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# Identification of enological yeasts and bacteria by MALDI-TOF mass spectrometry

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MALDI-TOF mass spectrometry has been adapted for use as an innovative tool for species identification of yeasts and bacteria isolated from various samples (musts, wines, beverages). Analysis of a large number of clones allows assessment of the diversity of yeast and acetic and lactic acid bacteria species present at pre-fermentation phases, during fermentation, during aging and after packaging. In the event of product deterioration, this innovative tool will contribute to better control of microbiological risks.

## Background

Species identification of microorganisms by MALDI-TOF (matrix-assisted laser desorption/ionization-Time Of Flight) mass spectrometry has been the reference method in the biomedical sector for more than fifteen years[1]. It allows quick and reliable identification of microorganisms and thus guides healthcare professionals towards appropriate therapeutic treatments. Its speed, reliability, simplicity and low cost make it a preferred alternative to identification methods based on DNA sequencing[2, 3]. It has already been adopted by other sectors, such as the food industry[4, 5]. It has been used in recent work to identify yeasts present in fermented products and beverages such as beer and wine[6, 7]. However, for reliable and robust identification of yeasts and bacteria of enological interest, the MALDI-TOF mass spectrometer must be used in conjunction with a database of specific protein spectra for enological yeast and bacteria strains. The work summarized in this article makes it possible to propose mass spectrometry as a routine method for the microbiological analysis of musts and wines.

## Principle of the analytical method

Identification of microorganisms takes place in five stages (Figure 1): 1/ The first essential is to have yeast or bacteria colonies isolated on an agar nutrient medium, from samples of must or wine. 2/ Each colony or colony fraction to be identified is then deposited on a suitable target allowing the simultaneous identification of more than 90 isolates in approximately one hour. 3/ After rapid preparation of all the colonies to be analyzed, the target is placed in the mass spectrometer and analyzed. Each colony is irradiated with a laser beam to destroy the cells, split their proteins into polypeptides and ionize them for analysis in the mass spectrometer. 4/ The set of polypeptides for each colony produces a protein spectrum, specific to a yeast or bacteria species. 5/ This spectrum is finally compared with those listed in the manufacturer's spectral database (DB). Species identification of the colony deposited on the target is thus possible in less than one hour if the comparison is satisfactory. However, if this colony belongs to a yeast or bacteria species that is absent from the database, or present but with reference strains too far removed from the enological environment, the species is not identified. The manufacturer's database used in this study contains more than 9000 protein spectra, from yeasts and bacteria strains that are rarely of enological origin.

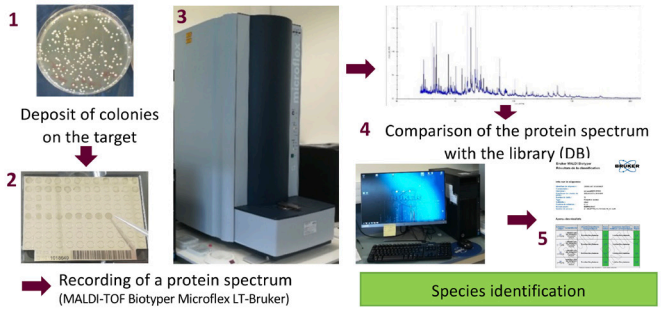


**FIGURE 1.** Stages in identification of a microorganism species by MALDI-TOF mass spectrometry.

## Database specific to enological microorganisms

The manufacturer's reference spectral database (DB) has proven to be quite effective in identifying certain species of interest such as *Hanseniaspora uvarum* and *Kluyveromyces lactis* yeasts (present in pre-fermentation phases) or the lactic acid bacterium *Lactiplantibacillus plantarum* (previously named *Lactobacillus plantarum*) that can carry out malolactic fermentation.

This work, initiated in 2015, proved less satisfactory for other species, like *Saccharomyces cerevisiae*[6] or the major agent of malolactic fermentation *Oenococcus oeni*, which were not correctly identified with sufficient consistency. For the spoilage yeast *Brettanomyces bruxellensis*, the identification efficiency was estimated at only 46% with this spectral database (DB) alone. Equally unsatisfactory results are observed for the yeasts *Torulaspora delbrueckii* and *Metschnikowia* spp. (used in must bioprotection) and *Zygosaccharomyces bailii* (causing spoilage) was even less well identified. Finally, identification is impossible for enological species not listed in this spectral database, such as the yeasts *Starmerella bacillaris* and *Trigonopsis cantarelli* or acetic acid bacteria of the genus *Acetobacter*.

To allow reliable identification of enological species, an "OENO" database was created listing the protein spectra of 217 yeast and bacteria isolates from the ISVV Biological Resource Center (CRB Oeno) and representative of the main must and wine species. It contains the protein spectra of 118 yeast isolates, corresponding to 35 different species (Figure 2-A), of which around fifteen were initially absent from the manufacturer's spectral database. Thanks to current knowledge, the 44 strains of *B. bruxellensis* listed belong to genetic groups that are more or less resistant to $SO_2$[8].



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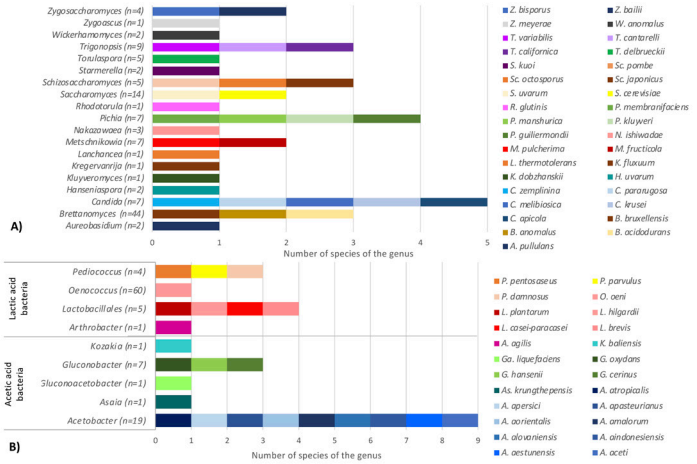


**FIGURE 2.** Number of species of the 19 yeast genera (A) (118 isolates) and the 9 bacteria genera (B) (99 isolates) added to the enological database (the number of strains of each genus is noted in brackets).

Around one hundred bacterial isolates including 17 species of acetic acid bacteria and 9 species of lactic acid bacteria (Figure 2-B) are now listed, of which 7 were initially absent, especially for the *Acetobacter* genus of acetic acid bacteria. The 60 strains of *Oenococcus oeni* listed in the "OENO" spectral database belong to different genetic groups, suitable for must or different types of wine[9].

## Validation of the method for must and wine analysis

To validate the effectiveness of the method, over 10,000 yeast and bacteria colonies from musts[10] and wines were analyzed using the manufacturer's database alone or supplemented with the new "OENO" spectral database in various studies conducted during the 2020 and 2021 vintages.

Figure 3 shows that the "OENO" database significantly improves the identification of all yeast and bacteria species, regardless of their origin. Addition of the "OENO" library allows successful identification of over 99% of yeast isolates compared with only one-third using the manufacturer's library (DB) alone (Figure 3-A). Just under half of enological bacteria are identified with the manufacturer's DB library alone, whereas 91% of bacterial isolates are successfully identifiable with the addition of the "OENO" spectral database.

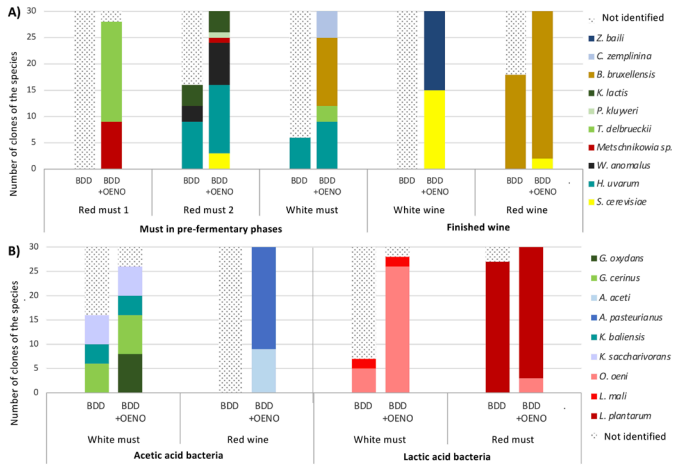


**FIGURE 3.** Proportion of each species of yeasts (A) and bacteria (B) among 30 clones analyzed by MALDI-TOF/MS and isolated from musts or wines using the manufacturer's spectral database (DB) alone, or with the addition of the specific enological microorganism library (DB+OENO).

This new database is essential for analysis of the microbial biodiversity of musts but also for quick identification of wine spoilage agents such as *B. bruxellensis*.

## Conclusion

Creating a new "OENO" database has made it possible to adopt MALDI-TOF mass spectrometry as a routine analytical tool for reliable identification in one hour of enological yeasts and bacteria previously isolated on agar nutrient media at the ISVV. This method can be used for the microbiological analysis of musts and wines, as well as for biodiversity studies, particularly in the context of reducing doses of $SO_2$. ■

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