

MALDI-TOF MS as an identification tool for food-borne pathogens

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At the beginning, confirmation and identification of bacteria in microbiology were mainly based on morphology. The biochemical tests in test tubes emerged later, which were afterwards miniaturized. PCR was later developed, followed by the MALDI-TOF MS (i.e. matrix-assisted laser desorption-ionization – time of flight mass spectrometry). MALDI-TOF MS has already been used for about twenty years in different fields of applications especially in chemical laboratories for the detection of different molecules like sugars, nucleic acid and proteins. More recently the technique is more and more used for veterinary diagnosis and in the environmental field, en is now booming in the microbiological laboratories as a good identification system. At global level, there are nowadays around 1000 installations in the clinical and pharmaceutical sectors and about a hundred ones in the food sector.

As far as food safety is concerned, this technique meets the necessary requirements for confirmation and identification of food-borne pathogens. MALDI-TOF MS is faster than the usual microbiology methods but has the same discrimination level, it is less expensive and requires less technical expertise than the genotyping methods. Moreover, MALDI-TOF MS can also be used in routine laboratories of food microbiology, where the time factor plays a key role since these laboratories mostly work with the food industry. This technique can also be used in the case of food-borne outbreaks.

The MALDI-TOF MS technology in microbiology, can identify microorganisms to the species level. The principle is based on ionisation of the bacterial proteins by a laser beam and on creation of typical peaks (spectrum). By means of a spectra database, the related software searches for the correspondence with the bacterium species according to a reliability index between both spectra. The MALDI-TOF MS does however not give information on the serotype and pathogenicity of the species (ex: *Vibrio parahaemolyticus*).

A mass spectrometer is typically composed of 3 elements: an ion source (MALDI), a separation of the molecules (the TOF) and the detection.

MALDI-MS:

The analyte is first co-crystallized with small organic compounds (matrix) in order to be protected against direct contact with the ionizing beam and to avoid its degradation. These compounds will absorb the UV laser radiation and transfer the energy to the proteins that become positively ionized. The generated ions will free themselves from the proteins. The charged molecules (+1) are then accelerated in an electric field.

TOF:

After the ions went through the electric field, they enter a tube not exposed to an electric field and are separated according to their mass-charge. The ions reach different speeds because the big molecules move more slowly than the small ones. A time-of-flight analyzer will then detect each passing ion and create the peaks on the spectrum, which will afterwards be compared with a database. Some components of the spectrum are specific to a genus, others to a species or even to a subspecies. According to the sample quality and purity and to the number of reference spectra in the database, the identification of the bacterium takes a few seconds or a few minutes.

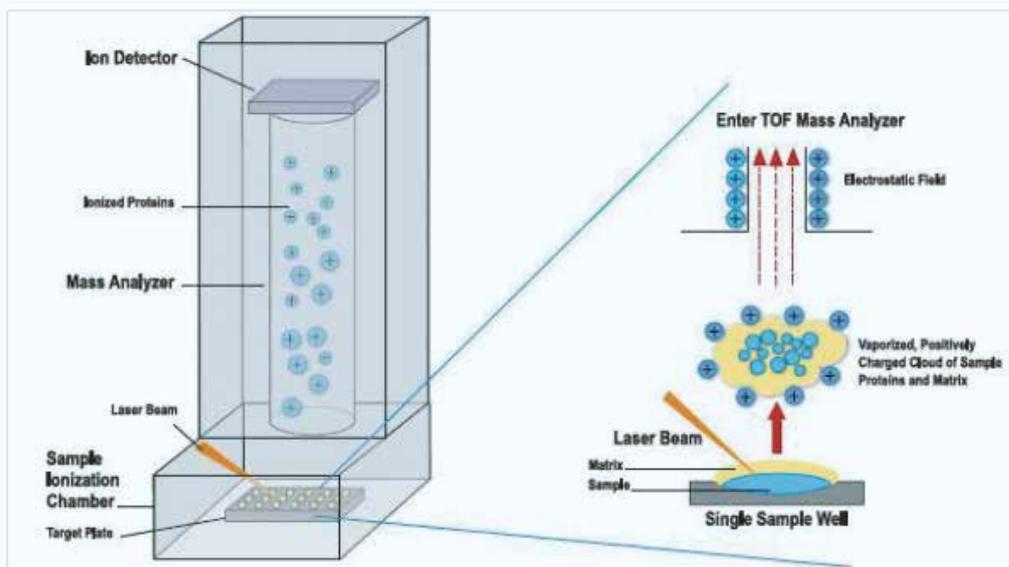


Figure 1: Working principle of a MALDI-TOF mass spectrometer

All sorts of food microorganisms can be screened by following the same protocol.

The preparation of the test sample is a critical step in the MALDI-TOF MS analyses. Indeed, in order to get reliable and reproducible identification results, it is necessary that the growth conditions of the bacterium (time and incubation temperature, culture medium) stay the same through the different tests. The test starts from a pure culture of bacteria. This culture can be used as such or be subjected to a pre-treatment. The tests must be carried out with fresh bacterial cultures because the stress caused by the cold or by a nutrient deficiency could alter the protein composition and so have an impact on the test result.

The quality of the database is also a critical point for a precise identification of the bacteria. The many surveys on identification of bacteria with MALDI-TOF MS have been carried out on clinical samples and the requirements specific to the food microbiology laboratories are usually not taken into account. Only a few reports do study the MALDI-TOF MS applied to food microbiology. Some surveys reveal that the major obstacle in the identification of food isolates is related to the database, which has to be regularly updated with reference spectra coming from food strains.

Table 1: The pros and cons of MALDI-TOF MS applied to food microbiology

PROS	CONS
- Sample preparation is universal for bacteria, yeasts and moulds	- Purchasing the device is expensive
- Protocol is simple	- Fresh cultures for the analyses
- Low operating costs (consumables, staff)	- Influence of growth medium on test result?
- No waste	The database must be large enough to discriminate properly very related bacteria, such as <i>E. coli</i> and <i>Shigella spp</i>
- Very rapid result	- Does not give any information on the pathogenicity of the species (ex: <i>Vibrio parahaemolyticus</i>)
- The database can easily be expanded	The species which are problematic for identification with 16S are sometimes also problematic with this technique

The MALDI-TOF MS will be integrated in the identification tests within the current revision of ISO 7218, besides the biochemical galleries, nucleic probes and agglutination tests.

To conclude, the MALDI-TOF MS is a promising tool for the identification of bacteria in the routine laboratories of food microbiology. The simple protocol, the rapid results and the low test costs are the main advantages of this technique, which is certainly fitted for a use in the food safety field.

Littérature:

<http://www.biomerieux.com/fr/spectrometrie-de-masse-maldi-tof>

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