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REVIEW ARTICLE

Application of the MALDI-TOF MS method for identification of *Vibrio* spp. in aquaculture

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ABSTRACT

Aquaculture is developing rapidly and plays an important role in providing animal protein to the world's growing population. However, increasing mortality from bacterial disease outbreaks in important species poses a challenge to production progress in this sector. In order to reduce the impact of these diseases, rapid and accurate pathogen identification is essential for disease management, early detection and the continued health of aquaculture. The aim of this review is to summarise studies on the identification and diagnosis of *Vibrio* pathogens in aquatic organisms by MALTI-TOF MS (Matrix-Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry), a rapid identification method based on protein profiling of bacteria. The profiles of bacterial protein obtained are compared with a global microbial protein library for identification. This study demonstrates the potential of using MALDI-TOF MS for the detection of *Vibrio* pathogens in aquaculture in studies published between 2015 and 2024. While purchasing a time-of-flight mass spectrometer is expensive when compared to conventional and molecular identification methods. It also appears to be much more efficient in terms of time spent on identification. MALDI-TOF MS has been shown to be simple to use in fish identification laboratories.

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Introduction

Aquaculture, a rapidly growing sector, suffers tremendous financial losses every year due to fish deaths caused by disease outbreaks and treatment expenses (Woo & Bruno, 2011). There are numbers of gram-negative, halophilic, flagellated and facultative anaerobic *Vibrio* species in Vibrionaceae family such as *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* cause diarrheal disease, septicaemia and serious wound infections in humans (Malainine et al., 2013; Burbick et al., 2018; Boonstra et al., 2023). *V. alginolyticus*, *V. anguillarum*, *V. parahaemolyticus*, *V. harveyi*, *V. splendidus* and *V. ordalii* are also known to be the causative agent of Vibriosis that causes symptoms of haemorrhagic septicaemia in various marine fish and freshwater fish (salmon, rainbow trout) (Silva-Rubio et al.,

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2008) as well as shellfish, crustaceans and bivalves (Tanrikul, 2007; Mougin et al., 2020). Lastly, bacterium *V. tapetis* causes Brown Ring Disease (BRD) in Manila clam (Paillard et al., 2006). Since bacterial infections are a major contributor to fish mortality in aquaculture, mitigating their impact is of great importance to the aquacultural and fisheries industry. It is essential to monitor these diseases by surveillance and quick bacterial detection in order to diagnose and treat aquatic animal diseases before they pose major hazards to animal welfare and country economies that depend on marine and inland aquaculture (Ashfaq et al., 2022).

Traditionally, bacterial fish pathogens have been identified and characterized using conventional microbiological, immunological and molecular biological approaches (Ruiz-Zarzuela et al., 2005; Altinok et al., 2008; Timur et al., 2009; Austin, 2019; Duman et al., 2022). Although these classical methods are frequently used in the identification of bacterial fish diseases, they also have the disadvantages of requiring too much effort and time and not being able to distinguish between some closely related species.

Consequently, the emphasis has been to develop quick, affordable substitute methods that have a significant level of sensitivity and specificity for identifying *Vibrio* agents. In the identification of bacterial disease pathogens, mass spectrometry with Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) is a recent breakthrough. By examining bacterial protein profiles, this proteomics-based method offers rapid and precise identification. It can be applied in supplementary to and as a validator of other microbial identification techniques (Lauková et al., 2018).

In this review, a brief overview of the general principles, applications and history of MALDI-TOF MS is provided, followed by an evaluation of the studies on the detection of *Vibrio* spp. by this method.

History, Applications and General Principles of MALDI-TOF MS

MALDI-TOF MS was first developed by Karas et al. (1985) and after Tanaka won the Nobel Prize in Chemistry for this development in 2002, MALDI-TOF MS has made significant advances in proteomics research, enabling rapid identification of many different microorganisms in medical microbiology research (Tanaka, 2003). In the following years, open access protein mass spectrum libraries were created to facilitate microbial (virus, bacteria, fungi and yeast) characterization for clinical studies (Böhme et al., 2012).

MALDI-TOF was first used in clinical microbiology (Seng et al., 2010; Erler et al., 2015; Patel, 2015; Anwer et al., 2022), followed by veterinary, soil, plant, food and water microbiology (Popović et al., 2017; Chun et al., 2022) and more recently for the aquatic pathogens (Jansson et al., 2020; Piamsomboon et al., 2020).

The basic principle of the MALDI-TOF MS method works by ionising and measuring the mass to charge ratios (*m/z*) of ribosomal proteins of microorganisms, resulting in a mass spectrum with a unique fingerprint (Singhal et al., 2015). The obtained microbial peptide mass fingerprints are compared with the mass spectral library database of pre-existing reference samples and identification is performed (Brauge et al., 2021). Figure 1 summarize the MALDI-TOF MS procedure steps for different *Vibrio* identification. Step A is sample preparation from *Vibrio* culture, step B individual colonies on MALDI-TOF device and step C is the analysis and step D is identification (Sandalakis et al., 2017; Kazazić et al., 2019a, 2019b). In the first step, a single colony is selected from culture petri dishes with *Vibrio* and spread on the target plate. The sample plate of the device is then coated with a matrix solution of 70% formic acid and allowed to dry at room temperature. As the matrix dries, it crystallises together with the sample and is then placed in the MALDI-TOF device and the analysis is initiated (Popović et al., 2017). Once the matrix plate is placed in the instrument, ionised *Vibrio* peptides are converted by laser beams into protonated ions which are accelerated by an electric field towards a detector with the time required to travel along the flight tube under vacuum and measured according to their mass to charge ratio (*m/z*). Smaller proteins, followed by progressively larger analytes, arrive at the detector, creating a characteristic mass spectrum, which allows molecules present in samples to be identified based on their unique mass fingerprint. The distinctive spectrum obtained for each species is recorded and compared with a reference spectrum database. Species identification is achieved by comparing *Vibrio* species with reference mass spectra in databases based on peaks and ranking them on a logarithmic scale from 0 to 3.0 The criteria for a reliable identification at the species level should be a number higher than 1.7 (Puk et al., 2018).

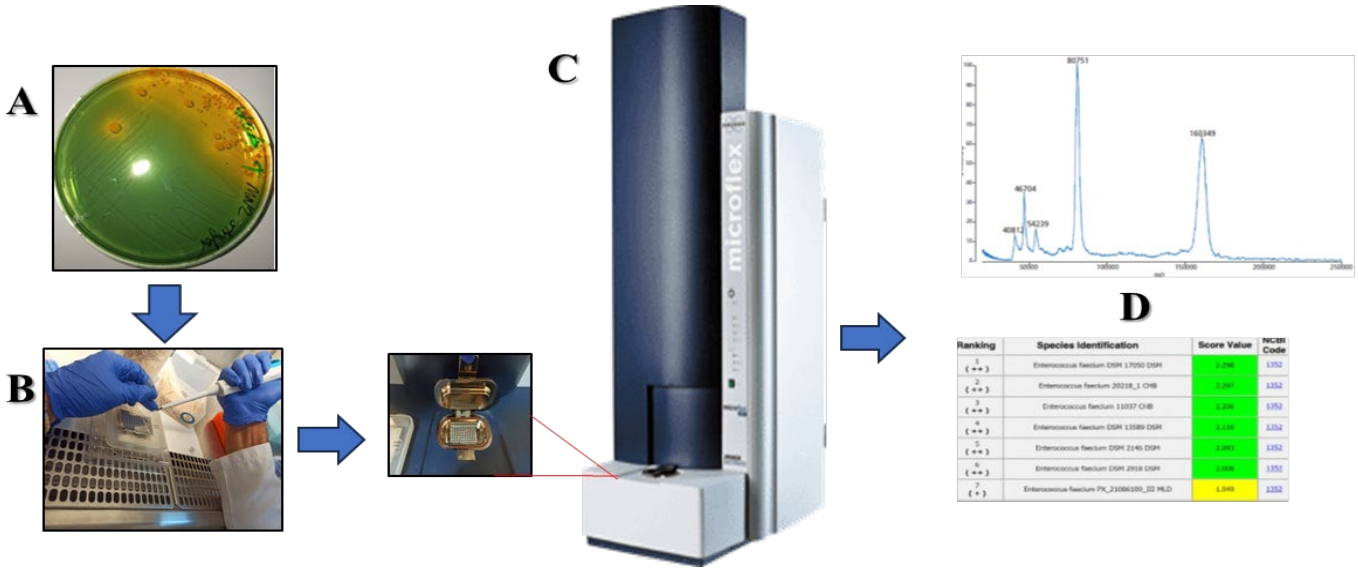

Figure 1. Schematic steps for MALDI-TOF MS **A)** Preparation of bacterial sample; **B)** Application of samples; **C)** MALDI-TOF MS analysis; **D)** Identification of *Vibrio* spp.

MALDI-TOF MS Analysis for Identification of *Vibrio* spp. and Other Fish Pathogens

Research has shown that the MALDI-TOF MS technique is capable of identifying a single bacterial disease agent or co-infectors, which is important for fish species used in both inland and marine aquaculture (Piamsomboon et al., 2020; Nissa et al., 2021; Moreira et al., 2021; Duman et al. 2022; Saticioglu et al., 2023). Besides Vibrionaceae (Burbick et al., 2018), several bacterial families of Mycobacteriaceae, Aeromonadaceae and Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae that cause disease in aquaculture have been shown to be identified by MALDI TOF (Singhal et al., 2015; Popović et al., 2017; Assis et al., 2017; López-Cortés et al., 2017).

This section reviews research articles on the identification of *Vibrio* species by MALDI-TOF MS from 2015 to 2024 retrieved using "MALDI-TOF MS" and "each *Vibrio* spp. separately" as keywords in the Google Scholar search engine (Figure 2). The bar graph in Figure 2 shows the number of research studies that utilised MALDI-TOF MS for the identification of *Vibrio* spp. during this period has been gradually increasing.

The reported applications and results of MALDI-TOF MS to identify *Vibrio* pathogens causing common vibriosis in fish, shellfish and shrimp are summarised in Table 1. A study by Dieckmann et al. (2010) showed that *Vibrio* spp. were identified from different samples by RNA polymerase beta subunit gene (*rpoB*) sequencing and compared with MALDI-TOF MS, which was shown to give a score mass of 4200 to 6500 Da for accurate identification. Bauer et al. (2018) identified eleven pathogenic *Vibrio* species from Pacific white shrimp samples (*Litopenaeus vannamei*) by protein spectrum MALDI-TOF MS analysis and compared these data with 16S rRNA sequencing and sequencing of the uridylate kinase encoding gene (*pyrH*). Burbick et al. (2018) reported that 29 out of 35 *Vibrio* spp. (83%) from different fish such as *Hippocampus abdominalis* (Big-belly seahorse), *Seriola lalandi* (yellowtail kingfish), *Atractoscion nobilis* (white seabass), *Pterapogon kauderni* (Banggai cardinal fish), *Paralichthys californicus* (California halibut) were correctly identified at species level using MALDI-TOF MS. *V. fluvalis* and *V. vulnificus* were identified by analysing peptide mass score values between 1.750 and 2.41 obtained from seawater (Haider et al., 2023) and *Anguilliformes* species (eels) (Boonstra et al., 2023), respectively. *V. anguillarum*, another important vibriosis causing bacterium, was identified from *Dicentrarchus labrax* (European sea bass) and *Sparus aurata* (sea bream) by MALDI-TOF MS with an average mass score of 2.12-2.50 (Kazazić et al., 2019b; Jansson et al., 2020; Mougin et al., 2021). Low et al. (2014) reported the identification of *V. alginolyticus* from *Epinephelus fuscoguttatus* (brown-marbled grouper) using MALDI TOF. Additionally, Rahmani et al. (2021) also came to the conclusion that *V. tapetis* isolated from Manila clams, were found as pathogenic based on the protein profiles which demonstrated the presence of a virulence gene. *V. parahaemolyticus*, the causative agent of vibriosis, was reported to be identified from *D. labrax*, *Haliotis tuberculata* (green ormer) and *Crassostrea gigas* (Pacific oyster) by Malainine et al. (2013) and Mougin et al. (2020) using MALDI TOF method. Finally, Yavuzcan et al. (2022) showed that *V. harveyi* from *Sarpa salpa* (dreamfish) was identified by the same method with a score of 2.248.

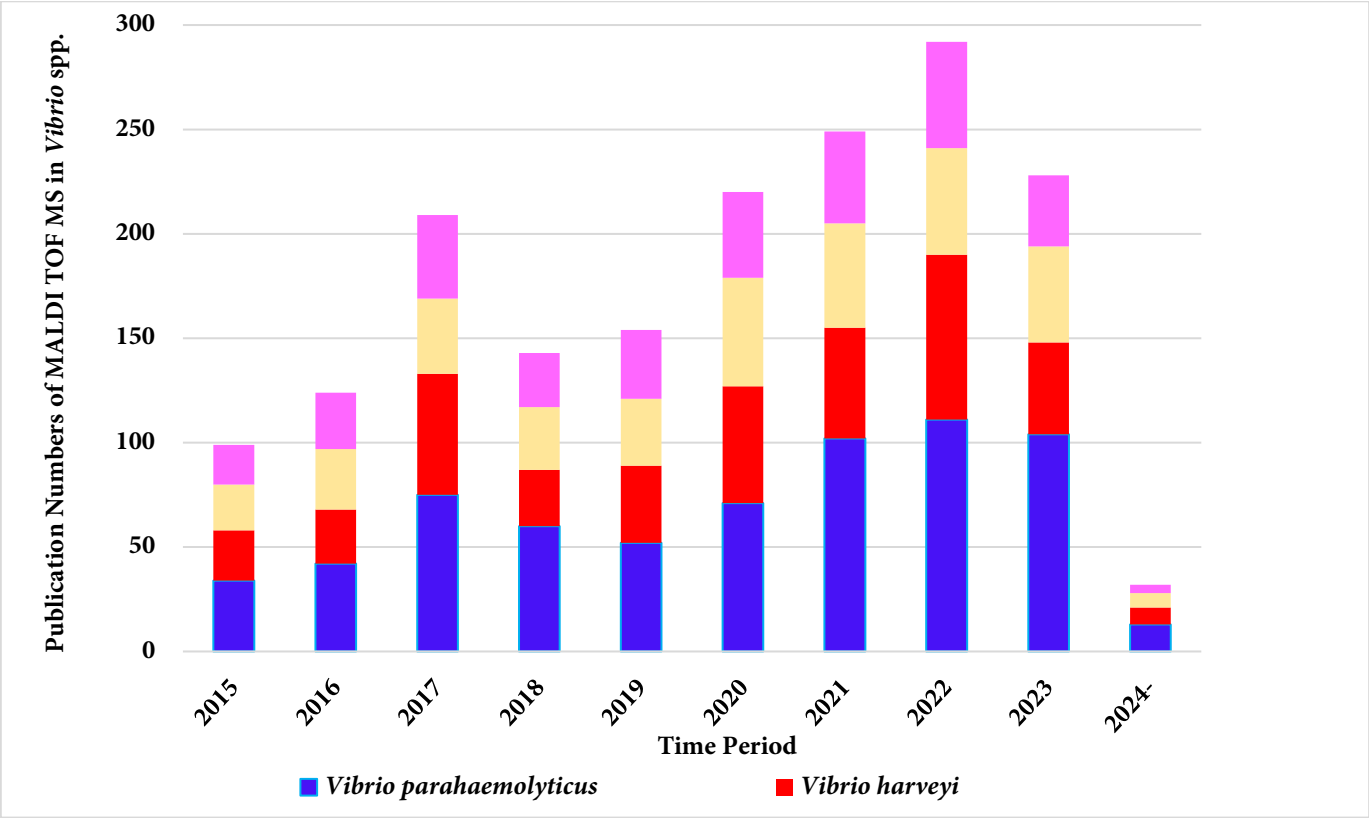

Figure 2. The number of publications over ten years related to found identification *Vibrio* pathogens with MALDI-TOF MS. The bar indicates the number of Web of Science search associated with *Vibrio* pathogens

Table 1. A list of *Vibrio* spp. identified with MALDI-TOF MS

Bacteria	Hosts	MALDI-TOF MS Peaks (Da)/Scores	References
Vibrio spp.	Molluscs	Scores 4200 to 6500	Dieckmann et al. (2010)
	Hippocampus abdominalis (big-belly seahorse), *Seriola lalandi* (yellowtail Kingfish), *Atractoscion nobilis* (White seabass), *Pterapogon kauderni* (Banggai cardinalfish), *Paralichthys californicus* (California halibut)	Scores 1.700 to 3.010	Burbick et al. (2018)
	Litopenaeus vannamei (Pacific white shrimp)	Scores 1.600 to 2.440	Bauer et al. (2018)
V. vulnificus	*Anguilliformes* spp. (Eels)	Score 2.000	Boonstra et al. (2023)
	Fish	Scores 2.218 to 2.418	Jansson et al. (2020)
V. anguillarum	Marine fish	Scores 2.123 to 2.318	Jansson et al. (2020)
	Dicentrarchus labrax (European seabass)	Score 2.500	Mougin et al. (2021)
	D. labrax, *S. aurata* (gilthead seabream)	Score 2.232	Kazazić et al. (2019b)
V. alginolyticus	*Epinephelus fuscoguttatus* (brown-marbled grouper)	Scores 910 to 2000 Da	Low et al. (2014)
V. fluvalis	Sea water	Score 1.750	Haider et al. (2023)
V. splendidus	Marine fish	Scores 1.780 to 2.030	Jansson et al. (2020)
V. harveyi	*Sarpa salpa* (dreamfish)	Score 2.248	Yavuzcan et al. (2022)
	D. labrax	Score 2.480	Mougin et al. (2021)
V. parahaemolyticus	*D. labrax, Haliotis tuberculate* (green ormer), *Crassostrea gigas* (Pacific oyster)	Scores 2.290 to 2.400	Mougin et al. (2020)
	shellfish, sea water and sediments	Scores 3000 to 11000 Da	Malainine et al. (2013)

Advantages, Limitations and Future Perspectives of MALDI-TOF MS for the Identification of Bacterial Fish Diseases

In terms of aquatic health, MALDI-TOF MS serves as a state-of-the-art diagnostic tool for identification of fish pathogens affecting aquatic organisms. There are a number of advantages to using MALDI-TOF MS analysis for identifying those pathogens, promoting sustainable practices in aquaculture and healthier fish populations. First of all, it provides rapid and accurate identification of the bacterial species causing fish disease, facilitating rapid detection of diseases affecting aquatic organisms. Furthermore, since MALDI-TOF MS has a high throughput and minimal sample preparation requirements, it can effectively screen a large number of field samples. Moreover, it is also an advantageous system as it reduces the need for costly reagents and labour-intensive steps involved in traditional techniques.

However, there are some limitations to take into account. The initial high cost of purchasing a MALDI-TOF MS device is a significant disadvantage that may prevent farming companies from using this technology. Alternatively, it can be suggested that instead of purchasing device, users who wants to bacterial identification, can obtain services from the devices available in clinical microbiology laboratories.

Another limitation of the current devices is that bacteria cannot be sampled directly from the diseased fish sample and identified in the equipment. Although MALDI-TOF MS can accurately identify most of the bacterial species, its inability to detect some species not found in proteomic databases may be one of the limitations of this method.

Overall, MALDI-TOF MS technology has ongoing innovations and system refinement for future user convenience. Efforts to optimise sample preparation protocols and streamline data analyses can make MALDI-TOF MS more accessible and user-friendly for aquaculture practitioners and hold great promise for advancing disease diagnosis and promoting sustainable aquaculture practices in the future. Nevertheless, more fish pathogens' score values and peak data should be uploaded to global protein databases for accurate discrimination and identification of fish pathogens.

Conclusion

In conclusion, the number of published papers between 2015 and 2024 applying MALDI-TOF MS increased significantly, demonstrating the technique's effectiveness and efficacy regarding the management of fisheries and aquatic health. With distinct fingerprints (spectral protein peaks) and mass score values obtained from cellular ribosomal proteins in *Vibrio*s, MALDI-TOF MS has demonstrated the ability to quickly and accurately detect, identify, and differentiate pathogens at the species level through comparison with reference mass spectrum databases. The use of this method in the aquaculture disease sector promotes sustainable practices and healthier fish populations in aquaculture by contributing to early detection of disease and the development of timely and effective intervention strategies.

Compliance With Ethical Standards

Authors' Contributions

İTÇ: Conceptualization, Writing - Original Draft, Writing-Review and Editing, Data Curation, Formal Analysis, Supervision

KG: Writing - Original Draft, Data Curation, Visualization

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

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