mass spectrometer (Micromass, UK) for rapid identification and subsequent categorization of different environmentally important bacteria (acquired from American Type Culture Collection, ATCC). Similarly, Ruelle et al. (2004) developed a method for the identification of environmental bacteria (*Escherichia coli, Salmonella,* and *Acinetobacter*) isolated from sewage sludge. In 2005, another two articles were published aiming to develop and validate the method for bacterial identification using MALDI-TOF MS from marine sponges (Dieckmann et al., 2005) and bioaerosol particles (Kim et al., 2005).

From water samples, the first two articles were published in 2007 by Siegrist et al. (2007) and Donohue et al. (2007). In the research of Siegrist et al. (2007), MALDI-TOF MS was evaluated for rapid

Table 3
Summary of different studies related to the application of MALDI-TOF MS in environmental studies.

Bacterial strain	Significance	Source	Reference
Environmental and Ecological studies (Categories: Ecology, Environmental monitoring, Environment, and Health)			
Gemmatimonas groenlandica sp.,	Capability of performing bacteriochlorophyll-based phototrophy (chlorophototrophy).	Surface water	Zeng et al. (2021)
Methylobacterium extorquens, M. adhaesivum, M. marchantiae	Known to be alpha-proteobacteria that inhibits the phyllosphere and consume the emitted methanol from the plants	Plants	Tani et al. (2012)
Staphylococcus edaphicus	Isolated from Antarctica, with special adaptation to extreme environments	Soil	Pantucek et al. (2018)
Enterococci faecium	Monitoring of water quality routinely in the water industry and can be used to assess the bacterial diversity of water intended for human consumption	Water	Laukova et al. (2019)
Aeromonas hydrophila, Bacillus cereus, Pseudomonas aeruginosa, and *Stenotrophomonas maltophilia*	Monitoring of groundwater being used as a drinking water	Water	Jancova et al. (2020)
Staphylococcus aureus	Examining the hygiene facilities provided including handwashing and hand-drying facilities in public washrooms	Surfaces	Suen et al. (2019)
Staphylococcus aureus, E. faecalis, A. viridans, and *Aspergillus* sp.	Investigate the presence of rarely described and underestimated species of bacteria on the surfaces of magazines found in waiting areas of the hospital	Surfaces	Ade et al. (2017)
Staphylococcus sp., *Escherichia* sp., *Enterococcus* sp., and *Bacillus* sp.	Rapid identification of various air pollutants	Air	Elbehiry et al. (2019a)
Aeromonas hydrophila, Aeromonas caviae and *Aeromonas sobria*	Rapid and fast identification and characterization of human pathogens isolated from chicken meat and water	Food and Water	Elbehiry et al. (2019b)
Bukhloderia pseudomallei	Categorization of *B. pseudomallei* based on its source i.e., clinical, or environmental and to identify the source-specific biomarkers for the strains	Soil and Patients	Niyompanich et al. (2014)
Escherichia coli, *Enterobacter cloacae*, and *Klebsiella pneumoniae*	Characterization of dust from laying hen farm for its microbial load and presence of antibiotic-resistant bacteria	Air (Dust)	Ahmed et al. (2020)
Staphylococcus aureus, S. warneri, *S. hominis*, *S. saprophyticus*, and *S. cohnii*	Identification of medically relevant *staphylococci* from the air samples taken from schoolrooms	Air	Fox et al. (2011)
Environmental Biotechnology and Bioremediation studies			
Lysinibacillus fusiformis, Bacillus mycoides, Bacillus pumilus, and *Bacillus cereus*	Isolation and identification of 25 bacterial strains from 1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene (DDT) contaminated site.	Soil	Lara et al. (2021)
Actinobacteria sp., *Firmicutes* sp. and *Proteobacteria* sp.	To remove polycyclic aromatic hydrocarbons (PAHs) from the environment	Surface water seawater, and marine sediments	Silva-Jimenez et al. (2018)
Bacillus sp., *Enterobacter* sp., *Burkholderia* sp., and *Klebsiella* sp.	To characterize the bacterial strains which showed resistance to copper contamination	Water	Avanzi et al. (2016)
Arthrobacter sp., *Serratia* sp., *Rhodococcus* sp., and *Rhizobium* sp.	Isolation of bacterial isolates that can utilize biphenyls for carbon and energy source and subsequently biodegrade them	Soil	Uhlik et al. (2011)
Plant sciences (Category: Agriculture science, Phytoremediation, Plant Pathology)			
Enterobacter cloacae and Burkholderia cepacia	Isolation and evaluation of the potential growth promoters present in plants and plant pathogenic fungi growth inhibitors	Plants	Junior et al. (2020)
Rhizoctonia solani and *Phytophthora infestans*	Investigation of their plant growth-promoting characteristics	Soil (Rhizosphere)	Ghyselinck et al., (2013)
Burkholderia sp.	Isolate and identify endophytic bacteria from locally cultivated sugarcane plants and screen the isolates for their plant growth-promoting activities.	Plant	Rajendran and Marimuthu, (2017)
Pseudomonas sp., *Enterobacter* sp., *Ewingella* sp., *Bacillus* sp., *Rahnella* sp., *Serratia* sp., *Yokenella* sp., *Enterobacter* sp., *Yersinia* sp., *Raoultella* sp., and *Klebsiella* sp.	Isolate and identify endophytic bacteria in bananas and investigate their potential to be developed as biofertilizers	Plant	Muthuri et al. (2012)
Enterobacter sp.	Screen out bacterial strains that are resistant to cadmium present in contaminated rice rhizosphere as well as evaluate its influence on the rice seedlings' growth under cadmium stress	Soil (Rhizosphere)	Mitra et al. (2018)
Stanleya pinnata and *Astragalus bisulcatus*	Characterization of selenium hyperaccumulator bacteria with respect to selenium related properties and plant growth promoting properties	Plant	Sura-de Jong et al. (2015)
Nicotiiana tabacum and *Armoracia rusticana*	Investigation of the effect of plants and added natural compounds such as naringin, caffeic acid, and their mixture as PCB degrading inducers on indigenous microbial communities and activity of degrading bacteria	Plant	Prouzova et al. (2012)
Pythium ultimum, Enterobacter cloacae, and *Aeromonas media*	Identify the most abundant bacterial species that were isolated from rhizoplane	Soil (Rhizosphere)	Oberhaensli et al. (2017)
Pseudomonas syringae	*Pseudomonas syringae* pathogenicity on *Chenopodium quinoa* partial leaves	Plants	Fonseca-Guerra et al. (2021)
Pseudomonas syringae	To prove the pathogenicity of *Pseudomonas syringae* towards the leaf blight on *Miscanthus sinensis*	Plants	Choi et al. (2017)
Burkholderia glumae, Burkholderia gladioli pv. gladioli, and *Erwinia chrysanthemi pv. Zeae*	To detect the plant pathogens responsible for the infection caused in rice seedlings	Plants	Kajiwara (2016)
Food Microbiology			
Brochothrix thermosphacta	Identifying and differentiating different foodborne pathogenic bacteria	Food	Illikoud et al. (2019)
Bacillus sp, *Staphylococcus* sp., *Lysinibacillus* sp., *Micrococcus* sp. *and Brevibacillus* sp.	Investigation of the bacteria present in different kinds of honey of variable geographical and botanical origins	Food	Pomastowski et al. (2019)
	Identification of beer spoilage bacteria	Food	Turvey et al. (2016)

(*continued on next page*)

Table 3 (*continued*)

Bacterial strain	Significance	Source	Reference
Actobacillus lindneri, Lactobacillus brevis and Pediococcus damnosus			
Water treatment and Disinfection technology			
Halomonas aquamarina, Halomonas elongata, Pseudomonas fragi, P. stutzeri	Isolation and screening of seawater microorganisms to determine their ability to use antiscalants as carbon and energy source.	Water	Ashfaq et al. (2019)
Proteobacteria sp., *Firmicutes* sp., *Bacteroidetes* sp., *and Actinobacteria* sp.	Assess the bacterial community in a drinking water treatment plant	Water	Sala-Comorera et al. (2017)
Acinetobacter lwoffii, Bacillus amyloliquefaciens, Bacillus endophyticus, Bacillus megaterium, Bacillus oceanisediminis, Bacillus oleronius, Pantoea agglomerans, Psychrobacillus psychrodurans, Staphylococcus haemolyticus, and *S. warner*	Investigate the application of a medical diode laser for the disinfection of small microbiologically contaminated spots on degraded collagenous materials	Surfaces	Rybitwa et al. (2020)

identification and differentiation of coliform bacteria (*Escherichia coli*) in surface waters. Donohue et al. (2007) developed the library comprising 40 strains representing 17 species of *Aeromonas*, potentially pathogenic to humans. Using MALDI-TOF MS, the identification of 52 isolates from drinking water was achieved and it was concluded that the technique could be used to identify microbial hazards in the water. Despite successful development and validation of technology for bacterial identification from the environmental origin, there were no further studies reported for another two years (2008 and 2009).

In 2010, the first plant-source and plant beneficial bacterium i.e., *Pantoea agglomerans* was identified using MALDI-TOF MS by Rezzonico et al. (2010). The results of MALDI-TOF MS clustering agreed with the gyrB sequencing method. Since 2010, there has been a steady increase in reported studies employing MALDI-TOF MS for the identification of bacteria from the environmental origin (water, soil, plants, and others) (Fig. 4).

4.2. Evolution of MALDI-TOF MS application in different environmental compartments

Based on the categorization of all selected studies, the evolution of MALDI-TOF MS over time in different subject areas is depicted in Fig. 5 for water, soil, plants, and miscellaneous environmental samples. According to Fig. 5a, the 85 articles published for "water" compartment comprised of studies related to Monitoring (47%), Methodology validation (15%), Environment and Health (14%), Water Treatment techniques (13%), and Ecology (11%). Fig. 5a also depicts the increase in researchers' interest with time as 75% of the total articles were published during the last 7 years (excluding 2021) that are related to water compartment. In addition, during the early stages of application (2007–2013), the researchers were more inclined towards the development and validation of methodology. However, with time, other applications emerged such as the identification of bacteria during treatment of water and wastewaters.

Moreover, for 64 articles published for "soil" compartment, they were mainly comprised of studies related to Environmental Biotechnology/Bioremediation (36%), Plant Biotechnology (20%), Ecology (19%), Environment and Health (12%"), Methodology validation (11%); and only 2% of the studies were related to the biomineralization. It is also noteworthy that 80% of the total articles were published during the last 6 years (2015–2020). Whereas, during the early stages of application (2010–2014), only 20% of the articles were published out of which mostly were related to the development and validation of the MALDI-TOF MS technique for identification of soil bacteria (Fig. 5b). However, with time, other applications of MALDI-TOF MS in soil sciences emerged such as in Plant Biotechnology, Environment and Health, and others.

Out of 65 published articles in which MALDI-TOF MS was employed for the identification of bacteria isolated from plant samples, 51% of these studies are related to Agriculture and Plant Sciences (26%) and Plant pathology (25%). While the studies related to Ecology, Methodology Validation, and Phytoremediation each comprised 23%, 22%, and 5%, respectively. During the last 5 years (2016–2020), the application in the plant has gained significant interest as 63% of the articles are published during this time with a focus on its utilization in Agriculture, Plant Pathology, and Ecology (Fig. 5c).

The published articles that do not belong to any of the environmental compartments discussed before i.e., water, soil, and plants, these articles were separately categorized under Miscellaneous Environmental samples. There were 104 articles under this category. According to Fig. 5d, these 104 articles are comprised of studies related to Methodology validation (30%), Food Microbiology (29%), and Monitoring (22%). In addition, 8% of the studies were related to the "Environment and Health" and 7% were related to "Ecology", 3% to "Bioremediation" and 2% to "Disinfection Technology". It can be concluded that during the initial phase from 2004 to 2012, most of the researchers' interest was towards the development and validation of the MALDI-TOF MS technique for the identification of environmental bacteria. However, other applications also emerged more recently, and the technique is now used more frequently and being applied in a variety of studies.

4.3. Bibliometric evolution in subject categories

The number of articles published based on different WOS categories is also depicted in Fig. 6. The WOS categories are simplified for easy representation based on a most related subject area such as Microbiology; Virology; Parasitology; Medical Laboratory Technology; and other similar categories were grouped under "Microbiology". In Fig. 6a, the thicker the edge is, the higher the number of articles published in a particular environmental compartment. Thus, the results presented in Fig. 6a and b showed that the greatest number of articles published in journals were related to "Microbiology (n = 62, 19.5%)", out of which, 19 articles belong to soil, 18 to miscellaneous environmental samples, 14 to water and 11 to plant samples. This is then followed by "Biotechnology & Applied Microbiology (n = 55, 17.3%)" comprising contributions of 19 from miscellaneous environmental samples, 13 each from soil and plants, and 10 from water samples. Such analysis helps to understand the aim, type, and scope of different studies published encompassing the use of MALDI-TOF MS for bacterial identification from water samples.

In terms of each environmental compartment, the most used WOS categories for "Water" samples include "Environmental Sciences & Engineering (n = 22)", "Microbiology (n = 14)", and "Biotechnology & Applied Microbiology (n = 10)". On the other hand, only one article was published in each of the "Agriculture & Plant Sciences", "Mineralogy and Mineral Processing", and "Medicine". Similarly for miscellaneous environmental samples, the most used were "Biotechnology & Applied Microbiology (n = 20)", "Microbiology (n = 18)", and "Food Science & Technology (n = 17)".

5. Categorical literature review

5.1. Application in environmental and ecological studies

5.1.1. Ecological studies

MALDI-TOF MS has been used for the identification and characterization of new novel strains from environmental samples, and for exploring the biodiversity of bacteria in different environments (Table 3). The technique was also used to identify the bacterial communities and diversity in the rhizosphere, phyllosphere and to identify and characterize the new bacterial species responsible for plant diseases.

The chlorophyll-based phototrophy (chlorophototrophy) is done by bacteria that belong to the phylum Gemmatimonadetes. Nevertheless, only one bacterium capable of performing chlorophototrophy has been isolated which has limited our understanding of the lifestyle and evolution of photoheterotrophy. Thus, Zeng et al. (2021) adopted a culturomics strategy that incorporated a high-throughput colony screening approach using MALDI-TOF MS and genome sequencing to isolate a new strain of Gemmatimonadetes bacteria from the stream of Northeast Greenland. The rapid screening approach helped to isolate and identify one strain from 330 isolated strains. Another study that aimed to isolate and identify the biosurfactant-producing bacteria, MALDI-TOF MS was used. It was found that the 8 isolates out of 234 were capable of producing biosurfactants and were identified as *Alcaligenes faecalis*, *Proteus mirabilis,* and *Providenia alcalifaciens* (Powthong and Suntornthiticharoen, 2018).

Sedlacek et al. (2020), isolated four psychrotrophic bacterial strains from soil samples collected from the northern part of the James Ross Island (Antarctica). In addition to the molecular biology techniques, MALDI-TOF MS was also utilized for the characterization of new strains. The combined results from different techniques showed that the strains belong to the genus *Hymenobacter*. The characterization of lipids and cellular fatty acids was also carried out. Based on the obtained results, two novel species were proposed and named *Hymenobacter terrestris*, and *Hymenobacter lapidiphilus*. In a similar study, Pantucek et al. (2018) isolated two strains of *Staphylococcus* from sandy soil in James Ross Island. The isolated strains were considered as novel species of *Staphylococcus* as the molecular techniques as well as MALDI-TOF MS categorized the strain separately from its closest *S. saprophyticus*. Thus, the novel strain was designated as *S. edaphicus* (Table 3). These studies show the usefulness of MALDI-TOF MS in ecological studies as it holds great potential in the identification and characterization of new species due to its high sensitivity and accuracy in protein mass fingerprinting.

Bacteria play an important role in the biogeochemical cycling of various elements; therefore, some researchers aim to explore the microbiota associated with different metal ores. One such study was carried out by Nosalova et al. (2021), to investigate the microbiota of gold ore. Cultivation analyses showed the occurrence of bacteria with colony-forming units (CFU) ranging from 2.18×10^5 to 3.16×10^5 per 1 g of dry ore material. The identification results of 473 bacterial isolates showed that 89% of the microbiota of gold ore mainly belongs to the four main genera: *Rhizobium, Microbacterium, Pseudomonas,* and *Acinetobacter*. A similar study was carried out by Malinicova et al. (2020) to explore the microbiota of gold ore in the Rozalia gold mine in Hodrusa-Hamre. It was found that the ore was comprised of mainly 18 different bacterial genera including *Acidovorax, Acinetobacter, Aerococcus, Rhizobium, Staphylococcus, Pseudomonas, Microbacterium,* and others.

In another study, a more challenging task was undertaken with MALDI-TOF MS as it was used as a dereplication tool to discriminate bacterial isolates. Four hundred bacterial strains including both pigmented and non-pigmented isolates obtained from seawater of the Norwegian Trondheimsfjord and neighboring coastal areas were used (Stafsnes et al., 2013). The dendrogram produced by MALDI-TOF MS identified bacteria belonging to a variety of taxa, which was in agreement with the pigment profile. Thus, the pigmentation could be linked to species-specific characteristics. Thus, it was demonstrated that MALDI-TOF MS could be used as a dereplication tool before pigment profiling to avoid massive redundant analysis. The study showed the application of MALDI-TOF MS as a rapid and cost-effective tool for characterizing a large number of environmental strains to reduce superfluous work typically done using culture-based techniques.

5.1.2. Environmental monitoring

The monitoring of different environments, foods, and surfaces is important to investigate microbial composition and diversity. It is done sometimes to monitor the spread of pathogenic bacteria or sometimes to track the source of bacterial contamination. MALDI-TOF MS has shown its potential to be utilized for continuous monitoring of such environments and provide useful information about the composition and biodiversity of bacteria. The technique has also been used for the bacterial analysis of natural waters, wastewater, and drinking water for environmental monitoring purposes. For example, a study was carried out recently to analyze bacterial constituents of tap and mineral water; the two commonly used sources of drinking water. A total of 11 tap water samples were collected from different locations and 10 mineral water samples from different brands were obtained (Sala-Comorera et al., 2020). The bacterial diversity obtained from two different drinking water types was different as bacteria belonging to *Alphaproteobacteria* were most common in tap water, while *Gammaproteobacteria* were frequently isolated from mineral water. In addition, the season in which the water was bottled also affected the bacterial diversity and presence, as some of the bacteria were identified only in one season. The research showed that the MALDI-TOF MS is a suitable technique for monitoring water quality routinely in the water industry and can be used to assess the bacterial diversity of water intended for human consumption. Laukova et al. (2019), reported the presence of *Enterococci faecium* ($n = 23$) using MALDI-TOF MS in cows' dung water (Table 3). Out of 23 strains, 56.5% were identified with the score ranging from 2.300 to 3.00 which means highly probable species-level identification. While the remaining 43.5% of the strains were identified with the score ranging from 2.000 to 2.299 which means highly probable genus-level identification and probable species-level identification.

Biogenic amines (BAs) are naturally occurring organic compounds, which can be toxic to human health at certain concentrations. Some bacteria produce decarboxylase enzyme, which causes decarboxylation of amino acids resulting in the formation of BAs. Since such microorganisms are commonly found in milk and dairy products, the wastewater from these industries can be a vector for the transportation of such bacteria in the environment. For this purpose, the wastewater from the dairy industry was collected for the bacterial analysis of water samples. From a total of 6 wastewater samples collected, 86 bacteria positive for decarboxylase activity were isolated and identified using MALDI-TOF MS. Results showed the identified bacteria belonged to different species of genera *Acinetobacter, Aeromonas, Enterobacter, Klebsiella, Kocuria, Lactococcus, Microbacterium, Pseudomonas,* and *Staphylococcus*.

The technique of MALDI-TOF MS was also used for the monitoring of groundwater being used as drinking water in the US (Jancova et al., 2020). The high-throughput method helped to identify a variety of opportunistic pathogens in groundwater samples namely, *Aeromonas hydrophila, Bacillus cereus, Pseudomonas aeruginosa,* and *Stenotrophomonas maltophilia,* which showed the risk from consumption of contaminated groundwater to human health. In a similar study, MALDI-TOF MS was also used for real-time study of different microbiomes and their variations in response to the anthropogenic activities (Suzuki et al., 2018). Thus, the groundwater samples were collected from rural areas where agricultural activities and oil, and gas exploration activities were common. The results of MALDI-TOF MS showed the presence of mainly denitrifying and heterotrophic bacteria belonging to the proteobacteria phylum.

Suen et al. (2019) used MALDI-TOF MS to evaluate the methods for sampling of *S. aureus* and other *Staphylococcus* species from indoor

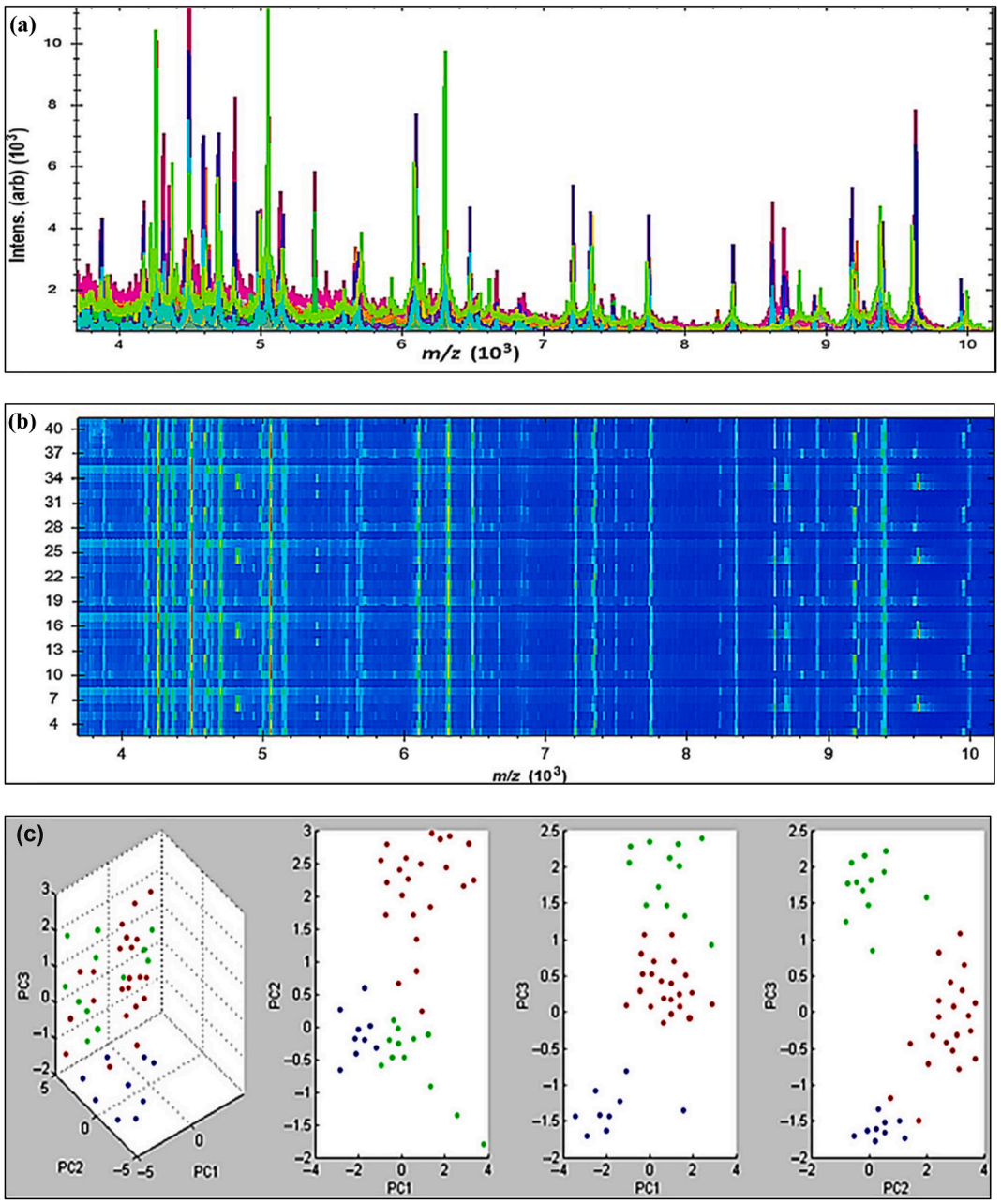


Fig. 7. (a) MALDI-TOF MS spectral profiles of 43 *Aeromonas* strains isolated from chicken meat and drinking water samples; (b) The gel profile for the comparison of different mass spectra; (c) PCA analysis showing classification of *A. hydrophila* (red), *A. caviae* (green), and *A. sobria* (blue) (Contributions from PC1 45%, PC2 17% and PC3 9%) (Adapted from Elbehiry et al., 2019b). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

surfaces. In total, 16 different *Staphylococcus* species were identified and 2 of the total 66 samples were positive for *S. aureus*. It was found that the pre-moistened eSwabs are 10 times more sensitive than dip slides, pre-moistened viscose, and cotton swabs. Another study evaluated the hygienic conditions of hand washing and hand drying facilities in public washrooms of various high to low-class communities. The samples were taken from a total of 55 public washrooms, from which 52 bacterial species were identified using MALDI-TOF MS. 97% of the pathogenic *Staphylococcus* species were also found to be multi-drug resistant organisms (MDRO). It was concluded that the public washrooms do not provide hygienic hand washing and hand drying facilities and they can act as a reservoir for transmission of various pathogenic bacteria. In a similar study, Ade et al. (2017) used MALDI-TOF MS to reveal the presence of rarely described and underestimated species of bacteria on the surfaces of magazines found in waiting areas of the hospital. The samples were taken from 15 magazines from 5 waiting rooms of a hospital. From the isolated bacteria, 60% of the bacteria were skin flora. However, some pathogenic bacteria were also identified such as *S. aureus, E. faecalis, A. viridans,* and *Aspergillus* sp. In addition, the bacteria like *A. iwolfii* and *M. osloensis, which* represent oropharyngeal flora, and fecal bacteria like *B. stercoris* were also isolated. Interestingly, there were some species not been described previously in the hospital environment were also identified which include *Paracoccus yeei* or *Kytococcus sedentarius*.

The contamination of air with pathogenic microorganisms is one of the rising concerns for public health and safety. To prevent and control air pollution, rapid, cost-effective, and high-throughput techniques for bacterial identification are required. For this purpose, Elbehiry et al.

(2019a) used MALDI-TOF MS for rapid identification of various air pollutants (Table 3). From the 400 air samples collected at various sites (hospital, university, and poultry slaughterhouses), 119 out of 129 isolates (92.5%) were identified using MALDI Biotyper. Out of these 119, 93 (72.10%) were gram-positive and 36 (27.90%) were gram-negative bacterial isolates. In general, the most common genera were *Staphylococcus* (n = 43, 33.33%), *Escherichia* (n = 16, 12.40%), *Enterococcus* (n = 15, 11.63%) and *Bacillus,* (n = 15, 11.63%). Overall, it was shown that the air is contaminated with various environmental microorganisms in the region and the technique of MALDI-TOF MS is effective for monitoring such air pollutants.

Another important research was conducted to investigate the source of psychrotrophic bacterial contamination in poultry food products at a large plant in Belgium (Samapundo et al., 2019). Both the air and surface samples were collected across the food processing area and food products (chicken legs and wings) before and after the expiry date were microbiologically analyzed. It was found that food contact surfaces such as cutting blades, leg hooks, Ertalon and polyurethane conveyor belts, working tables, and the hands of the operators could contaminate the food products mainly with *Carnobacterium maltaromaticum*. Other bacteria identified on expired wings and legs included *Carnobacterium divergens, Brocothrix thermosphacta, Lactobacillus curvatus,* and *Lactobacillus brevis*. Thus, the results showed that the cleaning and disinfection of the food processing area are inadequate. Moreover, polluted air can also be the source of food contamination with psychrotrophic bacteria. These studies demonstrate the potential of MALDI-TOF MS for use in monitoring water, groundwater, air, and surfaces.

5.1.3. Environment and Health

Several studies used the MALDI-TOF MS technique for rapid and fast identification and characterization of human pathogens in the environmental matrices. This was done to rapidly detect the pathogens in the environment, or characterize the pathogens on genus, species, and subspecies levels. For example, the identification of *Aeromonas*, a human pathogen, has posed certain challenges due to the phenotypic similarity between the species. Therefore, Elbehiry et al. (2019b), used MALDI-TOF MS to identify and differentiate *Aeromonas* spp. A total of 150 strains isolated from chicken meat and water were used. Results showed that MALDI-TOF MS identified all the strains with a high score of >2.000. The high sensitivity of MALDI-TOF MS also allowed researchers to identify four discriminating peaks for three *Aeromonas* species (Fig. 7a) (Elbehiry et al., 2019b). Moreover, principal component analysis (PCA) of the protein profiles successfully separated *A. hydrophila, A. caviae* and *A. sobria* into different groups (Fig. 7b).

Bukhloderia pseudomallei are a causative agent of endemic disease in Thailand and Australia. The contaminated water has been the source of infection from *Bukhloderia pseudomallei.* Therefore, Niyompanich et al. (2014) used MALDI-TOF MS for categorization of *B. pseudomallei* based on its source i.e., clinical, or environmental and to identify the source-specific biomarkers for the strains. A total of 11 strains from clinical (n = 6) and environmental (n = 5) sources were tested. Results showed that MALDI-TOF MS was able to correctly categorize 6 out of 11 strains based on their sources. By employing ClinProTools, 6 biomarker ions were identified for environmental strains, while 4 were identified for clinical strains.

Bacillus anthracis: classified as Tier 1 bioterrorism agent and has been used as a biological weapon in World Wars; is the causative agent for anthrax (a haemorrhagic and lethal disease in humans, wildlife, and domestic animals). Some studies have shown the presence of non-pathogenic *B. anthracis* in soil. Thus, the study was carried out to isolate and identify *B. anthracis* in soil samples collected in the state of Rio de Janeiro, Brazil (Salgado et al., 2020). In addition to the phenotypic and genotypic approaches, MALDI-TOF MS was also used for the identification of isolates. Results showed the presence of non-pathogenic *B. anthracis* (n = 1) in clay loam soil, *B. anthracis*-like strain (n = 1) in loamy sand soil, and 10 *Bacillus* sp. from loamy sand soil. This study was the first one of its kind that reports surveillance of *B. anthracis* in soil samples in Brazil. Another research was formulated to identify the occurrence and diversity of pathogenic bacteria in soil samples collected from cattle, sheep, and goat camps (Chitura et al., 2019). The identification done by MALDI-TOF showed the presence of *B. cereus, S. aureus, Listeria monocytogenes,* and *E. coli*. The frequency and distribution of each bacterium were different in different types of samples with *B. cereus* was happened to be the most commonly found bacteria in all soil types, while *E. coli* was mainly present in cattle camps and was absent in sheep and goat camps.

Ahmed et al. (2020), conducted a study in which the dust from laying hen farm was characterized for its microbial load and presence of antibiotic-resistant bacteria (ESBL producers, MRSA) (Table 3). The results showed that dust in Egyptian laying hen houses contains high concentrations of microorganisms and endotoxins, which might impair the health of birds and farmers when inhaled. Using MALDI-TOF MS, 100 ESBL suspected isolates were identified (*Escherichia coli*, n = 64; *Enterobacter cloacae*, n = 20; and *Klebsiella pneumoniae,* n = 16).

Some researchers have attempted to estimate the health risk of workers working in different environments that can be harmful to humans. For example, the wastewater treatment plant workers' exposure to airborne pathogenic bacteria was evaluated by Lu et al. (2020). It was noted that the identification of bacteria using MALDI-TOF MS to species level in personal samples was necessary for the risk assessment, and measurement of the microbial composition made the source tracking possible. The identified bacterial pathogens include *S. aureus, Y. enterocolitica, N. meningitidis, P. rettgeri, Enterobacter cloacae, P. aeruginosa, K. oxytoca,* and *E. coli* from aeration tank, grid house, and personal exposure samples. In another research carried out to evaluate the risk of greenhouse workers (Madsen et al., 2021), it was found that the exposure levels, as well as the microbial composition, were associated significantly with a grouped work task and season with high exposures during tasks in close contact with mature and old plants and in the autumn. The organisms identified using MALDI-TOF MS were *Halomonas elongata, Stenotrophomonas maltophilia, Podosphaera fusca, and Wallemia* spp. and others. Bacteria belonging to genera *Ralstonia,* and *Cladosporium* were most common. Hence, these studies showed the ability of MALDI-TOF MS to identify pathogenic bacteria from environmental matrice like water, soil, and air.

5.2. Application in environmental biotechnology and bioremediation

Bioremediation involves the use of indigenous or deliberately introduced microorganisms for the degradation of environmental pollutants. Recently, several studies have utilized MALDI-TOF MS for the isolation and identification of pollutant degrading microorganisms from different contaminated sites. Lara et al. (2021) reported the isolation and identification of 25 bacterial strains from 1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene (DDT) contaminated site using MALDI-TOF MS. The identified strains include *B. cereus, B. pumilus, B. marisflavi, Lysinibacillus fusiformis, S. maltophilia, P. nitroreducens* among others. As per the MALDI-TOF MS score, 21 of the organisms were identified till species level, while the remaining 4 were identified till genus level only. Similarly, another research used MALDI-TOF MS to identify a halotolerant strain as *Shewanella haliotis.* The identified bacteria were found to be capable of tolerating bisphenol A (BPA) concentration up to 150 mg/L and could biotransform 75 mg/L in 10 h in a liquid culture medium.

Hydrocarbons are also a class of environmental pollutants, and the isolation and characterization of new microorganisms with the ability to biodegrade these pollutants are important for effective biodegradation. In research conducted by Silva et al. (2019), 44 bacteria were isolated from compost and were identified by MALDI-TOF MS and 16S rRNA gene sequencing technique. MALDI-TOF MS identified 36 bacteria at the genus level (*Acinetobacter, Pseudomonas, Stenotrophomonas,* and *Gordonia*) or species level (*Klebsiella pneumoniae* and *Bacillus shackletonii*).

However, six isolates could not be identified by MALDI-TOF MS which were then identified through molecular techniques as *Aquamicrobium* spp., *Chryseobacterium* spp., *Gordonia paraffinivorans, Gordonia cholesterolivorans,* and *Gordonia sihwensis.* Silva-Jimenez et al. (2018) isolated the diversity of bacteria from surface water, seawater, and marine sediments from the coast of Rosarito Port, B.C., Mexico (Table 3). The study employed MALDI-Biotyper (Bruker Daltonics) as a powerful analytical tool to identify a total of 52 bacteria that were capable of growing in 25 mg/L pyrene as a sole carbon and energy source. Thus, the technique was able to identify hydrocarbon-degrading bacteria belonging to various phylogenetic groups i.e., *Actinobacteria, Firmicutes* and *Proteobacteria*.

Avanzi et al. (2016), employed MALDI-TOF MS to characterize the bacterial strains which showed resistance to copper contamination. A total of sixteen strains were identified as *Bacillus* (n = 4), *Enterobacter* (n = 3), *Burkholderia* (n = 1), and *Klebsiella* (n = 1). For the four strains that were not identified by MALDI biotyper, the 16S rDNA technique was able to identify those strains as *Pseudomonas* sp. (n = 2), *Cupriavidus* sp. (n = 1), and *Citrobacter* sp. (n = 1). Similarly, 27 strains were isolated from oil-contaminated soil for identification by MALDI-TOF and comparison with16 rDNA method (Rabodonirina et al., 2019). Out of those 27 strains, 8 strains with high capability to biodegrade polyaromatic hydrocarbons (PAHs) and use them as carbon and energy source were selected. By MALDI-TOF MS and 16 rDNA sequencing techniques, two strains were identified as *B. simplex,* and *B. pumilus,* and another two as *P. stutzeri*. One out of 8 tested strains were identified up to the genus level only i.e., *Bacillus* by MALDI-TOF, which was later identified as *B. cereus* by 16 rDNA technique. There were three strains not identified by MALDI-TOF that were later identified as *B. pumilus* (n = 2) and *P. stutzeri* by the 16 rDNA technique (Rabodonirina et al., 2019). Failure to obtain identification through MALDI-TOF could be due to the unknown spectrum attributed to new species or due to insufficient quality of the spectrum (Rabodonirina et al., 2019).

A study concluded by Lovecka et al. (2015), collected isolates from contaminated soils from the Czech Republic polluted sites, where they had the ability to degrade DDT (dichlorodiphenyltrichloroethane), HCB (hexachlorobenzene), and lindane. The degradation performed by microorganisms was examined through the amplification of the genes linA and bphA1 that are involved in pesticide degradation. The identification of seven selected isolates was done by MALDI-TOF MS, which identified them as *Aeromonas* sp., *Rhodococcus* sp., *Stenotrophomonas* sp., and three *Bacillus* sp., but one strain was not identified. The identification results of MALDI-TOF were complying with the 16S rRNA sequencing technique, where the latter technique was also able to identify the unidentified isolate as *Lysinibacillus fusiformis*.

So far, there is only one study, which shows the application of MALDI-TOF MS in identifying biomineralizing bacteria from soil. In a study conducted by Bibi et al. (2018), 18 strains were isolated from soil adapted to harsh conditions (high temperature, and hydrocarbon-polluted soil). The strains, which were found to be positive for urease activity (ureolytic bacteria), were then identified and differentiated using MALDI-TOF MS. The ureolytic bacteria were identified as *B. cereus, B. subtilis,* and *B. licheniformis.* Results of identification confirmed through molecular methods for 6 strains showed 100% matching which demonstrated the use of the MALDI-TOF MS technique for rapid identification of biomineralizing strains from the soil. In conclusion, all these examples from the literature showed that the MALDI-TOF MS technique holds potential for the identification of different bacteria that are adapted to harsh and contaminated environments.

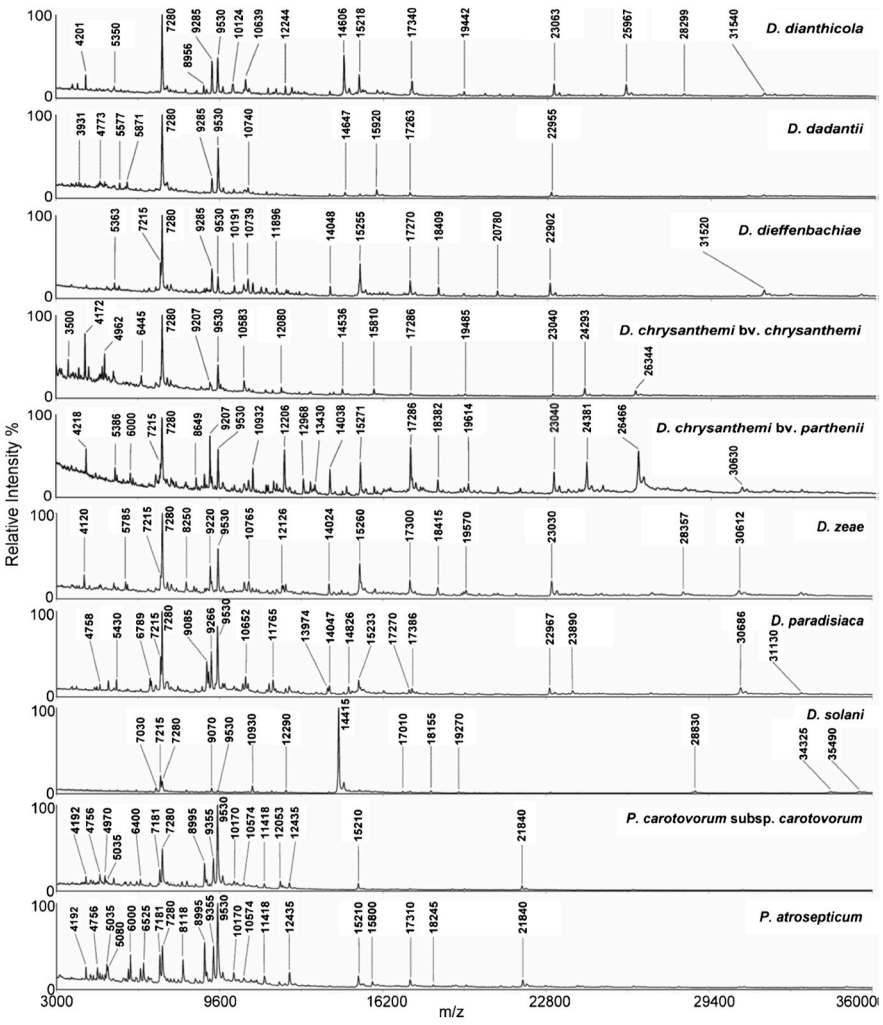
5.3. Application in plant sciences

5.3.1. Agricultural sciences

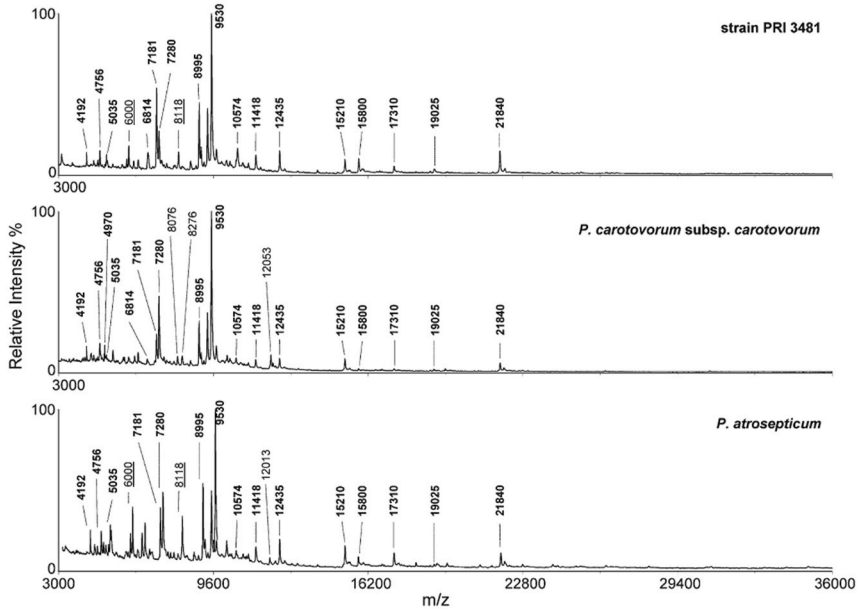
Various researchers have employed MALDI-TOF MS for the identification of plant growth-promoting (PGP) bacteria and pathogenic fungi growth inhibitors to protect the crops and increase the crop yield. Since Garlic (Allium sativum L.) has gained great economic importance in Brazil, the endophytic bacteria present in the garlic roots could be considered as a cost-efficient alternative for cost reduction (Junior et al., 2020). Therefore, researchers investigated the isolation and evaluation of the potential growth promoters present in plants and plant pathogenic fungi growth inhibitors by utilizing endophytic bacteria from garlic roots and bacteria from the Agricultural Microbiology Culture Collection at the Federal University of Lavras. MALDI-TOF MS was used to identify 48 endophytic bacteria based on the protein profile of each isolate. Among these 48 isolates, 4 were chosen based on their capability of fixing nitrogen, producing auxin as well as solubilizing phosphate. The improved growth and physiological attributes of garlic due to its inoculation with *Enterobacter cloacae* and *Burkholderia cepacian* indicated its feasible application as plant growth promoters for commercial cultivation. Another study was conducted by Muthuri et al. (2012) to isolate and identify endophytic bacteria in bananas and investigate their potential to be developed as biofertilizers. In this study, bacteria from bananas were isolated via five different isolation media after collecting the banana from 2 banana cultivars in 5 different regions. Profiling of the microorganisms was analyzed through MALDI-TOF MS. A total of 43 isolates demonstrated PGP activities such as nitrogen fixation, phosphate solubilization, and siderophores production. *Pseudomonas, Enterobacter, Ewingella, Bacillus, Rahnella, Serratia, Yokenella, Enterobacter, Yersinia, Raoultella, and Klebsiella* species were among the identified isolates.

Furthermore, plant growth promoters can be extracted from rhizobacteria in which plant growth-promoting rhizobacteria (PGPR) – mediated bioremediation can be used to detoxify heavy metals present in the soil as well as promote the growth of plants under the stress of heavy metals. Nowadays, agricultural lands are suffering from cadmium phytotoxicity causing a poor yield. Therefore, using PGPR is a convenient strategy for its detoxification. Pramanik et al. (2017), used MALDI-TOF MS to identify K5 strain as *Klebsiella pneumoniae* and It was found that this strain has various PGP traits namely, the production of IAA (3413 μg/ml), solubilization of phosphate (80.25 ppm), ACC deaminase activity (40 ng alpha-ketobutyrate/mg protein/h), ability to fix N_2 (1.84 μg N_2 fixed/h) and several other traits. Furthermore, it was found that among all the reported cadmium resistant PGPR, this strain has the highest cadmium resistance value of 4000 μg/ml. In another research, Costerousse et al., (2017) proposed an easy approach to isolate, identify and characterize several zine-solubilizing bacteria using MALDI-TOF MS. The novel approach helped to isolate and identify bacteria such as *Curtobacterium* (n = 1), *Plantibacter* (n = 2), *Pseudomonas* (n = 3), *Stenotrophomonas* (n = 1), and *Streptomyces* (n = 1). Similarly, other researches have shown the potential of MALDI-TOF MS to identify salt-tolerant PGPR for their applications in agriculture and plant growth (Sarkar et al., 2018; Macedo-Raygoza et al., 2019)

Furthermore, insect pest is a serious problem that leads to significant economic losses in agricultural production. Karut et al. (2020) conducted a study to investigate the bacteria-insect interactions as well as the defense mechanism of whitefly. Hence, this study determined the bacterial flora of *Bemisia tabaci* and their entomopathogenic potential to control the pest biologically. Among the collected sesame and melon plants samples, 9 bacterial strains were identified by MALDI-TOF MS, morphological, and molecular identification techniques up to the species level. Five bacterial strains of the sesame plant samples were identified as *Microbacterium* sp., *Sphingomonas* sp., *Methylobacterium* sp., *Serratia marcescens,* and *Bacillus* sp., while 4 melon bacterial strains were identified as *Staphylococcus hominis, Acinetobacter Iwoffii, Staphylococcus warned,* and *Bacillus cereus*. The biological efficiency towards *B. tabaci* was determined by feeding the whitefly with sucrose and water as insect food (control). A yield of 72% against the whitefly was achieved by the entomopathogenic *S. marcescens* bacterial isolate, however, this was not the case with the other strains which had <25% efficiency against whitefly. Therefore, further studies are required to be carried out on the



(a)



(b)

Fig. 8. (a) Protein profiles of the identified *Dickeya* and *Proteobacterium* species; (b) Assignment of unidentified isolate through protein profile comparison (Salplachta et al., 2015).

entomopathogenic bacterial pathology in pest insects.

5.3.2. Phytoremediation

There are mainly three research studies in which MALDI-TOF MS was used for the identification of bacteria that can help in promoting plant growth with simultaneous removal of pollutants (phytoremediation) from the environment (Mitra et al., 2018; Jong et al., 2015; Prouzova et al., 2012) (Table 3). Phytoremediation and rhizoremediation are promising technologies to remediate soil contaminants. Various plant-derived chemicals stimulate microorganisms in the biodegradation process and serve as inducers for the bioremediation process Prouzova et al. (2012). Mitra et al. (2018), screened out bacterial strains that are resistant to cadmium present in contaminated rice rhizosphere as well as evaluated its influence on the rice seedlings' growth under cadmium stress. Screening based on different PGP traits and resistance toward various heavy metals including lead, cadmium, and arsenic with a minimum inhibitory concentration of 2500, 3500, and 1050, respectively, was carried out only for isolate S2 among other isolates. Phenotypic characterization, MALDI-TOF MS analysis, and 16S rDNA sequence homology were used to identify S2 strain as *Enterobacter* sp. Moreover, it was noted that 95% removal efficiency was achieved by this strain. Various morphological and biochemical characteristics of the growth of the rice seedlings inoculated with this strain were enhanced compared to the grown un-inoculated rice seedlings in a cadmium-stressed environment. Furthermore, this strain decreased the cadmium accumulation in seedlings, reduced the ethylene stress, exhibited alleviation of cadmium-induced oxidative stress, as well as conferred plants with cadmium tolerance.

The selenium-rich plants can be used to clean up selenium-contaminated waters or soils. Sura-de Jong et al. (2015), targeted the endophytic bacterial plants that hyperaccumulate selenium up to 0.5%–1% dry weight. The identification and characterization were carried out for the cultivable endophytes from hyperaccumulators *Stanleya pinnata* and *Astragalus bisulcatus*. MALDI-TOF MS Biotyper analysis and 16S rRNA gene sequencing for the 66 bacterial morphotypes showed that it includes various strains such as *Variovorax, Bacillus, Staphylococcus, Pseudomonas, Paenibacillus, Advenella, Pantoea,* and *Arthrobacter*. All isolates reduced nitrite, reduced selenite to red elemental Se, as well as produced siderophores, while most of the isolates were highly resistant to up to 200 mM selenite and selenate. Among all the isolates, 7 isolates were found to have plant growth-promoting properties both when co-cultivated with either *Brassica juncea* (Brassicaceae) or *Medicago sativa* (Fabaceae) as well as in pure culture. Results showed that selenium accumulation on plants was not affected. It can be concluded that the hyperaccumulator endophytes have high selenium resistance characteristics, as well as the ability to produce elemental selenium and plant growth-promoting properties.

5.3.3. Plant Pathology

It is important to identify plant bacterial pathogens to control their impact on plant growth and health. Various studies have demonstrated the use of MALDI-TOF MS technology to identify plant bacterial pathogens (Fonseca-Guerra et al., 2021; Oberhaensli et al., 2017; Choi et al., 2017) (Table 3). Kajiwara (2016), utilized MALDI-TOF MS to detect the plant pathogens responsible for the infection caused in rice seedlings namely *Burkholderia glumae, Burkholderia gladioli pv. gladioli,* and *Erwinia chrysanthemi pv. Zeae* without culturing in an artificial medium. MALDI-TOF MS analysis showed that the extracts of infected rice seedlings had peaks originating from bacteria in which the spectral peaks had significantly high scores regardless of minor spectral differences. MALDI-TOF MS direct analysis was not affected by the spectral peaks originating from host plant tissues. Salplachta et al. (2015), classified *Dickeya* and *Pectobacterium* species by using various analytical techniques including MALDI-TOF MS, capillary zone electrophoresis (CZE), and capillary isoelectric focusing (CIEF) due to the insufficiency of the cultural, serological, and molecular methods that are usually used for bacterial classification. *Dickeya* and *Pectobacterium* species are responsible for blackleg and soft rot diseases on various plants since they are representing a crucial broad-host-range group of phytopathogens. In this study, 43 strains of various *Dickeya* and *Pectobacterium* species were selected, including *Pectobacterium carotovorum, Dickeya dianthicola, Dickeya dadantii, Dickeya dieffenbachiae, Dickeya chrysanthemi, Dickeya zeae, Dickeya solani, and Dickeya paradisiaca, Pectobacterium atrosepticum*. Some selected protein profiles of identified bacteria are given in Fig. 8a. Moreover, among all selected bacteria, one bacterium was not classified by traditional microbiological methods. However, through comparison with the protein profile of the unidentified bacterium in MALDI-TOF MS, the isolate was assigned to *Pectobacterium carotovorum* (Fig. 8b).

Wang et al. (2012), used MALDI-TOF MS analysis to differentiate between the 2 closely related plant pathogenic bacteria *Acidovorax oryzae* and *Acidovorax citrulli* which is normally difficult to differentiate between them by using the traditional methods such as ELISA detection tests, and carbon source utilization profile. This study characterized and compared the results of 10 strains of each plant pathogenic bacteria by MALDI-TOF MS, traditional bacteriological methods, and FTIR spectroscopy. Results showed that MALDI-TOF MS and FTIR spectroscopy showed significant differences in the spectra between both bacteria. MALDI-TOF MS results showed that 22 peaks were specific to *Acidovorax oryzae* and 18 peaks were specific to *Acidovorax citrulli*.

Similarly, Sawada et al. (2019), used the MALDI-TOF MS technique to identify and characterize the bacteria that grew on turnips and caused the rot disease in Japan. After isolation of bacteria that formed colonies with beige to cream color and were determined as pathogenic, the isolates were identified as *Pseudomonas grimontii*. The identification has been done based on MALDI-TOF MS analysis, biochemical and physiological characterization, as well as multilocus sequence analysis using concatenated sequences of 16S rRNA, rpoD, gyrB, and rpoB genes. This study indicated that *P. grimontii* has strong virulence, and can cause rot disease; hence, it is crucial to record it as a novel pathogen that needs vigilance since it threatens the plants. Such examples from the literature demonstrate the wide potential of the MALDI-TOF MS technique in the plant and agriculture sciences, its ability to identify plant growth promoters, plant pathogens, and other plant-associated bacteria.

5.4. Applications in food microbiology

Microorganisms are associated with food in a variety of ways. They may be used in the manufacturing of food products, they may cause food spoilage, and they may also be transmitted by food. Food can be considered as a medium for the growth of bacteria. Food can become a source of various disease outbreaks; therefore, food microbiology has gained a lot of attention. MALDI-TOF MS technique can play its role in the field of food microbiology through identifying and differentiating different foodborne pathogenic bacteria, lactic acid bacteria, and other fermentative bacteria (Table 3). The diversity of *Brochothrix thermosphacta*; one of the major bacteria involved in the spoilage of meat and seafood; was characterized by MALDI-TOF MS and other techniques (Illikoud et al., 2019). A total of 161 strains of *B. thermosphacta* were differentiated using the MALDI technique which showed significant intra-species diversity, while the classifications did not relate to the origin of strains. Pomastowski et al. (2019) investigated the bacteria present in different kinds of honey of variable geographical and botanical origins. Through MALDI-TOF MS, a variety of bacteria such as *B. subtilis, B. megaterium, B. pumilus, B. cereus, B. mycoides, P. alvie, L. boronitolerans*, and *M. luteus* were accurately identified up to the species level. Using PCA, the physicochemical characteristics of kinds of honey were also correlated to the bacterial composition and the results showed that the pH, electrical conductivity, and total acidity were the significant factors affecting the bacterial composition of honey.

In the brewing industry, there is a need for constant quality checks of its products for any microbial contamination that can spoil the beers. For

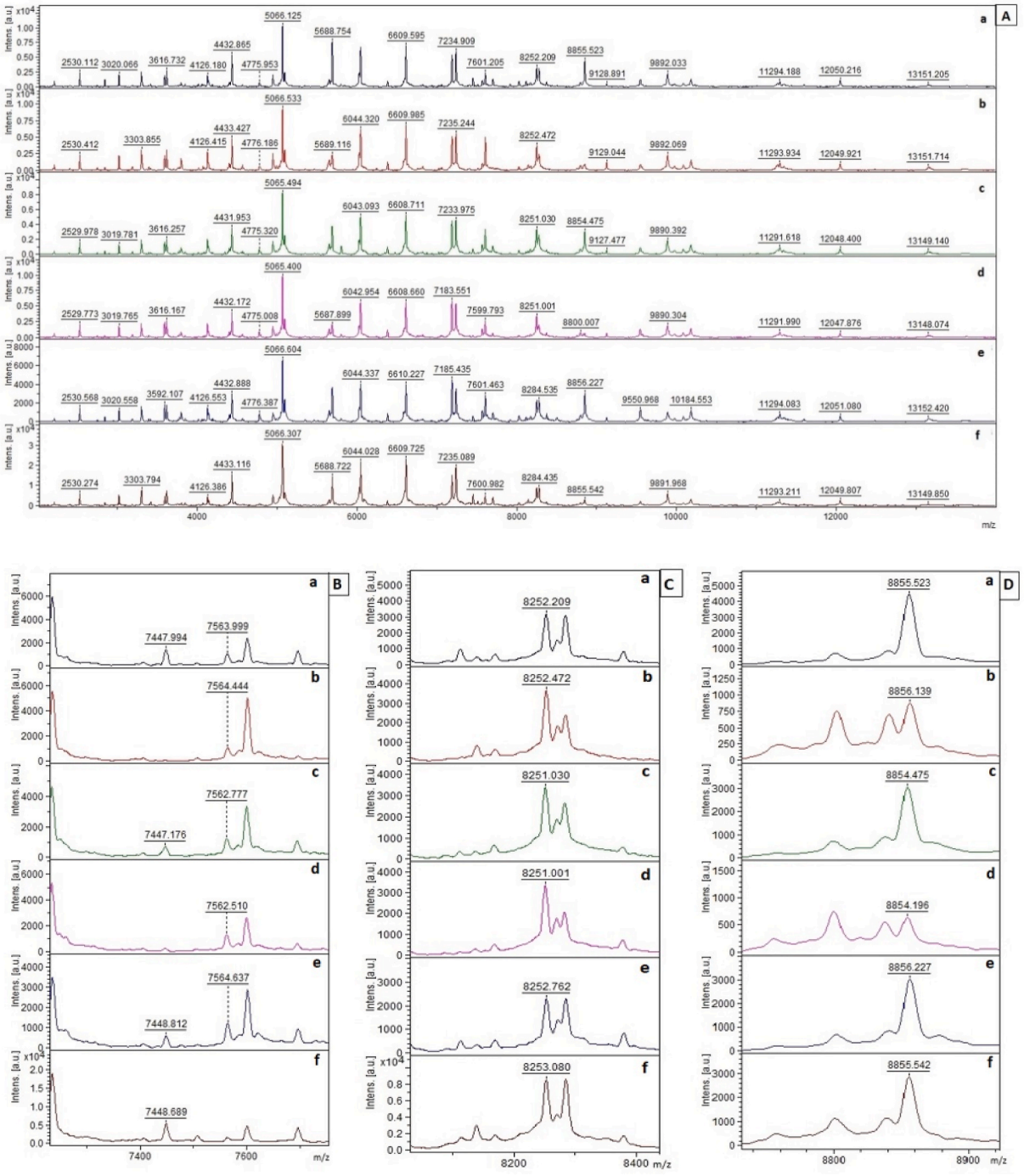


Fig. 9. MALDI-TOF MS spectra profiles obtained for different *P. fragi* strains from offshore and onshore seawater samples (A) Main spectra (2000–13000 m/z); Magnified regions (B) 7400–7700 m/z (C) 8100–8400 m/z (D) 8700–8900 m/z, showing the differences between the strains (Ashfaq et al., 2019).

this purpose, Turvey et al. (2016) investigated the potential of MALDI-TOF MS for its ability to identify beer spoilage bacteria. For this purpose, the protein profiles of the three most found bacteria in beer (*L. lindneri, L. brevis,* and *P. damnosus*) were included in the Biotyper reference library. It was noted that the technique can accurately identify and distinguish each of the three bacterial species from one another and over 5600 other bacteria present in the reference library. The study showed that the technique is a promising candidate for biological quality control testing within the brewing industry as a more rapid, high-throughput, and cost-effective technology that can be tailored for the detection of brewery-specific spoilage organisms from the local environment. Sapugahawatte et al. (2020) used MALDI-TOF as a high-throughput technique for the identification of pathogens (MDROs) from fish and pork acquired from the wet markets across Hong Kong. 730 food animal samples were analyzed, and it was found that there is a high prevalence of Extended spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E) (ranging from 0.5% to 52.4%) and carbapenemase-producing Enterobacteriaceae (CPE) (0%–9.9%) in various food animal samples.

The quality of raw milk is important in determining the quality of manufactured dairy products. In a study carried out by Zhang et al. (2020), the numbers, and types of psychrotrophs in raw milk with and without refrigerated enrichment (5 days at 7 °C) were compared to understand the effect of extended refrigeration on the changes in raw

milk microbial ecology. In fresh raw milk, 119 isolates were identified that were belonging to 12 different genera and 23 species. The highest relative abundance of 74.79% of the total was of *Pseudomonas,* while the remaining 25.21% belong to 11 genera (*Acinetobacter, Candida, Chryseobacterium, Carnobacterium, Enterobacter, Enterococcus, Raoultella, Lactococcus, Hafnia, Filobasidium,* and *Serratia*). After refrigerating milk for 5 days, the chilled milk was also analyzed, and 127 isolates were identified that were belonging to 9 genera and 20 species. The results showed that the diversity of psychrotrophic bacteria is less in chilled stored milk as compared to fresh raw milk. Among the identified strains from chilled milk, 93.68% of the total was represented by *Pseudomonas*, while the remaining 6.32% was contributed by 8 genera (*Acinetobacter, Bacillus, Buttiauxella, Carnobacterium, Lactococcus, Hafnia, Rahnella,* and *Serratia*). Many of the isolated bacteria have demonstrated their potential to spoil the milk and therefore, it is important to investigate the biodiversity of both fresh raw and stored milk to ensure the safety of milk and dairy products.

MALDI-TOF MS was used as a tool for the dereplication of a large number of bacteria to a smaller set for easy downstream characterization (Gantzias et al., 2020). A total of 88 non-starter lactic acid bacteria (NSLAB) were isolated from 18 samples of four different artisanal cheeses produced in Greece. The technique was able to identify 95.5% of the total isolates and the identified bacteria include *Lactobacillus brevis, Lactobacillus plantarum, Lactococcus lactis, Leuconostoc mesenteroides Lactobacillus paracasei, Lactobacillus rhamnosus, Pediococcus pentosaceus,* and *Enterococcus faecium*.

Probiotics are used as supplements to improve health, positively affect health, and treat various diseases. A research was conducted to isolate new probiotic bacteria from different food items that are already known to possess probiotic features (Dogan and Ozpinar, 2017). For this purpose, a total of 144 strains were isolated from 130 food samples including 60 raw milk samples, 40 cheese, 20 kefir, and 10 boza samples. The strains were identified as *Enterococcus faecium* (n = 127), *Lactobacillus plantarum* (n = 7), *Lactobacillus para-plantarum* (n = 5) and *Lactobacillus brevis* (n = 5). When these isolated strains were characterized further for their probiotic features, only 6 (4.1%) of the bacteria demonstrated their potential to be used as probiotics. Among these 6 strains, there were 3 *Lactobacillus brevis* strains isolated from boza, and the remaining 3 were *Lactobacillus plantarum* species isolated from kefir. From milk and cheese samples, there were no probiotics isolated.

5.5. Applications in water treatment and disinfection technology

MALDI-TOF MS technology has also found its application in water treatment technology. It has been used as a fast and cost-effective tool to identify bacteria at different treatment stages or to investigate the efficiency of treatment for bacterial removal. For example, Ashfaq et al. (2019) identified and differentiated various antiscalant-degrading microorganisms from seawater of the Arabian Gulf using MALDI-TOF MS (Table 3). The seawater samples collected from various sites including from the inlet of the desalination plant contained various bacteria capable of using antiscalants (chemicals used to control scaling in the desalination industry) as a carbon and energy source. The bacteria were identified as *Halomonas aquamarina, Halomonas elongata, P. stutzeri* and *P. fragi* among others. Fig. 9 shows the differentiation of protein profiles for various P. fragi strains isolated from seawater. Similarly, in a study conducted by Loff et al. (2014), MALDI – TOF MS was utilized for the routine detection of multiple pathogens, namely E. *coli, Listeria,* and *Salmonella* present in wastewater. In this study, samples were collected from the wastewater treatment plant for a period of 8 weeks from the influent point, effluent point, and aeration tank. 47 isolates of the presumptive *E. coli* strains, *Listeria* spp. and *Salmonella* spp. were obtained and identified by MALDI – TOF MS. Results revealed that MALDI – TOF MS identified 20 out of the 21 presumptive *E. coli* isolates (95%) at both genus and species levels. However, none of the presumptive *Listeria* (n = 17) and *Salmonella* (n = 9) isolates were correctly identified, which

Table 4
Comparison between MALDI-TOF MS and other established techniques for identification of different environmental bacteria - few examples from the literature.

#	Bacterial strains studied	Sources	Summary of Findings	Reference
1	*Alteromonas* sp., *Bacillus* sp., *Colwellia* sp., *Erythrobacter* sp., *Marinobacter* sp., *Marinococcus* sp., *Pseudoalteromonas* sp., *Pseudomonas* sp., *Roseobacter* sp., *Sphingomonas* sp. and *Vibrio* sp.	Marine sponges	The bacterial identification by MALDI-TOF was identical to those determined by analysis of partial rDNA sequences. Although there are considerable intra- and inter-species classification problems, which 16S rDNA sequencing fails to resolve, MALDI-TOF MS comparatively achieved higher-level resolution and was able to discriminate organisms.	Dieckmann et al., (2004)
2	*Acinetobacter, Burkholder, Citrobacter, Cupriavidus, Klebisella, Pseudomonas, Stenotrophomonas* and others	Water and Soil	In comparison with 16S rDNA, 72 were correctly identified by MALDI-TOF MS, while 16 were differently identified. 4 strains, which belonged to *Pseudomonas, Cupriavidus,* and *Citrobacter* genera were not identified by MALDI-TOF MS	Avanzi et al. (2017)
3	*Enterococcus* sp.,	Recreational waters	100% of the isolates were identified by MALDI-TOF MS. In comparison with the 16S rRNA method, 74% were identified correctly. While API method only identified 11% correctly.	Christ et al. (2017)
4	*Pseudomonas* sp.	Fish	At species-level identification, 60% of the identification results were not in accordance with the two methods (MALDI-TOF MS and 16s rDNA sequence)	Kacaniova et al. (2019)
5	*Raoultella* sp,	75 isolates (source not mentioned), 3 reference strains	97.4% strains were identified by MALDI-TOF MS, whereas ID32E and VITECK2 Compact only identified 81.4%	Sekowska et al. (2018)

(*continued on next page*)

Table 4 (*continued*)

#	Bacterial strains studied	Sources	Summary of Findings	Reference
			and 93.3% strains, respectively.	
6	*Legionella* sp.	Water (Hospitals, Hotels, Private apartments, Fitness & Wellness centers, Bathhouses)	Comparison with culture and MIP gene sequencing technique, MALDI-TOF MS identified 45% strains up to species level.	Pascale et al. (2020)
7	*Lactobacillus*-related genera, *Weissella*, and *Leuconostoc* sp.	Kimchi	All the isolates were identified up to species level by MALDI-TOF MS, while the 16S rRNA technique identified most of the strains up to genus level.	Kim et al. (2021)

could be attributed to the unavailability of reference mass spectra in the database used for these strains. The MALDI-TOF identification of *Listeria* and *Salmonella* spp. has already been reported in other studies (Jadhav et al., 2014; Sparbier et al., 2012).

In another research, MALDI-TOF MS was used to assess the bacterial community in a drinking water treatment plant (Sala-Comorera et al., 2017) (Table 3). Water samples were collected from influent (raw surface water) and effluent from different treatment stages during a period of 1 year. The technique was useful in detecting changes in the bacterial diversity at different stages i.e., raw water, and after sand filtration, ultrafiltration, reverse osmosis, and chlorination. Thus, the technique is useful to detect abnormal or recurrent strains in drinking water treatment plants using the limited laboratory resources available in the water company. Moreover, the knowledge related to the bacterial community at different stages will help to develop and improve the water treatment strategies for the production of clean and healthy drinking water.

To combat the spread of infection, various disinfection techniques are used to clean the surfaces and kill the pathogenic bacteria that may be present in the environment and surfaces. The research was conducted to investigate the application of a medical diode laser for the disinfection of small microbiologically contaminated spots on degraded collagenous materials (Rybitwa et al., 2020) (Table 3). The surfaces were microbiologically examined, and microorganisms were identified using MALDI-TOF MS. The bacteria identified include *Acinetobacter lwoffii, Bacillus amyloliquefaciens, Bacillus endophyticus, Bacillus megaterium, Bacillus oceanisediminis, Bacillus oleronius, Pantoea agglomerans, Psychrobacillus psychrodurans, Staphylococcus haemolyticus,* and *Staphylococcus warner.* Another study was conducted to investigate the effect of enhanced cleaning on the presence of MDROs. MALDI-TOF MS was employed for screening 1042 MDROs before and after cleaning. The results showed that the cleaning reduced the rate of MDRO detection to 13.32% from 31.77%.

6. Development and validation of MALDI-TOF MS for environmental bacteria

To demonstrate the suitability of the MALDI-TOF MS technique for the identification of bacteria, several studies were undertaken to compare the results of bacterial identification with other developed methods (Table 4). Through bibliometric analysis, it was noted that earlier studies focused more on evaluating the potential of MALDI-TOF MS for the identification, differentiation, and dereplication of environmental bacteria. Krader and Emerson et al., (2004) evaluated the use of MALDI-TOF MS for the identification of different archea and environmentally important bacteria such as sulfate-reducing bacteria, thermophiles, and others. The researchers used 8 archaea (10 genera, 20 spp.) and 42 bacteria (25 genera, 37 spp.) and found that the MALDI-TOF MS provides accurate, reproducible, and reliable results in the form of mass spectral profiles. The technique was able to distinguish halophilic bacteria (such as *Halococcus dombrowski, Halobacterium salinarium,* and *Haloarcula marismortui*).

Similarly, in another research, Dieckmann et al., (2004) evaluated the dereplication potential of the MALDI-TOF MS technique. The researchers utilized Intact-Cell MALDI-TOF (ICM) mass spectrometry to perform proteometric clustering of 456 isolates. The cluster analysis was performed for the obtained protein mass spectral profiles which categorized 456 isolates into 11 major groups; representing species of *Pseudoalteromonas* (n = 297), *Pseudomonas* (n = 56), *Colwellia* (n = 31), *Erythrobacter* (n = 19), *Alteromonas* (n = 15), *Marinobacter* (n = 14), *Vibrio* (n = 10), *Marinococcus* (n = 6), *Bacillus* (n = 3), *Roseobacter* (n = 3), and *Sphingomonas* (n = 2) which was also verified by 16S rDNA analysis. Siegrist et al. (2007) obtained E. coli isolates from various animal, human sources, and wastewater treatment plants for the purpose to characterize the strains based on their sources to help in identifying the source of contamination for surface waters. Results suggested that in comparison to rep – PCR, MALDI – TOF MS is more effective in grouping *E. coli* environmental isolates based on their respective sources although it has lower repeatability levels. Moreover, source-specific biomarkers were identified through computer-aided analysis of MALDI – TOF MS spectra, which can speed up the identification of the fecal inputs impacting the quality of water.

Kim et al. (2021) evaluated the culture-dependent MALDI-TOF MS technique for analysis of microbial community during fermentation of kimchi. The results were compared with the culture in-dependent 16S rRNA technique. Both methods identified *Lactobacillus*-related genera, *Leuconostoc*, and *Weissella* as predominant microorganisms. Although the 16S rRNA technique could also identify non-lactic acid bacteria, it identified most of the important microorganisms until the genus level. In contrast, MALDI-TOF MS identified bacteria up to species level showing its high accuracy and precision (Table 4). *Pseudomonas* species are present in freshwater ecosystems and can be pathogenic to humans and other aquatic organisms. Thus, research was conducted to develop effective and reliable methods for the identification of *Pseudomonas* species from water. For this purpose, *Pseudomonas* spp. (n = 161) were isolated from freshwater and were identified using MALDI-TOF MS (Kacaniova et al., 2019). The identified strains were *P. extremorientalis, P. fluorescens, P. fragi P. proteolytica, P. veronii,* and others. However, 9 out of 15 strains used for comparison with the 16s rDNA gene sequencing method were not in accordance with the two methods. Overall, results showed the high discrimination power of MALDI-TOF MS for *Pseudomonas* identification from environmental samples.

Raoultella is a commonly found gram-negative bacterium mostly isolated from water, soil, and plants. The identification of species of *Raoultella* has gained significant interest due to its involvement in a number of clinical cases. Its identification is challenging due to its close phylogenetic relationship with *Klebsiella* sp. Hence, research was conducted to evaluate three different methods for the identification of *Raoultella* spp. and their differentiation from *Kelbsiella* spp (Sekowska et al., 2018). The three methods used were manual test ID32E (bioMerieux), automatic test VITEK2 Compact (bioMerieux), and MALDI-TOF MS. It was found that manual test ID32E identified only 81.4%, while the VITECK2 Compact test identified 93.3% of the strains. Comparatively, MALDI-TOF MS gave the best results as 97.4% of the strains were identified and correctly differentiated from *Klebsiella* sp. Among the identified strains were *R. planticola, R. terrigena,* and *R. ornithinolytica.*

Similarly, MALDI-TOF MS was also evaluated for the identification and characterization of *Enterococcus* spp., a coliform bacterium from marine recreational waters (Christ et al., 2017). The results of

Table 5
Comparison of 3 studies focusing on identification of *Burkholderia cepacia* complex by MALDI-TOF MS (Furlan et al., 2019; Vicenzi et al., 2016; Fehlberg et al., 2013).

#	Source	Strains	Identification by			Concordance (%)	
			recA gene sequencing	MALDI-TOF MS			
				Genus level	Species level	Genus level	Species level
01	*Clinical samples*	*B. cenocepacia*	70	70	68	100	97.15
02		*B. multivorans*	16	16	13	100	81.25
03		*B. contaminans*	12	12	0	100	0
04		*B. vietnamiensis*	22	22	21	100	95.45
05		*B. cepacia*	07	07	03	100	42.85
06	*Environmental soil samples*	*B. lata*	02	00	00	0	0
07		*B. cenocepacia*	15	07	00	46.67	0
08		*B. cepacia*	03	03	03	100	100
09		*B. ambifaria*	02	02	00	100	0

MALDI-TOF MS were compared with the identification carried out by bioMerieux's API (R) 20 Strep and biochemical methods. From the isolated strains of *Enterococcus* (n = 127), the biochemical test identified 92% (n = 117), while MALDI-TOF identified 100% of the strains. When the results were compared with the reference method i.e., 16s rRNA gene sequencing technique, the MALDI identified 74% correctly, while API identified only 11% (Table 4). Thus, it was concluded that MALDI-TOF MS is a more reliable method for the identification of *Enterococcus* spp. from environmental matrices.

In an attempt to understand the diversity of the microbial community in copper contaminated sites to explore the impact of the metal on bacterial diversity, MALDI-TOF MS was evaluated and compared with the 16s rDNA method (Avanzi et al., 2017). It was revealed that the MALDI-TOF MS could be considered as a reliable and fast method for the identification of copper-resistant bacteria from water samples as 84% of the identification results were in agreement with the reference method. The copper-resistant bacteria identified include *Acinteobacter, Bacillus, Burkholderia, Citrobacter, Ralstonia, Stenotrophomonas,* and *Pseudomonas* among others. Moreover, further differentiation of *P. aeruginosa* strains based on their protein profiles was also made possible using MALDI-TOF MS showing the capability and sensitivity of this technique for differentiation of closely related strains.

MALDI-TOF MS has also been evaluated to produce and differentiate protein profiles for biological samples, which have been treated differently since this technique provides a reliable, cost-efficient, and fast approach to investigate the protein profiles. Ribeiro et al. (2020), conducted a comparison study between the Xanthomonas campestris pv. *Campestris* (Xcc) grown in vivo (recovered from a plant) and in a culture medium. Explants at various somatic embryogenesis (SE) stages were included after analyzing the plant samples, in addition to the *Brassica oleracea* and *Arabidopsis thaliana* leaves which were inoculated with Xcc at different times. From the obtained results, the unequivocal differentiation of bacteria and highly divergent plant samples from embryogenic stages can be observed in which the proteomic analysis by 2-DE was used for clustering. The obtained findings demonstrated the significance of MALDI-TOF MS profiling in which it can be used for the selection and prioritization of samples to be analyzed before using more complicated techniques such as transcriptomics and proteomics. Moreover, it was clearly shown that during the interactions between plant and pathogen, when subtle differences were obtained, MALDI-TOF MS profiling could mainly contribute to the assessment of experimental variability. This is relevant since reproducibility is a hard problem to address when dealing with complicated experimental conditions such as plant-pathogen interactions. The usage of MALDI-TOF MS profiling is to help to minimize the experimental variability that is not related to the analyzed conditions. Moreover, MALDI-TOF MS can be used for tracing certain microorganisms in a plant environment and their interaction in the presence of another microbiota.

To enhance the application of MALDI-TOF MS in the dereplication of bacteria, Dumolin et al. (2019) evaluated a software called SPeDE for high-throughput dereplication and accurate classification of microorganisms using MALDI-TOF MS spectra data. The data set of 5228 spectra comprising 167 bacterial strains of 132 genera across six phyla was used for dereplication. The results showed that the SPeDE is a valuable tool to achieve dereplication and classification of a large amount of MALDI-TOF MS data set. It could identify redundant spectra and obtain distinguishing features between the spectra with high efficiency, thus allowing rapid classification of a large set of data. In a similar study, Ghyselinck et al. (2011) used 249 unidentified bacterial isolates from the rhizosphere of potato plants to evaluate the identification and dereplication potential of MALDI-TOF MS and rep-PCR. Profile inspection and cluster analysis illustrate that for 82% of the isolates (n = 204), the taxonomic resolution was comparable for both MALDI-TOF MS and rep-PCR, while one of both techniques had a higher taxonomic resolution for 18% of the isolates (n = 45). Higher reproducibility was achieved by MALDI-TOF MS than rep-PCR, in which it was more promising in terms of high-throughput analysis, time, automation, and cost-efficiency. Hence, there are various attempts undertaken to compare and validate the MALDI-TOF MS technique for the identification of environmental bacteria and the results have been encouraging. With the further development and enhancement in the reference databases, this technique can prove to be useful for the identification of environmental bacteria at a wider scale.

7. Challenges and progress in identification of environmental bacteria

Bacteria present in different environments may possess different phenotypic traits and therefore, their protein – spectral-based identification by MALDI-TOF MS can be affected. However, there is a very limited number of studies that have demonstrated the effect of bacterial living environment with the identification accuracy. Niestepski et al. (2019) investigated the identification of *Bacteroides fragilis* group (BFG) from different environmental samples i.e. human and rat feces, hospital wastewater, untreated and treated sewage from a wastewater treatment plant (WWTP) by MALDI-TOF MS. The results showed that the accuracy of identification was reduced from 100% for human and rat feces to 40% (treated wastewater) and 20% (hospital wastewater and untreated wastewater). This could be attributed to the similarity of strains from humans and rate feces to the clinical isolates, for which the database of MALDI-TOF MS is optimized. On the other hand, the strains from water and wastewater samples were identified with less accuracy due to the different character of those samples, higher bacterial diversity, and different phenotypic traits of the bacteria present in these environments. Therefore, the MALDI-TOF MS database requires optimization for BFG strains isolated from environmental samples.

Another study was conducted to investigate the MALDI-TOF MS accuracy of identification for *Burkholderia cepacia* complex, which represents 21 different species widely found in the environment. The species belonging to *B. cepacia* complex have shown their significance in

Table 6
Examples of some environmental bacteria reported to be missing from commercial databases or wrongly identified due to an inadequate spectral database (Brauge et al., 2021; Pinar-Mendez et al., 2021; Abdel Samad et al., 2020; Ha et al., 2019; Kim et al., 2019; Timperio et al., 2017; Emami et al., 2016; Christie-Oleza et al., 2013).

#	Genus	Species	Missing/Wrong identification
1	*Aquabacterium*	-	Not available in Bruker BioTyper Library (DB-7311)
2	*Arthrobacter*	*soli*	Not available in Bruker BioTyper Library (V7.0.0)
3	*Bacillus*	*oceanisediminis*	Not available in Bruker BioTyper Library (V7.0.0)
		stratosphericus	
		drentensi	Not reliable identification (score <1.7)
		nealsoni	Not reliable identification (score <1.7) or misidentified as *B. circulans* (score 1.71)
		safensis	Not reliable identification (score <1.7) or misidentified as *B. pumilus* (score 1.72)
		aquimaris	Not reliable identification (score <1.7) or misidentified as *B. vietnamensis* (score 1.86)
		firmus	Not reliable identification (score <1.7)
		vallismortis	Not reliable identification (score <1.7)
		pumilus	Misidentified as *B. altitudinis* (score 1.88)
		thuringiensis	Differentiation between *B. cereus* and *B. thuringiensis*
4	*Bosea*	-	Not available in Bruker BioTyper Library (DB-7311)
5	*Brachybacterium*	*paraconglomeratum*	Not reliable identification (score <1.7)
6	*Cloacibacterium*	-	Not available in Bruker BioTyper Library (DB-7311)
7	*Desemzia*	*incerta*	Not available in Bruker BioTyper Library (V7.0.0)
8	*Domibacillus*	-	Not available in Bruker BioTyper
9	*Dyadobacter*	-	Library (DB-7311)
10	*Exiguobacterium*	*aestuarii*	Not available in Bruker BioTyper
		acetylicum	Library (V7.0.0)
		profundum	
		oxidotolerans	Not available in Bruker BioTyper Library
11	*Halobacillus*	*sp.*	Not reliable identification (score <1.7)
12	*Macrococcus*	*sp.*	Not reliable identification (score <1.7)
13	*Microbacterium*	*esteraromaticum*	Not available in Bruker BioTyper Library (V7.0.0)
		esteraromaticum	Not reliable identification (score <1.7)
14	*Planococcus*	*sp.*	Not available in Bruker BioTyper Library (V7.0.0)
15	*Psychrobacter*	*celer*	Not available in Bruker BioTyper
		faecalis	Library (V7.0.0)
16	*Pseudomonas*	*costantinii*	Not available in Bruker BioTyper Library
17	*Pseudoalteromonas*	*sp.*	Lack of spectral database
18	*Phyllobacterium*	-	Not available in Bruker BioTyper
19	*Polaromonas*	-	Library (DB-7311)
20	*Porphyrobacter*	-	
21	*Ruegeria*	*sp.*	Lack of spectral database
22	*Stenotrophomonas*	*rhizophila*	Not reliable identification (score <1.7)
23	*Salinicoccus*	*sp.*	Not available in Bruker BioTyper
24	*Shewanella*	*indica*	Library (V7.0.0)
25	*Staphylococcus*	*lentus*	Wrong identification at species level.
26	*Virgibacillus*	*marismortui*	Not available in Bruker Biotyper
27	*Virgibacillus*	*salarius*	Library (MBT-8468)
28	*Vagococcus*	*sp.*	Not reliable identification (score <1.7)

bioremediation, and biotechnology as well as in clinical settings. But, their accuracy of identification has been affected due to the very high similarity between the species in this group. Combining 3 different studies for clinical samples (Vicenzi et al., 2016; Fehlberg et al., 2013) and environmental soil samples (Furlan et al., 2019) related to the application of MALDI-TOF MS for identification of *B. cepacia* complex, it can be concluded that the accuracy of identification for environmental strains is significantly lower as compared to strains from clinical samples (Table 5). In comparison to the *recA* gene method, a gold standard method for identification of *B. cepacia*, none of the environmental strains belonging to B. *lata, B. cenocepacia,* and *B. ambifaria* was correctly identified up to species level. Moreover, it was found that most of the strains of *B. cenocepaica* and *B. lata* were wrongly identified as *Ochrobactrum anthropic* by MALDI-TOF MS. Thus, it can be concluded that the source of environmental bacteria can affect their identification by MALDI-TOF MS and therefore, the databases need further optimization as per the environmental isolates.

Another study aiming to utilize MALDI-TOF MS for differentiation of *V. alginolyticus* from other *Vibrio* species also showed significant differences in the protein profiles of strains isolated from environmental (water, and sediment) and clinical sources (Hazen et al., 2009). In contrast, Sulaiman et al. (2018) analyzed a total of 50 *Staphylococcus* strains from clinical, environmental, food, cosmetic products, a medical device, and American Type Culture Collection (ATCC). The results showed that the Vitek MS system could reliably identify all of the strains up to species level demonstrating the potential of this technique for the identification of *Staphylococcus* strains irrespective of the sources.

Another challenge faced by MALDI-TOF MS in environmental microbiology is the lack of spectra in the commercial databases for many environmental bacteria. Table 6 shows the list of different environmental bacteria either reported to be missing from commercial databases or misidentified by MALDI-TOF MS due to the inadequate robustness of the reference spectra. Several researchers have reported the inadequate database for the identification of *Virgibacillus* sp. such as *V. marismortui*, and *V. salarius* (Rim et al., 2020; Konate et al., 2021; Brauge et al., 2021). While the identification of *V. halodenitrificans* has been reported by Brauge et al. (2021) isolated from seafood and seawater. In addition, there are some other bacteria for which misidentification by MALDI-TOF MS has been reported and therefore, the database of such bacteria requires further optimization. These include *Bacillus drentensi, Bacillus safensis, Brachybacterium paraconglomeratum, Vagococcus* sp. to name few (Table 6).

One of the most adopted approaches for the inadequate database is the construction of an in-house database (Table 7). The most recent and excellent example of an in-house database is the construction of a drinking water library by Pinar-Mendez et al. (2021). The researchers developed this library by targeting various bacteria present in water intended for human consumption. The drinking water library contained 319 different bacterial strains belonging to 96 different bacterial generals, 44 of which were not present in the Bruker database. The use of the drinking water library significantly improved the identification of environmental bacteria from water by 76.2% from 54.8%. For *Pseudoalteromonas* sp. present in seawater, the inadequacy of the MALDI-TOF database has been reported previously (Emami et al., 2012). To resolve this problem, researchers developed an in-house database for the accurate identification of *Pseudoalteromonas* species. When tested using 10 environmental isolates of *Pseudoalteromonas* from water, the identification was achieved up to the genus level. It was concluded that the isolates belong to new species and therefore, further update in the database is required for accurate identification up to species level. Similarly, Fergusson et al. (2020) has also reported the development of an in-house library for the identification of *Burkholderia, Caballeronia*, and *Paraburkholderia*. When tested with the unknown group of strains, 39 out of 49 isolates were correctly identified to genus level. Moreover, it was noted that the strains with 3 or more representatives in the reference library were more correctly assigned as compared to the

Table 7
Summary of some studies reporting the creation of in-house databases for better identification of environmental bacteria.

#	Bacteria	No. of strains	Strain source	Spectra per MSP	Culture conditions	Results	Reference
1	*Alcanivorax, Aquabacterium, Chromobacterium* and many others	44	30 from Spanish Type Culture Collection, 14 treated water and non-treated water	24	Water Plate Count Agar at 22 °C	The identification performance of drinking water library was enhanced from 54.8% to 76.2%.	Pinar-Mendez et al. (2021)
2	*Burkholderia, Caballeronia,* and *Paraburkholderia*	95	Rhizosphere	24	LB agar for 72 h at 30 °C	39/49 genus level identification, 24/39 up to species level identification	Fergusson et al. (2020)
3	*Legionella* sp.	216	Clinical, Water, Soil, and Cooling towers	50	Buffered charcoal yeast extract agar at 36 °C for 48–72 h.	235/237 tested strains were reliably identified up to species level	Gaia et al. (2011)
4	*Pectinatus, Megasphaera,* and *Selenomonas,*	8	–		-	Species level identification increased from 47% to 85%	Vavrova et al. (2018)
5	*Pediococus*	141	130 isolated from kimchi or jeotgal, 11 reference strains from American Type Culture Collection (ATCC, USA), the Korean Agricultural Culture Collection (KACC, Korea) and the Korean Collection for Type Cultures (KCTC, Korea).	12	n/a	84% strains identified up to species level, 46% identified up to genus level	Cho et al. (2017)
6	*Pseudoalteromonas* sp. (29 different species)	31	DSMZ (German Collection of Microorganisms and Cell Cultures or JCM (Japan collection of Microorganisms).	30	Marine agar as per manufacturer instructions	13 strains from seawater and seaweed were tested and all were identified up to genus level	Emami et al. (2016)
7	*Ralstonia pseudosolanacearum, Ralstonia solanacearum, Ralstonia syzygii*	6	Plants	>20	Nutrient agar incubated at 28 °C for 48 h	All 53 tested strains were identified up to species level with no false positive identifications	van de Bilt et al. (2018)
8	*Rhizobiaceae (Ensifer, Rhizobium, Shinella)*	56	Water, Sludge, Soil, Plant roots/ nodules	36	TY and YMA medium incubated at 28 °C for 24 and 48 h	100% tested strains identified up to species level	Ferreira et al. (2011)
9	*Vibrionaceae family (more than 20 species)*	997	Collected from various organizations such as Governmental Institute of Public Health of Lower Saxony (NLGA), Centre for Environment, Fisheries & Aquaculture Science (CEFAS), National Institute for Public Health and the Environment (RIVM) and others	32	Marine broth medium, containing 50% or 75% seawater (1.6% or 2.4% sodium chloride) incubated at 37 °C or as per recommendation by DSMZ.	All tested strains identified up to species level. The identification score increased by more than 70%.	Erler et al. (2015)
10	*Vibrio (Harveyi clade)*	85	38 from DSMZ, the Spanish Type Culture Collection (CECT), and the Institut Pasteur Collection (CIP). 35 from a fish farm, 12 from French Research Institute for the Exploitation of the Sea (IFREMER)	18	Marine agar for 24 h at 37 °C/ 25 °C	All 47 strains were correctly identified to species level, 19 misidentified strains with commercial database were identified correctly with in-house database	Mougin et al. (2020)

strains with two or fewer representatives which emphasizes the importance of having multiple representatives for each species in the database library. Table 7 summarizes the in-house database developed for various environmental bacteria, and how it affected the results of identification.

Various approaches have been used to improve the identification accuracy of environmental bacteria other than using in-house databases. For example: to improve the accuracy of identification for *Ruegeria* sp., Christie-Oleza et al. (2013) used proteins identified by shotgun nanoLC-MS/MS and used them to propose 5 potential biomarkers which give rise to 10 m/z peak signals derived from the mono- and doubly protonated proteins. To investigate the application of these biomarkers, 30 seawater bacterial isolates were screened, out of which one bacterium was identified as *Reugeria* sp., which was also confirmed by the 16S RNA sequencing technique. Recently, the publicly available database of protein sequences (UniProt) was used to identify bacteria and this approach helped to identify 84.1% of the bacteria (n = 403) at the genus level (Cheng et al., 2018). The source code of the algorithm is available at https://github.com/dipcarbon/BacteriaMSLF.

There are also different open sources software such as BacteriaMS and BacteriaMS-Mixture introduced to enhance the accuracy of identification. Yang et al. (2017) tested the mass spectra of 1991 bacterial strains by adopting spectral similarity and novel bootstrapping procedure (BacteriaMS can be accessed at https://github.com/lmsac/BacteriaMS). In addition to the significant improvement in both genus and species level identification, the research group was also able to discriminate 40 strains of *Bacillus cereus* which were previously considered difficult to resolve by MALDI TOF MS.

The inability of MALDI-TOF MS to identify a mixture of bacteria was also recently tackled by introducing a new framework (BacteriaMS-Mixture, available at https://github.com/lmsac/BacteriaMS-mixture). The framework combined a synthetic mixture model based on a non-negative linear combination of candidate reference spectra and a statistical assessment by in silico-generated spectra through a jackknife resampling (Yang et al., 2018). To validate the new framework, a mixture of 97 bacterial samples, 8 cocultured bacterial samples (containing 2–6 bacteria in each mixed culture), and a reference database of 1081 strains were used. A sensitivity of more than 60% and an error rate of less than 5% were achieved for all types of mixed samples with more than 80% sensitivity for binary and ternary mixtures. Such a framework can help to resolve the issue of identifying mixed bacterial communities and can help in fostering MALDI-TOF usage in both clinical and environmental research.

From Table 7, it can be concluded that the creation of in-house databases has significantly helped researchers to optimize the processing of samples and obtain the desired results with high accuracy. However, this does not resolve the problem at the international level since these databases are not accessible to other researchers. To enhance the potential of this technique for the identification of environmental bacteria, efforts at an international level are needed to develop open-access databases through global collaboration. Initial efforts have been made by some researchers like the "FoodBIMS" database that comprises 26 species of food-borne pathogens (Mazzeo et al., 2006), and "SpectraBank" for 70 bacterial species (Bohme et al., 2012).

With the development of such open-access global databases, continuous efforts would be needed to keep such databases updated through the introduction of new mass spectral profiles into the database like done for GenBank. Moreover, the sample processing guidelines and analysis of spectral profiles (baseline subtraction, normalization, and others) need to be standardized to improve the spectral representation of the bacterial species. To enhance the robustness of the reference spectra, the number of spectra in the database should also be maximized. It is highly expected that with the availability of highly curated, open-access, and robust databases, the extensive application of MALDI-TOF MS in environmental microbiology, ecology, biotechnology, plant, and agricultural sciences can be achieved.

8. Conclusion

Numerous studies have validated the performance of MALDI-TOF for the identification of environmental bacteria through comparison with molecular techniques and have concluded that this technique is useful and applicable to environmental bacteria. With the further improvement in the database libraries of reference bacteria, its routine application in environmental studies should be encouraged due to its time saving and cost-effectiveness. The bibliometric analysis of the literature showed that the application of technology in environmental studies has gained significant attention during the past decade. The technique has been used with high accuracy and precision for the identification of various types of bacteria such as pollutant-degrading, biomineralizing, fermentative, foodborne/waterborne pathogens, coliforms, lactic acid, plant-associated and plant growth-promoting bacteria with environmental and biotechnological importance. Moreover, the technique has found its application in routine environmental monitoring which has helped the rapid identification of bacteria present in water, air, and different surfaces.

There are still untapped applications of MALDI-TOF MS technology that are not availed. These applications can include the use of the technique for the identification of proteins/enzymes produced by bacteria for their specific environmental function such as pollutant degradation, biomineralization, biosurfactants production, and others. With the further development in technology and bioinformatics tools, the technique can be used to explain the role of bacterial proteins behind the environmental function, thereby, identifying the responsible proteins/genes using such tools. Ultimately, there is a gap between the phylogenetic identity of bacteria and their potential environmental function/s. Further advancements and research should focus on bridging this gap, which will not only enhance the potential of this technology in environmental studies but will also ensure rapid progress in research through time and cost savings.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abdel Samad, R., Al Disi, Z., Ashfaq, M.Y., Wahib, S.M., Zouari, N., 2020. The use of principle component analysis and MALDI-TOF MS for the differentiation of mineral forming Virgibacillus and Bacillus species isolated from sabkhas. RSC Adv. 10, 14606–14616.

Abu-Dieyeh, M.H., Alduroobi, H.A., Al-Ghouti, M.A., 2019. Potential of mercury-tolerant bacteria for bio-uptake of mercury leached from discarded fluorescent lamps. J. Environ. Manag. 237, 217–227.

Ade, M., Burger, S., Cuntzmann, A., Exinger, J., Meunier, O., 2017. Magazines in waiting areas of hospital: a forgotten microbial reservoir? Ann. Biol. Clin. 75 (6), 673–681. https://doi.org/10.1684/abc.2017.1283, 1.

Ahmed, M.F.E., Ramadan, H., Seinige, D., Kehrenberg, C., Abd El-Wahab, A., Volkmann, N., Kemper, N., Schulz, J., 2020. Occurrence of extended-spectrum beta-lactamase-producing Enterobacteriaceae, microbial loads, and endotoxin levels in dust from laying hen houses in Egypt. BMC Vet. Res. 16 https://doi.org/10.1186/s12917-020-02510-4.

Alsayegh, S.Y., AlDisi, Z., Al-Ghouti, M.A., Zouari, N., 2021. Evaluation by MALDI-TOF MS and PCA of the diversity of biosurfactants and their producing bacteria, as adaption to weathered oil components. Biotechnology Reports 31, e00660.

Ashfaq, M.Y., Al-Ghouti, M.A., Qiblawey, H., Rodrigues, D.F., Hu, Y., Zouari, N., 2019. Isolation, identification and biodiversity of antiscalant degrading seawater bacteria using MALDI-TOF-MS and multivariate analysis. Sci. Total Environ. 656, 910–920. https://doi.org/10.1016/j.scitotenv.2018.11.477.

Ashfaq, M.Y., Al-Ghouti, M.A., Al Disi, Z.A., Zouari, N., 2020. Interaction of seawater microorganisms with scalants and antiscalants in reverse osmosis systems. Desalination 487, 114480.

Avanzi, I.R., Gracioso, L.H., Baltazar, M.D., Karolski, B., Perpetuo, E.A., do Nascimento, C.A., 2017. Rapid bacteria identification from environmental mining samples using MALDI-TOF MS analysis. Environ. Sci. Pollut. Res. Int. 24 (4), 3717–3726. https://doi.org/10.1007/s11356-016-8125-8.

Benagli, C., Demarta, A., Caminada, A., Ziegler, D., Petrini, O., Tonolla, M., 2012. A rapid MALDI-TOF MS identification database at genospecies level for clinical and environmental Aeromonas strains. PLoS One 7 (10), e48441. https://doi.org/10.1371/journal.pone.0048441.

Bibi, S., Oualha, M., Ashfaq, M.Y., Suleiman, M., Zouari, N., 2018. Isolation, differentiation and biodiversity of ureolytic bacteria of Qatari soil and their potential in microbially induced calcite precipitation (MICP) for soil stabilization. RSC Adv. 8, 5854–5863. https://doi.org/10.1039/C7RA12758H.

Biswas, S., Rolain, J.M., 2013. Use of MALDI-TOF mass spectrometry for identification of bacteria that are difficult to culture. J. Microbiol. Methods 92 (1), 14–24. https://doi.org/10.1016/j.mimet.2012.10.014.

Braga, R.M., Dourado, M.N., Araujo, W.L., 2016. Microbial interactions: ecology in a molecular perspective. Braz. J. Microbiol. 47 (Suppl. 1), 86–98.

Braga, P.A., Tata, A., dos Santos, V.G., Barreiro, J.R., Schwab, N.V., dos Santos, M.V., Eberlin, M.N., Ferreira, C.R., 2013. Bacterial identification: from the agar plate to the mass spectrometer. RSC Adv. 3, 994–1008.

Brauge, T., Trigueros, S., Briet, A., Debuiche, S., Leleu, G., Gassilloud, B., et al., 2021. MALDI-TOF Mass Spectrometry fingerprinting performance versus 16S rDNA sequencing to identify Bacterial Microflora from Seafood products and Sea water samples. Front. Mar. Sci. 8.

Bright, J.J., Claydon, M.A., Soufian, M., Gordon, D.B., 2002. Rapid typing of bacteria using matrix-assisted laser desorption ionisation time-of-flight mass spectrometry and pattern recognition software. Journal of Microbiology Methods 48 (2–3), 127–138.

Bohme, K., Fernandez-No, I.C., Barros-Velazquez, J., Gallardo, J.M., Canas, B., Calo-Mata, P., 2012. Spectra Bank: an open access tool for rapid Microbial identification by MALDI-TOF MS fingerprinting. Electrophoresis 33, 2138–2142. https://doi.org/10.1002/elps.201200074.

Carbonnelle, E., Mesquita, C., Bille, E., Day, N., Dauphin, B., Beretti, J.L., Ferroni, A., Gutmann, L., Nassif, X., 2011. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. Clin. Biochem. 44, 104–109.

Cheng, D., Qiao, L., Horvatovich, P., 2018. Toward spectral library-free matrix-assisted laser desorption/ionization time-of-flight mass spectrometry bacterial identification. J. Proteome Res. 17 (6), 2124–2130. https://doi.org/10.1021/acs.jproteome.8b00065.

Chitura, T., Ncube, I., Heerden, H., 2019. Contamination of the environment by pathogenic bacteria in a livestock farm in Limpopo province, South Africa. Appl. Ecol. Environ. Res. 17, 2943–2963. https://doi.org/10.15666/aeer/1702_29432963.

Cho, Y., Kim, E., Lee, Y., Han, S., Kim, C., Choo, D., et al., 2017. Rapid and accurate identification of species of the genus Pediococcus isolated from Korean fermented foods by matrix-assisted laser desorption/ionization time-of-flight MS with local database extension. Int. J. Syst. Evol. Microbiol. 67 (3), 744–752.

Choi, O., Kang, B., Cho, S.K., Park, J., Lee, Y., Kim, W., Marunga, J., Hwang, I., Kim, J., 2017. Identification of Pseudomonas syringae pv. syringae causing bacterial leaf

blight of Miscanthus sinensis. J. Plant Dis. Prot. 124, 97–100. https://doi.org/10.1007/s41348-016-0058-4.
Christ, A., Ramos, S.R., Cayo, R., Gales, A.C., Hachich, E.M., Sato, M., 2017. Characterization of Enterococcus species isolated from marine recreational waters by MALDI-TOF MS and Rapid ID API® 20 Strep system. Mar. Pollut. Bull. 118 (1–2), 376–381. https://doi.org/10.1016/j.marpolbul.2017.03.025.
Christie-Oleza, J.A., Pina-Villalonga, J.M., Guerin, P., Miotello, G., Bosch, R., Nogales, B., Armengaud, J., 2013. Shotgun nanoLC-MS/MS proteogenomics to document MALDI-TOF biomarkers for screening new members of the Ruegeria genus. Environ. Microbiol. 15 (1), 133–147. https://doi.org/10.1111/j.1462-2920.2012.02812.x.
Clark, A.E., Kaleta, E.J., Arora, A., Wolk, D.M., 2013. Matrix-assisted laser desorption ionization–time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. Clin. Microbiol. Rev. 26, 547–603.
Costello, M.J., Chaudhary, C., 2017. Marine biodiversity, biogeography, deep-sea gradients, and conservation. Curr. Biol. 27, 511–527.
Costerousse, B., Schönholzer-Mauclaire, L., Frossard, E., Thonar, C., 2017. Identification of heterotrophic zinc mobilization processes among bacterial strains isolated from wheat rhizosphere (Triticum aestivum L.). Appl. Environ. Microbiol. 84 (1), e01715–e01717. https://doi.org/10.1128/AEM.01715-17.
De Carolis, E., Vella, A., Vaccaro, L., Torelli, R., Spanu, T., Fiori, B., Posteraro, B., Sanguinetti, M., 2014. Application of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. The Journal of Infection in Developing Countries 8, 1081–1088.
Dekker, J.P., Branda, J.A., 2011. MALDI-TOF mass spectrometry in the clinical microbiology laboratory. Clin. Microbiol. Newsl. 33, 87–93.
Dieckmann, R., Malorny, B., 2011. Rapid screening of epidemiologically important *Salmonella enterica subsp. enterica* serovars by whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl. Environ. Microbiol. 77, 4136–4146.
Dieckmann, R., Graeber, I., Kaesler, I., Szewzyk, U., Dohren, H., 2005. Rapid screening and dereplication of bacterial isolates from marine sponges of the Sula Ridge by Intact-Cell-MALDI-TOF mass spectrometry (ICM-MS). Appl. Microbiol. Biotechnol. 67, 539–548. https://doi.org/10.1007/s00253-004-1812-2.
Dogan, M., Ozpinar, H., 2017. Investigation of probiotic features of bacteria isolated from some food products. Kafkas Univ Vet Fak Derg 23 (4), 555–562. https://doi.org/10.9775/kvfd.2016.17273.
Donohue, M.J., Best, J.M., Smallwood, A.W., Kostich, M., Rodgers, M., Shoemaker, J.A., 2007. Differentiation of Aeromonas isolated from drinking water distribution systems using matrix-assisted laser desorption/ionization-mass spectrometry. Anal. Chem. 79 (5), 1939–1946.
Dumolin, C., Aerts, M., Verheyde, B., Schellaert, S., Vandamme, T., Van der Jeugt, F., De Canck, E., Cnockaert, M., Wieme, A.D., Cleenwerck, I., Peiren, J., Dawyndt, P., Vandamme, P., Carlier, A., 2019. Introducing SPeDE: high-throughput dereplication and accurate determination of microbial diversity from matrix-assisted laser desorption-ionization time of flight mass spectrometry data. mSystems 4 (5). https://doi.org/10.1128/mSystems.00437-19 e00437-19.
Elbehiry, A., Al-Dubaib, M., Marzouk, E., Albejaidi, F.M., Radwan, M.A.A., Alzaben, F., Alharbi, A., 2019a. Proteomic analysis and molecular characterization of airborne bioaerosols in indoor and outdoor environment in Al-qassim region, Saudi arabia. J. Pure Appl. Microbiol. 13 (2), 1041–1052. https://doi.org/10.22207/JPAM.13.2.42.
Elbehiry, A., Marzouk, E., Abdeen, E., Al-Dubaib, M., Alsayeqh, A., Ibrahem, M., Hamada, M., Alenzi, A., Moussa, I., Hemeg, H.A., 2019b. Proteomic characterization and discrimination of Aeromonas species recovered from meat and water samples with a spotlight on the antimicrobial resistance of Aeromonas hydrophila. Microbiol. 8 (11), e782. https://doi.org/10.1002/mbo3.782.
Emami, K., Nelson, A., Hack, E., Zhang, J., Green, D., Caldwell, G., Mesbahi, E., 2016. MALDI-TOF Mass spectrometry discriminates known species and marine environmental isolates of Pseudoalteromonas. Front. Microbiol. 7 (104).
Emami, K., Askari, V., Ullrich, M., Mohinudeen, K., Anil, A.C., Khandeparker, L., Burgess, J.G., Mesbahi, E., 2012. Characterization of bacteria in ballast water using MALDI-TOF Mass spectrometry. PLoS One 7, e38515. https://doi.org/10.1371/journal.pone.0038515.
Erler, R., Wichels, A., Heinemeyer, E., Hauk, G., Hippelein, M., Reyes, N., Gerdts, G., 2015. VibrioBase: a MALDI-TOF MS database for fast identification of Vibrio spp. that are potentially pathogenic in humans. Syst. Appl. Microbiol. 38 (1), 16–25.
Fehlberg, L., Andrade, L., Assis, D., Pereira, R., Gales, A., Marques, E., 2013. Performance of MALDI-ToF MS for species identification of Burkholderia cepacia complex clinical isolates. Diagn. Microbiol. Infect. Dis. 77 (2), 126–128.
Fenselau, C., 2012. Rapid characterization of microorganisms by mass spectrometry—what can Be learned and how? J. Am. Soc. Mass Spectrom. 24, 1161–1166.
Fergusson, C., Coloma, J., Valentine, M., Haeckl, F., Linington, R., 2020. Custom matrix-assisted laser desorption ionization–time of flight mass spectrometric database for identification of environmental isolates of the genus Burkholderia and related genera. Appl. Environ. Microbiol. 86 (11).
Ferreira, L., Sánchez-Juanes, F., García-Fraile, P., Rivas, R., Mateos, P., Martínez-Molina, E., et al., 2011. MALDI-TOF mass spectrometry is a fast and reliable platform for identification and ecological studies of species from family rhizobiaceae. PLoS One 6 (5), e20223.
Fonseca-Guerra, I., Chiquillo, C., Padilla, M.J., Benavides-Rozo, M., 2021. First report of bacterial leaf spot on Chenopodium quinoa caused by Pseudomonas syringae in Colombia. J. Plant Dis. Prot. 128, 871–874. https://doi.org/10.1007/s41348-021-00435-0.
Fox, K., Fox, A., Rose, J., Walla, M., 2011. Speciation of coagulase negative staphylococci, isolated from indoor air, using SDS PAGE gel bands of expressed proteins followed by MALDI TOF MS and MALDI TOF-TOF MS-MS analysis of tryptic peptides. J. Microbiol. Methods 84 (2), 243–250. https://doi.org/10.1016/j.mimet.2010.12.007.
Furlan, J., Pitondo-Silva, A., Braz, V., Gallo, I., Stehling, E., 2019. Evaluation of different molecular and phenotypic methods for identification of environmental Burkholderia cepacia complex. World J. Microbiol. Biotechnol. 35 (3).
Gaia, V., Casati, S., Tonolla, M., 2011. Rapid identification of Legionella spp. by MALDI-TOF MS based protein mass fingerprinting. Syst. Appl. Microbiol. 34 (1), 40–44.
Gantzias, C., Lappa, I.K., Aerts, M., Georgalaki, M., Manolopoulou, E., Papadimitriou, K., De Brandt, E., Tsakalidou, E., Vandamme, P., 2020. MALDI-TOF MS profiling of non-starter lactic acid bacteria from artisanal cheeses of the Greek island of Naxos. Int. J. Food Microbiol. 323, 108586. https://doi.org/10.1016/j.ijfoodmicro.2020.108586.
Ghyselinck, J., Van Hoorde, K., Hoste, B., Heylen, K., De Vos, P., 2011. Evaluation of MALDI-TOF MS as a tool for high-throughput dereplication. J. Microbiol. Methods 86 (3), 327–336. https://doi.org/10.1016/j.mimet.2011.06.004.
Ha, M., Jo, H., Choi, E., Kim, Y., Kim, J., Cho, H., 2019. Reliable identification of Bacillus cereus group species using low mass biomarkers by MALDI-TOF MS. J. Microbiol. Biotechnol. 29 (6), 887–896.
Hanson, B., Zhou, Y., Bautista, E.J., Urch, B., Speck, M., Silverman, F., Muilenberg, M., Phipatanakul, W., Weinstock, G., Sodergren, E., Gold, D.R., Sordillo, J.E., 2016. Characterization of the bacterial and fungal microbiome in indoor dust and outdoor air samples: a pilot study. Environ. Sci. Process Impacts 18, 713–724.
Hazen, T., Martinez, R., Chen, Y., Lafon, P., Garrett, N., Parsons, M., et al., 2009. Rapid identification of Vibrio parahaemolyticus by whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl. Environ. Microbiol. 75 (21), 6745–6756.
Holland, R.D., Wilkes, J.G., Rafii, F., Sutherland, J.B., Persons, C.C., Voorhees, K.J., Lay Jr., J.O., 1996. Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom.: RCM (Rapid Commun. Mass Spectrom.) 10 (10), 1227–1232. https://doi.org/10.1002/(SICI)1097-0231(19960731)10:10<1227::AID-CM659>3.0.CO;2-6.
Hou, T.Y., Chiang-Ni, C., Teng, S.H., 2019. Current status of MALDI-TOF mass spectrometry in clinical microbiology. J. Food Drug Anal. 27 (2), 404–414. https://doi.org/10.1016/j.jfda.2019.01.001.
Illikoud, N., Rossero, A., Chauvet, R., Courcoux, P., Pilet, M.-F., Charrier, T., Jaffres, E., Zagorec, M., 2019. Genotypic and phenotypic characterization of the food spoilage bacterium Brochothrix thermosphacta. Food Microbiol. 81, 22–31. https://doi.org/10.1016/j.fm.2018.01.015. PMID: 30910085.
Jadhav, S., Sevior, D., Bhave, M., Palombo, E.A., 2014. Detection of Listeria monocytogenes rom selective enrichment broth using MALDI-TOF Mass Spectrometry. J. Proteome 97, 100–106.
Jancova, P., Pachlova, V., Cechov, E., Cedidlova, K., Sera, J., Pistekova, H., Bunka, F., Bunkova, L., 2020. Occurrence of biogenic amines producers in the wastewater of the dairy industry. Molecules 25 (21), 5143. https://doi.org/10.3390/molecules25215143.
Jang, K.S., Kim, Y.H., 2018. Rapid and robust MALDI-TOF MS techniques for microbial identification: a brief overview of their diverse applications. J. Microbiol. 56 (4), 209–216. https://doi.org/10.1007/s12275-018-7457-0.
Junior, P.C., Cardoso, F.P., Martins, A.D., Buttros, V.H.T., Pasqual, M., Dias, D.R., Schwan, R.F., Doria, J., 2020. Endophytic bacteria of garlic roots promote growth of micropropagated meristems. Microbiol. Res. 241, 126585. https://doi.org/10.1016/j.micres.2020.126585.
Kacaniova, M., Kluga, A., Kantor, A., Medo, J., Ziarovska, J., Puchalski, C., Terentjeva, M., 2019. Comparison of MALDI-TOF MS Biotyper and 16S rDNA sequencing for the identification of Pseudomonas species isolated from fish. Microb. Pathog. 132, 313–318. https://doi.org/10.1016/j.micpath.2019.04.024.
Kajiwara, H., 2016. Direct detection of the plant pathogens Burkholderia glumae, Burkholderia gladioli pv. gladioli, and Erwinia chrysanthemi pv. zeae in infected rice seedlings using matrix assisted laser desorption/ionization time-of-flight mass spectrometry. J. Microbiol. Methods 120, 1–5. https://doi.org/10.1016/j.mimet.2015.08.014.
Karut, S.T., Karut, K., Aysan, Y., 2020. Culturable bacterial strains isolated from Bemisia tabaci (Gennadius, 1889) (Hemiptera: aleyrodidae) populations of Adana and Mersin Provinces of Turkey and their entomopathogen characteristics. Turkish J. Entomol. 44, 13–22. https://doi.org/10.16970/entoted.578542.
Kim, E., Cho, E.J., Yang, S.M., Kim, M.J., Kim, H.Y., 2021. Novel approaches for the identification of microbial communities in kimchi: MALDI-TOF MS analysis and high-throughput sequencing. Food Microbiol. 94, 103641. https://doi.org/10.1016/j.fm.2020.103641.
Kim, E., Kim, H., Yang, S., Kim, C., Choo, D., Kim, H., 2019. Rapid identification of Staphylococcus species isolated from food samples by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J. Microbiol. Biotechnol. 29 (4), 548–557.
Kim, J.-K., Jackson, S.N., Murray, K.K., 2005. Matrix-assisted laser desorption/ionization mass spectrometry of collected bioaerosol particles. RCM (Rapid Commun. Mass Spectrom.) 19 (12), 1725–1729. https://doi.org/10.1002/rcm.1982.
Konate, S., Camara, A., Lo, C., Tidjani Alou, M., Hamidou Togo, A., Niare, S., et al., 2021. Virgibacillus doumboii sp. nov., a halophilic bacterium isolated from the stool of a healthy child in Mali. New Microbes and New Infections 42, 100890.
Kostrzewa, M., Sparbier, K., Maier, T., Schubert, S., 2013. MALDI-TOF MS: an upcoming tool for rapid detection of antibiotic resistance in microorganisms. Proteonomics Clin. Appl. 7, 767–778.
Krader, P., Emerson, D., 2004. Identification of archaea and some extremophilic bacteria using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass

spectrometry. Extremophiles 8 (4), 259–268. https://doi.org/10.1007/s00792-004-0382-7.
Krishnamurthy, T., Ross, P.L., Rajamani, U., 1996. Detection of pathogenic and nonpathogenic bacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom. 10 (8), 883–888.
Lara, B.G., Wrobel, K., Corrales Escobosa, A.R., Serrano Torres, O., Enciso Donis, I., Wrobel, K., 2021. Mass spectrometry-based identification of bacteria isolated from industrially contaminated site in Salamanca (Mexico) and evaluation of their potential for DDT degradation. Folia Microbiol. 66 (3), 355–369. https://doi.org/10.1007/s12223-020-00848-8.
Lartigue, M.F., Arnaud, E.H., Haguenoer, E., Domelier, A.S., Schmit, P.O., Marquet, N.V., Lanotte, P., Mereghetti, L., Kostrzewa, M., Quentin, R., 2009. Identification of Streptococcus agalactiae isolates from various phylogenetic lineages by matrix-assisted laser desorption ionization – time of flight mass spectrometry. Society 47, 2284–2287.
Laukova, A., Strompfova, V., Scerbova, J., Pogany, S., Monika, P.S., 2019. Virulence factor genes incidence among Enterococci from sewage sludge in eastern Slovakia following safety aspect. BioMed Res. Int. 1, 5. https://doi.org/10.1155/2019/2735895.
Lovecka, P., Pacovska, I., Stursa, P., Vrchotova, B., Kochankova, L., Demnerova, K., 2015. Organochlorinated pesticide degrading microorganisms isolated from contaminated soil. New biotechnology 32 (1), 26–31. https://doi.org/10.1016/j.nbt.2014.07.003.
Lu, R., Frederiksen, M.W., Uhrbrand, K., Li, Y., Ostergaard, C., Madsen, A.M., 2020. Wastewater treatment plant workers' exposure and methods for risk evaluation of their exposure. Ecotoxicol. Environ. Saf. 205, 111365. https://doi.org/10.1016/j.ecoenv.2020.111365.
Macedo-Raygoza, G.M., Valdez-Salas, B., Prado, F.M., Prieto, K.R., Yamaguchi, L.F., Kato, M.J., Canto-Canche, B.B., Carrillo-Beltran, M., Di Mascio, P., White, J.F., Beltran-Garcia, M.J., 2019. Enterobacter cloacae, an endophyte that establishes a nutrient-transfer symbiosis with banana plants and protects against the black sigatoka pathogen. Front. Microbiol. 10, 804. https://doi.org/10.3389/fmicb.2019.00804.
Madsen, A.M., White, J.K., Markouch, A., Kadhim, S., de Jonge, N., Thilsing, T., Hansen, V.M., Bælum, J., Nielsen, J.L., Vogel, U., Tendal, K., 2021. A cohort study of cucumber greenhouse workers' exposure to microorganisms as measured using NGS and MALDI-TOF MS and biomarkers of systemic inflammation. Environ. Res. 192, 110325. https://doi.org/10.1016/j.envres.2020.110325.
Malinicova, L., Nosalova, L., Timkova, I., Pristas, P., Sedlakova-Kadukova, J., 2020. Hidden microcosmos in Slovak gold mine Rozalia – microbial gold miners? Journal of the Polish Mineral Engineering Society 2. https://doi.org/10.29227/IM-2020-01-47.
Martiny, D., Busson, L., Wybo, I., El Haj, R.A., Dediste, A., Vandenberg, O., 2012. Comparison of the Microflex LT and Vitek MS systems for routine identification of bacteria by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. 50 (4), 1313–1325. https://doi.org/10.1128/JCM.05971-11.
Mauchline, T.H., Malone, J.G., 2017. Life in earth - the root microbiome to the rescue? Curr. Opin. Microbiol. 37, 23–28.
Mazzeo, M.F., Sorrentino, A., Gaita, M., Cacace, G., Di Stasio, M., Facchiano, A., Comi, G., Malorni, A., Siciliano, R.A., 2006. Matrix-assisted laser desorption ionization time of flight mass spectrometry for the discrimination of food-borne microorganisms. Appl. Environ. Microbiol. 72 (2), 1180–1189.
Mitra, S., Pramanik, K., Sarkar, A., Ghosh, P.K., Soren, T., Maiti, T.K., 2018. Bioaccumulation of cadmium by Enterobacter sp. and enhancement of rice seedling growth under cadmium stress. Ecotoxicol. Environ. Saf. 156, 183–196. https://doi.org/10.1016/j.ecoenv.2018.03.001.
Mougin, J., Flahaut, C., Roquigny, R., Bonnin-Jusserand, M., Grard, T., Le Bris, C., 2020. Rapid identification of Vibrio species of the harveyi clade using MALDI-TOF MS profiling with main spectral profile database implemented with an in-house database: luvibase. Front. Microbiol. 11.
Muthuri, C., Nyambura, N.C., Njeri, V.M., Tani, A., Wangari, M.C., 2012. Isolation and identification of endophytic bacteria of bananas (Musa spp.) in Kenya and their potential as biofertilizers for sustainable banana production. Afr. J. Microbiol. Res. 6, 6414–6422.
Niestepski, S., Harnisz, M., Korzeniewska, E., Osinska, A., 2019. Isolation of anaerobic bacteria of the Bacteroides fragilis group from environmental samples. E3S Web of Conferences 100, 00058.
Niyompanich, S., Jaresitthikunchai, J., Srisanga, K., Roytrakul, S., Tungpradabkul, S., 2014. Source-identifying biomarker ions between environmental and clinical Burkholderia pseudomallei using whole-cell matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). PLoS One 9 (6), e99160. https://doi.org/10.1371/journal.pone.0099160.
Nosalova, L., Malinicova, L., Kiskova, J., Timkova, I., Sedlakova-Kadukova, J., Pristas, P., 2021. Cultivable microbiota associated with gold ore from the rozália gold mine, hodruša-hámre, Slovakia. Geomicrobiol. J. 38, 1–11. https://doi.org/10.1080/01490451.2021.1871685.
Oberhaensli, T., Hofer, V., Tamm, L., Fuchs, J.G., Koller, M., Herforth-Rahme, J., Maurhofer, M., Thuerig, B., 2017. Aeromonas media in compost amendments contributes to suppression of Pythium ultimum in cress. Acta Hortic. 1164, 353–360. https://doi.org/10.17660/ActaHortic.2017.1164.45.
Okafor, N., 2011. Environmental Microbiology of Aquatic and Waste Systems. Springer, Dordrecht Heidelberg London New York, p. 303. https://doi.org/10.1007/978-94-007-1460-.
Pantucek, R., Sedlacek, I., Indrakova, A., Vrbovska, V., Maslanova, I., Kovarovic, V., Svec, P., Kralova, S., Kristofova, L., Keklakova, J., Petras, P., Doskar, J., 2018. Staphylococcus edaphicus sp. nov., isolated in Antarctica, harbours mecC gene and genomic islands with suspected role in adaptation to extreme environment. Appl. Environ. Microbiol. 84 https://doi.org/10.1128/AEM.01746-17. AEM.01746-17.
Pascale, M., Mazzotta, M., Salaris, S., Girolamini, L., Grottola, A., Simone, M., et al., 2020. Evaluation of MALDI–TOF mass spectrometry in diagnostic and environmental surveillance of Legionella species: a comparison with culture and mip-gene sequencing technique. Front. Microbiol. 11.
Pinar-Mendez, A., Fernández, S., Baquero, D., Vilaró, C., Galofré, B., González, S., Rodrigo-Torres, L., Arahal, D.R., Macián, M.C., Ruvira, M.A., Aznar, R., Caudet-Segarra, L., Sala-Comorera, L., Lucena, F., Blanch, A.R., Garcia-Aljaro, C., 2021. Rapid and improved identification of drinking water bacteria using the Drinking Water Library, a dedicated MALDI-TOF MS database. Water Res. 203, 117543. https://doi.org/10.1016/j.watres.2021.117543.
Pomastowski, P., Złoch, M., Rodzik, A., Ligor, M., Kostrzewa, M., Buszewski, B., 2019. Analysis of bacteria associated with honeys of different geographical and botanical origin using two different identification approaches: MALDI-TOF MS and 16S rDNA PCR technique. PLoS One 14 (5), e0217078. https://doi.org/10.1371/journal.pone.0217078.
Popovic, N.T., Kazazic, S.P., Strunjak-Peroviv, I., Coz-Rakovac, R., 2017. Differentiation of environmental aquatic bacterial isolates by MALDI-TOF MS. Environ. Res. 152, 7–16. https://doi.org/10.1016/j.envres.2016.09.020.
Powthong, P., Suntornthiticharoen, P., 2018. Isolation and characterization of biosurfactant-producing bacteria isolated from agriculture area in Thailand. Bulgarian Journal of Agricultural Science 24, 623–630.
Pramanik, K., Mitra, S., Sarkar, A., Soren, T., Maiti, T.K., 2017. Characterization of cadmium resistant Klebsiella pneumoniae MCC 3091 promoted rice seedling growth by alleviating phytotoxicity of cadmium. Environ. Sci. Pollut. Res. Int. 24 (31), 24419–24437. https://doi.org/10.1007/s11356-017-0033-z.
Prouzova, P., Hoskovcova, E., Musilova, L., Strejcek, M., Uhlík, O., Demnerova, K., Mackova, M., 2012. The effect of Plants and natural compounds on bacterial population in the contaminated soil. In: Phytoremediation and Rhizoremediation. Proceedings, 5th International Symposium on Biosorption and Bioremediation. (June 24 – 28, 2012).
Rabodonirina, S., Rasolomampianina, R., Krier, F., Drider, D., Merhaby, D., Net, S., Ouddane, B., 2019. Degradation of fluorene and phenanthrene in PAHs-contaminated soil using Pseudomonas and Bacillus strains isolated from oil spill sites. J. Environ. Manag. 232, 1–7. https://doi.org/10.1016/j.jenvman.2018.11.005.
Rajendran, A., Marimuthu, P., 2017. Studies on endophytic Burkholderia sp. from sugarcane and its screening for plant growth promoting potential. Journal of Experimental Biology and Agricultural Sciences 5, 242–257. https://doi.org/10.18006/2017.5(2).242.257.
Rezzonico, F., Vogel, G., Duffy, B., Tonolla, M., 2010. Application of whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid identification and clustering analysis of Pantoea species. Appl. Environ. Microbiol. 76 (13), 4497–4509.
Ribeiro, D.G., Carmo, L., Santos, I.R., Almeida, R.F., Silva, L.P., Oliveira-Neto, O.B., Scherwinski-Pereira, J.E., Mehta, A., 2020. MALDI TOF MS-profiling: applications for bacterial and plant sample differentiation and biological variability assessment. J. Proteonomics 213, 103619. https://doi.org/10.1016/j.jprot.2019.103619.
Rizzardi, K., Wahab, T., Jernberg, C., 2013. Rapid subtyping of Yersinia enterocolitica by matrix-Assisted laser desorption ionization-time of flight mass spectrometry (MALDITOF MS) for diagnostics and surveillance. J. Clin. Microbiol. 51, 4200–4203.
Ruelle, V., El Moualij, B., Zorzi, W., Ledent, P., Pauw, E.D., 2004. Rapid identification of environmental bacterial strains by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom.: RCM (Rapid Commun. Mass Spectrom.) 18 (18), 2013–2019. https://doi.org/10.1002/rcm.1584.
Rybitwa, D., Wawrzyk, A., Rahnama, M., 2020. Application of a medical diode laser (810 nm) for disinfecting small microbiologically contaminated spots on degraded collagenous materials for improved biosafety in objects of exceptional historical value from the auschwitz-birkenau state museum and protection of human health. Front. Microbiol. 11, 596852. https://doi.org/10.3389/fmicb.2020.596852.
Sala-Comorera, L., Blanch, A.R., Vilaro, C., Galofre, B., García-Aljaro, C., 2017. Heterotrophic monitoring at a drinking water treatment plant by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry after different drinking water treatments. J. Water Health 15 (6), 885–897. https://doi.org/10.2166/wh.2017.090.
Sala-Comorera, L., Caudet-Segarra, L., Galofre, B., Lucena, F., Blanch, A.R., García-Aljaro, C., 2020. Unravelling the composition of tap and mineral water microbiota: divergences between next-generation sequencing techniques and culture-based methods. Int. J. Food Microbiol. 334, 108850. https://doi.org/10.1016/j.ijfoodmicro.2020.108850.
Salgado, J.R.S., Rabinovitch, L., Gomes, M., Allil, R.C., Werneck, M.M., Rodrigues, R.B., Picao, R.C., Luiz, F.B., Vivoni, L.A.M., 2020. Detection of Bacillus anthracis and Bacillus anthracis-like spores in soil from state of Rio de Janeiro, Brazil. Mem. Inst. Oswaldo Cruz, 115.
Salplachta, J., Kubesova, A., Horky, J., Matouskova, H., Tesarova, M., Horka, M., 2015. Characterization of Dickeya and Pectobacterium species by capillary electrophoretic techniques and MALDI-TOF MS. Anal. Bioanal. Chem. 407 (25), 7625–7635. https://doi.org/10.1007/s00216-015-8920-y.
Samapundo, S., de Baenst, I., Aerts, M., Cnockaert, M., Devlieghere, F., Damme, P., 2019. Tracking the sources of psychrotrophic bacteria contaminating chicken cuts during processing. Food Microbiol. 81, 40–50. https://doi.org/10.1016/j.fm.2018.06.003.
Sandrin, T.R., Goldstein, J.E., Schumaker, S., 2012. MALDI TOF MS profiling of bacteria at the strain level: a review. Mass Spectrom. Rev. 32, 188–217.
Sapugahawatte, D.N., Li, C., Zhu, C., Dharmaratne, P., Wong, K.T., Lo, N., Ip, M., 2020. Prevalence and characteristics of extended-spectrum-β-lactamase-producing and

carbapenemase-producing Enterobacteriaceae from freshwater fish and pork in wet markets of Hong Kong. mSphere 5 (2), e00107–e00120. https://doi.org/10.1128/mSphere.00107-20.

Sarkar, A., Pramanik, K., Mitra, S., Soren, T., Maiti, T.K., 2018. Enhancement of growth and salt tolerance of rice seedlings by ACC deaminase-producing Burkholderia sp. MTCC 12259. J. Plant Physiol. 231, 434–442. https://doi.org/10.1016/j.jplph.2018.10.010.

Santos, I.C., Hildenbrand, Z.L., Schug, K.A., 2016. Applications of MALDI-TOF MS in environmental microbiology. Analyst 141 (10), 2827–2837. https://doi.org/10.1039/c6an00131a.

Sawada, H., Horita, H., Misawa, T., Takikawa, Y., 2019. Pseudomonas grimontii, causal agent of turnip bacterial rot disease in Japan. J. Gen. Plant Pathol. 85, 413–423. https://doi.org/10.1007/s10327-019-00869-3.

Sedo, O., Sedlaek, I., Zdrahal, Z., 2011. Sample preparation methods for MALDI-MS profiling of bacteria. Mass Spectrom. Rev. 30 (3), 417–434. https://doi.org/10.1002/mas.20287.

Sedlacek, I., Pantucek, R., Zeman, M., Holochova, P., Sedo, O., Stankova, E., Svec, P., Kralova, S., Vídenska, P., Micenkova, L., Urvashi, Korpole, S., Lal, R., 2020. Hymenobacter terrestris sp. nov. and Hymenobacter lapidiphilus sp. nov., isolated from regoliths in Antarctica. Int. J. Syst. Evol. Microbiol. 70 (12), 6364–6372. https://doi.org/10.1099/ijsem.0.004540.

Sekowska, A., Mikucka, A., Gospodarek-Komkowska, E., 2018. Identification of Raoultella spp.: comparison of three methods. Indian J. Med. Microbiol. 36 (2), 197–200. https://doi.org/10.4103/ijmm.IJMM_17_99.

Seng, P., Drancourt, M., Gouriet, F., La Scola, B., Fournier, P.E., Rolain, J.M., Raoult, D., 2009. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin. Infect. Dis. 49, 543–551.

Siegrist, T.J., Anderson, P.D., Huen, W.H., Kleinheinz, G.T., McDermott, C.M., Sandrin, T.R., 2007. Discrimination and characterization of environmental strains of Escherichia coli by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). J. Microbiol. Methods 68 (3), 554–562. https://doi.org/10.1016/j.mimet.2006.10.012.

Silva, N.M., de Oliveira, A., Pegorin, S., Giusti, C.E., Ferrari, V.B., Barbosa, D., Martins, L. F., Morais, C., Setubal, J.C., Vasconcellos, S.P., da Silva, A.M., de Oliveira, J., Pascon, R.C., Viana-Niero, C., 2019. Characterization of novel hydrocarbon-degrading Gordonia paraffinivorans and Gordonia sihwensis strains isolated from composting. PLoS One 14 (4), e0215396. https://doi.org/10.1371/journal.pone.0215396.

Silva-Jimenez, H., Araujo-Palomares, C., Macías-Zamora, J., Ramírez-Alvarez, N., Garcia, B., Escobosa, C.A., 2018. Identification by MALDI-TOF MS of environmental bacteria with high potential to degrade pyrene. Journal of the Mexican Chemical Society 62. https://doi.org/10.29356/jmcs.v62i2.411.

Singhal, N., Kumar, M., Kanaujia, P.K., Virdi, J.S., 2015. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Front. Microbiol. 6, 791. https://doi.org/10.3389/fmicb.2015.00791.

Sparbier, K., Weller, U., Boogen, C., Kostrzewa, M., 2012. Rapid detection of Salmonella spp. by means of a combination of selective enrichment broth and MALDI-TOF MS. Eur. J. Clin. Microbiol. Infect. Dis. 31, 767–773.

Stafsnes, M.H., Dybwad, M., Brunsvik, A., Bruheim, P., 2013. Large scale MALDI-TOF MS based taxa identification to identify novel pigment producers in a marine bacterial culture collection. Antonie Leeuwenhoek 103 (3), 603–615. https://doi.org/10.1007/s10482-012-9844-6.

Suen, L., Siu, G., Guo, Y.P., Yeung, S., Lo, K., O'Donoghue, M., 2019. The public washroom - friend or foe? An observational study of washroom cleanliness combined with microbiological investigation of hand hygiene facilities. Antimicrob. Resist. Infect. Control 8, 47. https://doi.org/10.1186/s13756-019-0500-z.

Sulaiman, I., Banerjee, P., Hsieh, Y., Miranda, N., Simpson, S., Kerdahi, K., 2018. Rapid detection of Staphylococcus aureus and related species isolated from food, environment, cosmetics, a medical device, and clinical samples using the VITEK MS microbial identification system. J. AOAC Int. 101 (4), 1135–1143.

Sura-de Jong, M., Reynolds, R.J., Richterova, K., Musilova, L., Staicu, L.C., Chocholata, I., Cappa, J.J., Taghavi, S., van der Lelie, D., Frantik, T., Dolinova, I., Strejcek, M., Cochran, A.T., Lovecka, P., Pilon-Smits, E.A., 2015. Selenium hyperaccumulators harbor a diverse endophytic bacterial community characterized by high selenium resistance and plant growth promoting properties. Front. Plant Sci. 6, 113. https://doi.org/10.3389/fpls.2015.00113.

Suzuki, Y., Niina, K., Matsuwaki, T., Nukazawa, K., Iguchi, A., 2018. Bacterial flora analysis of coliforms in sewage, river water, and ground water using MALDI-TOF mass spectrometry. J. Environ. Sci. Health - Part A Toxic/Hazard. Subst. Environ. Eng. 53 (2), 160–173. https://doi.org/10.1080/10934529.2017.1383128.

Tani, A., Sahin, N., Matsuyama, Y., Enomoto, T., Nishimura, N., Yokota, A., Kimbara, K., 2012. High-throughput identification and screening of novel Methylobacterium species using whole-cell MALDI-TOF/MS analysis. PLoS One 7 (7), e40784. https://doi.org/10.1371/journal.pone.0040784.

Timperio, A., Gorrasi, S., Zolla, L., Fenice, M., 2017. Evaluation of MALDI-TOF mass spectrometry and MALDI BioTyper in comparison to 16S rDNA sequencing for the identification of bacteria isolated from Arctic sea water. PLoS One 12 (7), e0181860.

Tsuchida, S., Umemura, H., Nakayama, T., 2020. Current status of matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) in clinical diagnostic microbiology. Molecules 25 (20), 4775. https://doi.org/10.3390/molecules25204775.

Turvey, M.E., Weiland, F., Meneses, J., Sterenberg, N., Hoffmann, P., 2016. Identification of beer spoilage microorganisms using the MALDI Biotyper platform. Appl. Microbiol. Biotechnol. 100 (6), 2761–2773. https://doi.org/10.1007/s00253-016-7344-8.

Uhlik, O., Strejcek, M., Junkova, P., Sanda, M., Hroudova, M., Vlcek, C., Mackova, M., Macek, T., 2011. Matrix-assisted laser desorption ionization (MALDI)-time of flight mass spectrometry- and MALDI biotyper-based identification of cultured biphenyl-metabolizing bacteria from contaminated horseradish rhizosphere soil. Appl. Environ. Microbiol. 77 (19), 6858–6866. https://doi.org/10.1128/AEM.05465-11.

van de Bilt, J., Wolsink, M., Gorkink-Smits, P., Landman, N., Bergsma-Vlami, M., 2018. Application of matrix-assisted laser desorption ionization time-of-flight mass spectrometry for rapid and accurate identification of Ralstonia solanacearum and Ralstonia pseudosolanacearum. Eur. J. Plant Pathol. 152 (4), 921–931.

Vavrova, A., Matoulkova, D., Balazova, T., Sedo, O., 2018. MALDI-TOF MS analysis of anaerobic bacteria isolated from biofilm-covered surfaces in brewery bottling Halls. J. Am. Soc. Brew. Chem. 72 (2), 95–101.

Vicenzi, F., Pillonetto, M., Souza, H., Palmeiro, J., Riedi, C., Rosario-Filho, N., Dalla-Costa, L., 2016. Polyphasic characterisation of *Burkholderia cepacia* complex species isolated from children with cystic fibrosis. Memórias Do Instituto Oswaldo Cruz 111 (1), 37–42.

Wang, Y., Zhou, Q., Li, B., Liu, B., Wu, G., Ibrahim, M., Xie, G., Li, H., Sun, G., 2012. Differentiation in MALDI-TOF MS and FTIR spectra between two closely related species Acidovorax oryzae and Acidovorax citrulli. BMC Microbiol. 12, 182. https://doi.org/10.1186/1471-2180-12-182.

Weikl, F., Tischer, C., Probst, A.J., Heinrich, J., Markevych, I., Jochner, S., Pritsch, K., 2016. Fungal and bacterial communities in indoor dust follow different environmental determinants. PLoS One 11, e0154131.

Yang, Y., Lin, Y., Qiao, L., 2018. Direct MALDI-TOF MS identification of bacterial mixtures. Anal. Chem. 90 (17), 10400–10408. https://doi.org/10.1021/acs.analchem.8b02258.

Yang, Y., Lin, Y., Chen, Z., Gong, T., Yang, P., Girault, H., Liu, B., Qiao, L., 2017. Bacterial whole cell typing by mass spectra pattern matching with bootstrapping assessment. Anal. Chem. 89 (22), 12556–12561. https://doi.org/10.1021/acs.analchem.7b03820.

Zancarini, A., Echenique-Subiabre, I., Debroas, D., Taib, N., Quiblier, C., Humbert, J.F., 2017. Deciphering biodiversity and interactions between bacteria and microeukaryotes within epilithic biofilms from the Loue River. France. Sci. Rep. 7, 4344.

Zeng, Y., Nupur, Wu, N., Madsen, A.M., Chen, X., Gardiner, A.T., Koblízek, M., 2021. Gemmatimonas groenlandica sp. nov. Is an aerobic anoxygenic phototroph in the phylum Gemmatimonadetes. Front. Microbiol. 11, 606612. https://doi.org/10.3389/fmicb.2020.606612.

Zhang, D., Palmer, J., Teh, K.H., Flint, S., 2020. Identification and selection of heat-stable protease and lipase-producing psychrotrophic bacteria from fresh and chilled raw milk during up to five days storage. LWT- Food Science and Technology 134. https://doi.org/10.1016/j.lwt.2020.110165.