Development of a MALDI-TOF/MS method for monitoring grape and wine microorganims

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MICROFLORA
L'EXPERTISE EN MICROBIOLOGIE
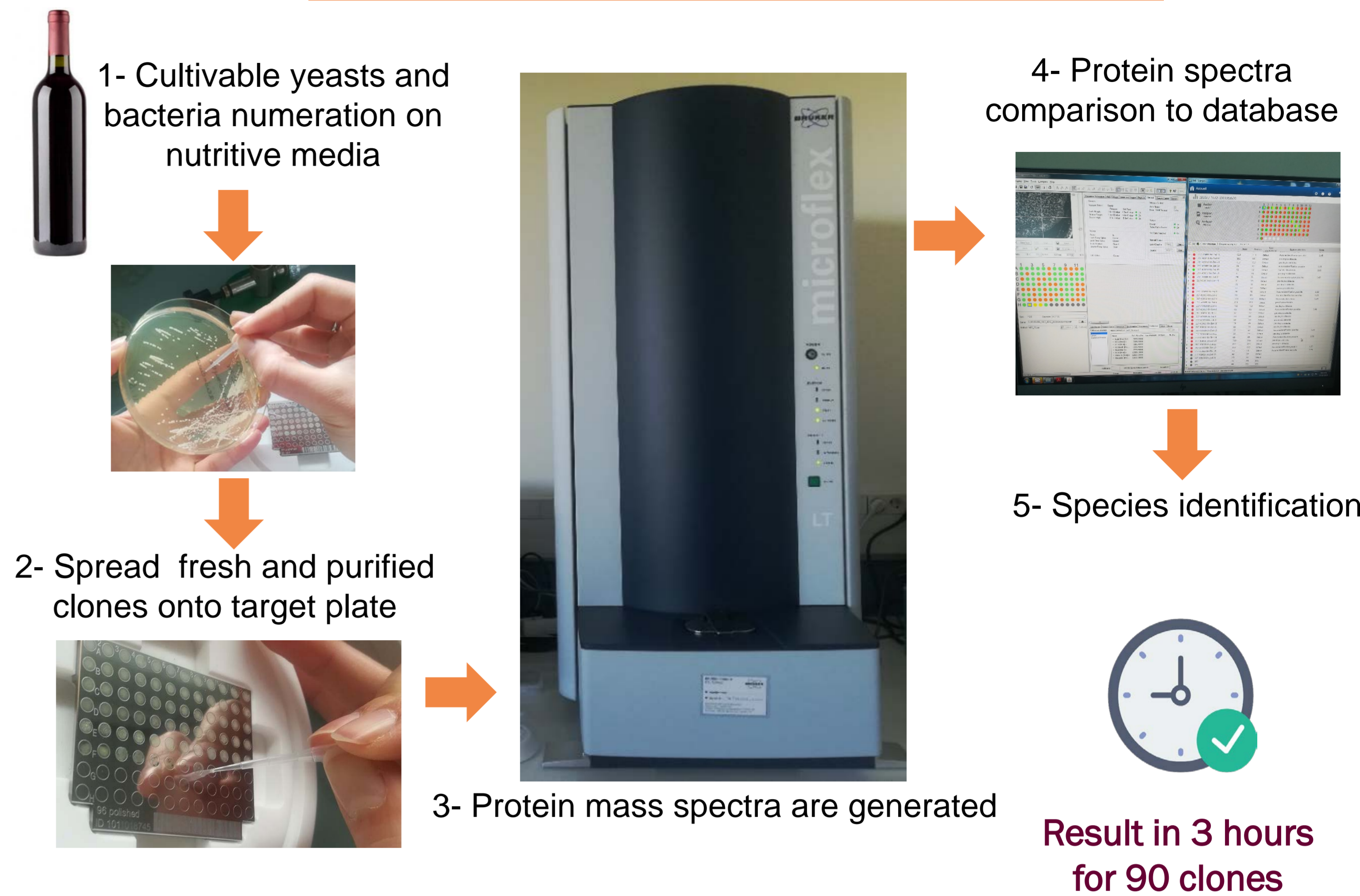Unité de Recherche Œnologie

Aim of study

Since the last 15 years, Matrix-Assisted Laser Desorption Ionization-Time of Flight/Mass Spectrometry (MALDI-TOF/MS) is a widely used method for microorganism identification in medical sector. In comparison with conventional microbiological methods used to identify yeasts and bacteria isolated from grape and wine, MALDI-TOF/MS offers numerous benefits: reliable, quick, cheap and easy to use. This new tool allows evaluation of cultivable microbial community by analysing a large number of colonies.

Recently, MALDI-TOF/MS has been used to study microorganisms from fermented products such as beer and wine (1,2,3). However, this method should be optimized to obtain reliable identification of microorganisms from wine related environment.
As MALDI-TOF/MS is based on a comparison to a reference database, currently mainly composed of medical microorganisms, it is necessary to create an oenological database of wine yeasts and bacteria.
This work reports the first application of MALDI-TOF/MS for analysing the wine microbial community.

MALDI-TOF/MS species identification

1- Cultivable yeasts and bacteria numeration on nutritive media

2- Spread fresh and purified clones onto target plate

3- Protein mass spectra are generated

4- Protein spectra comparison to database

5- Species identification

Result in 3 hours for 90 clones

Oenological Database

Why a specific Oenological Database?

1- To identify species absent in Brucker Database
Currently, more than 7854 Entries
(89% bacteria and 11% yeast)

2- To improve score identification of microbial community of grape and wine
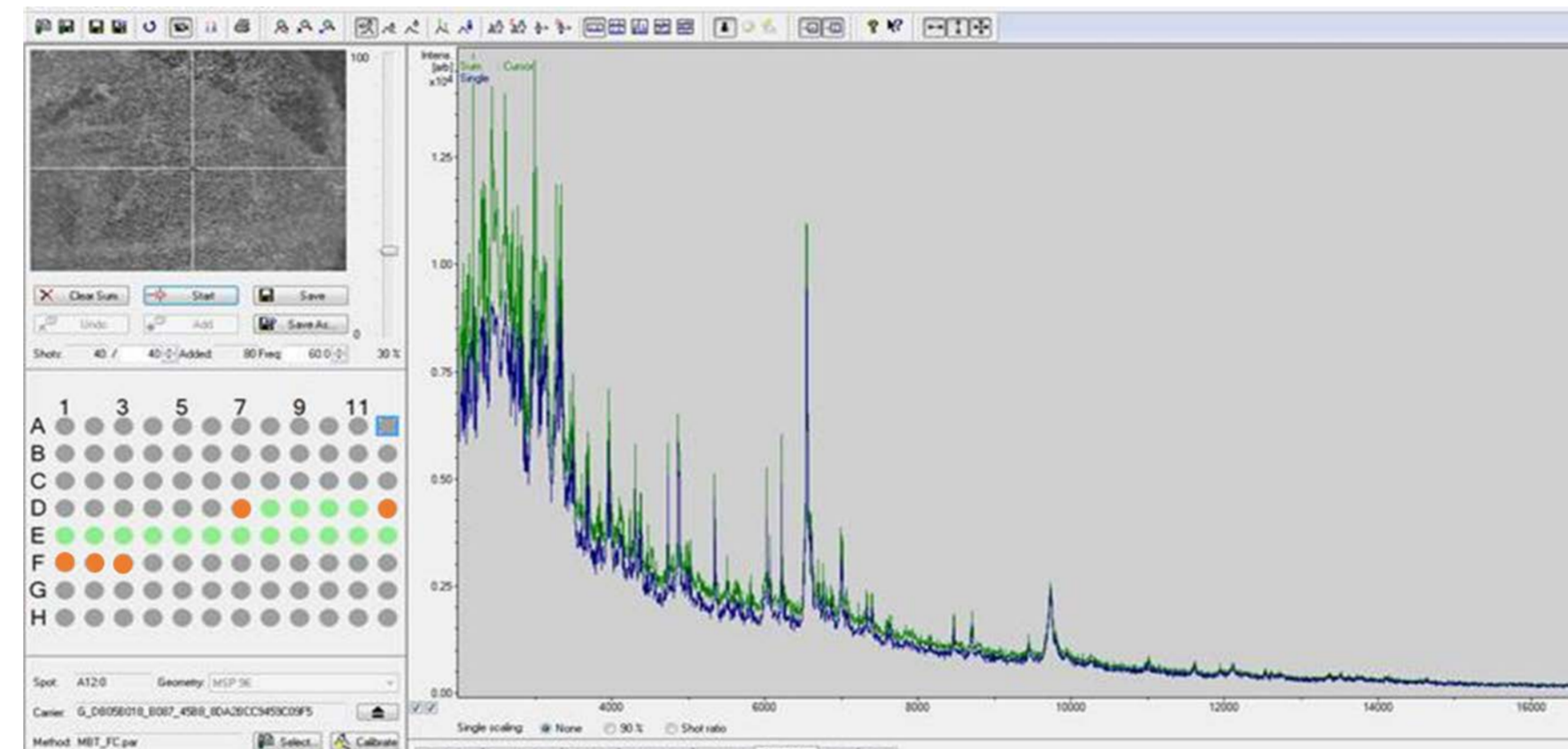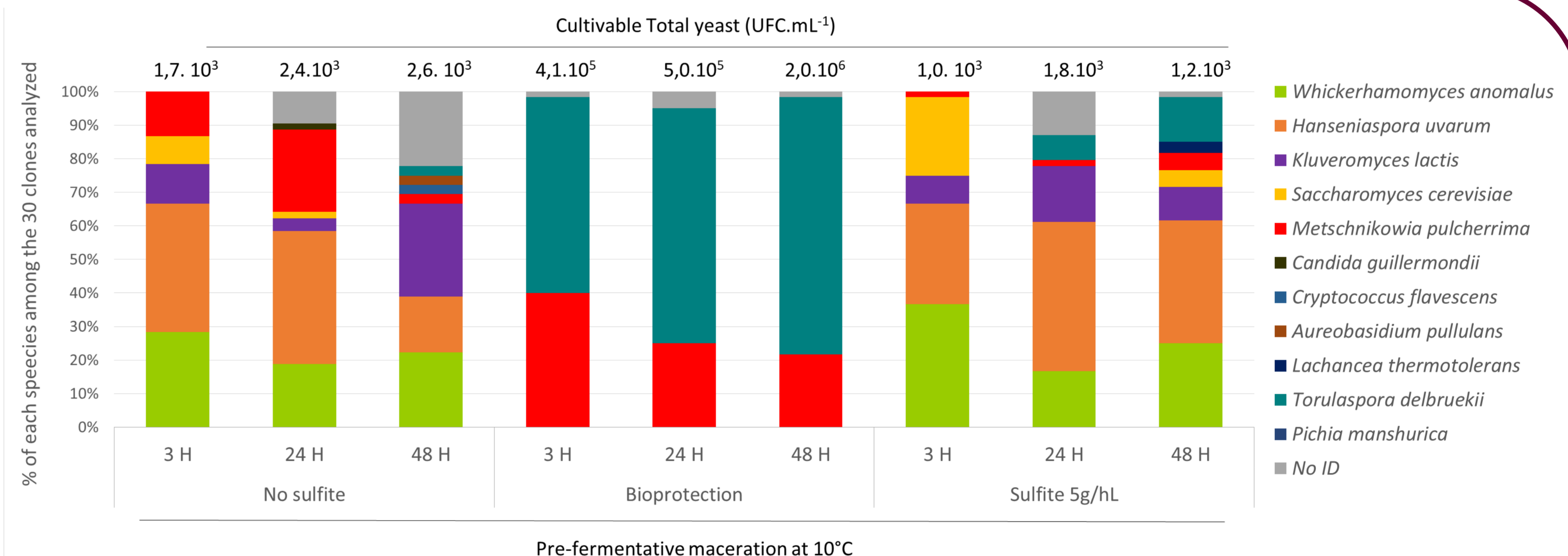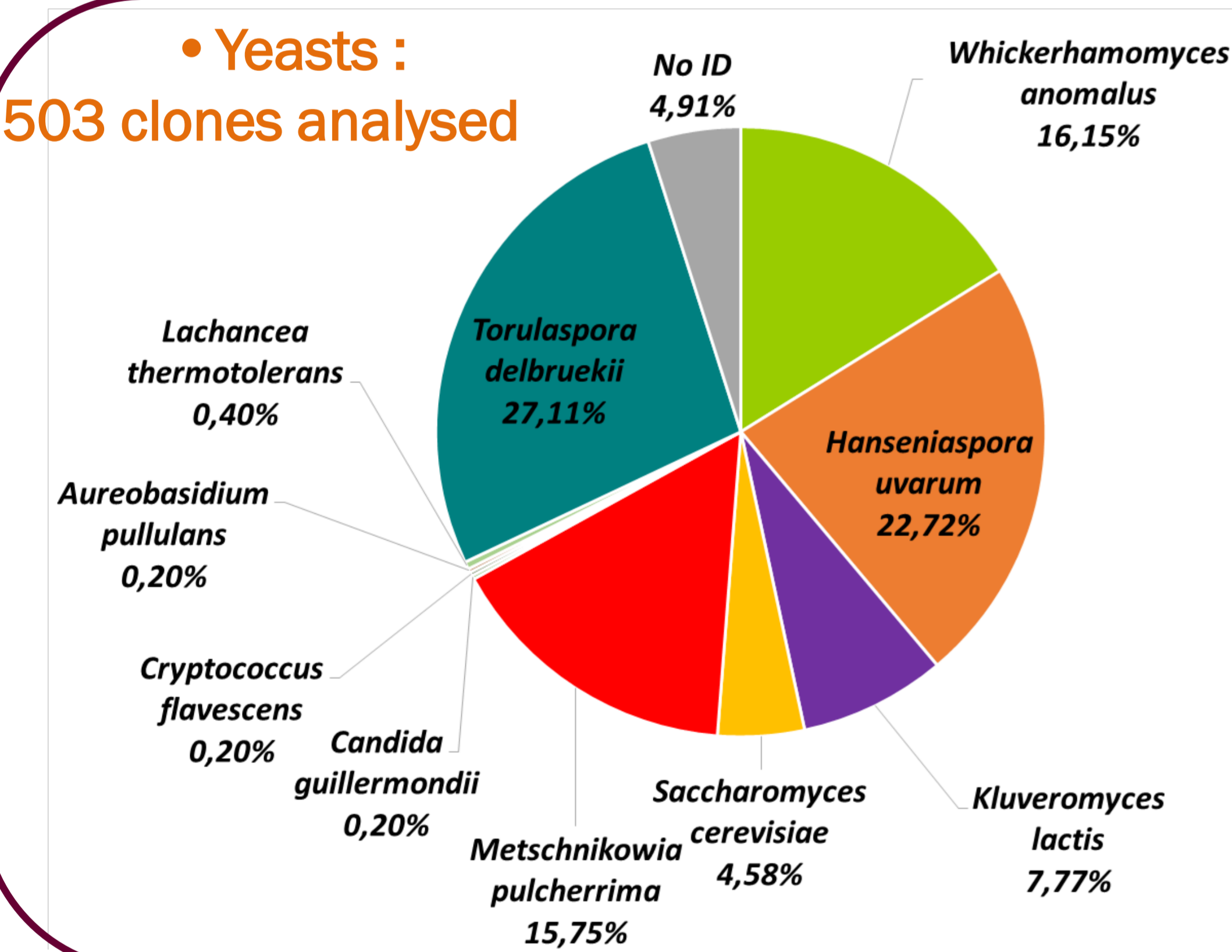
Oenological Database under development

Microorganisms from BRC Oeno
(Biological Ressource Center Œnology)

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- **Yeasts: 27 entries**
(13 distinct species)

- **Bacteria: 25 entries**
(16 distinct species)

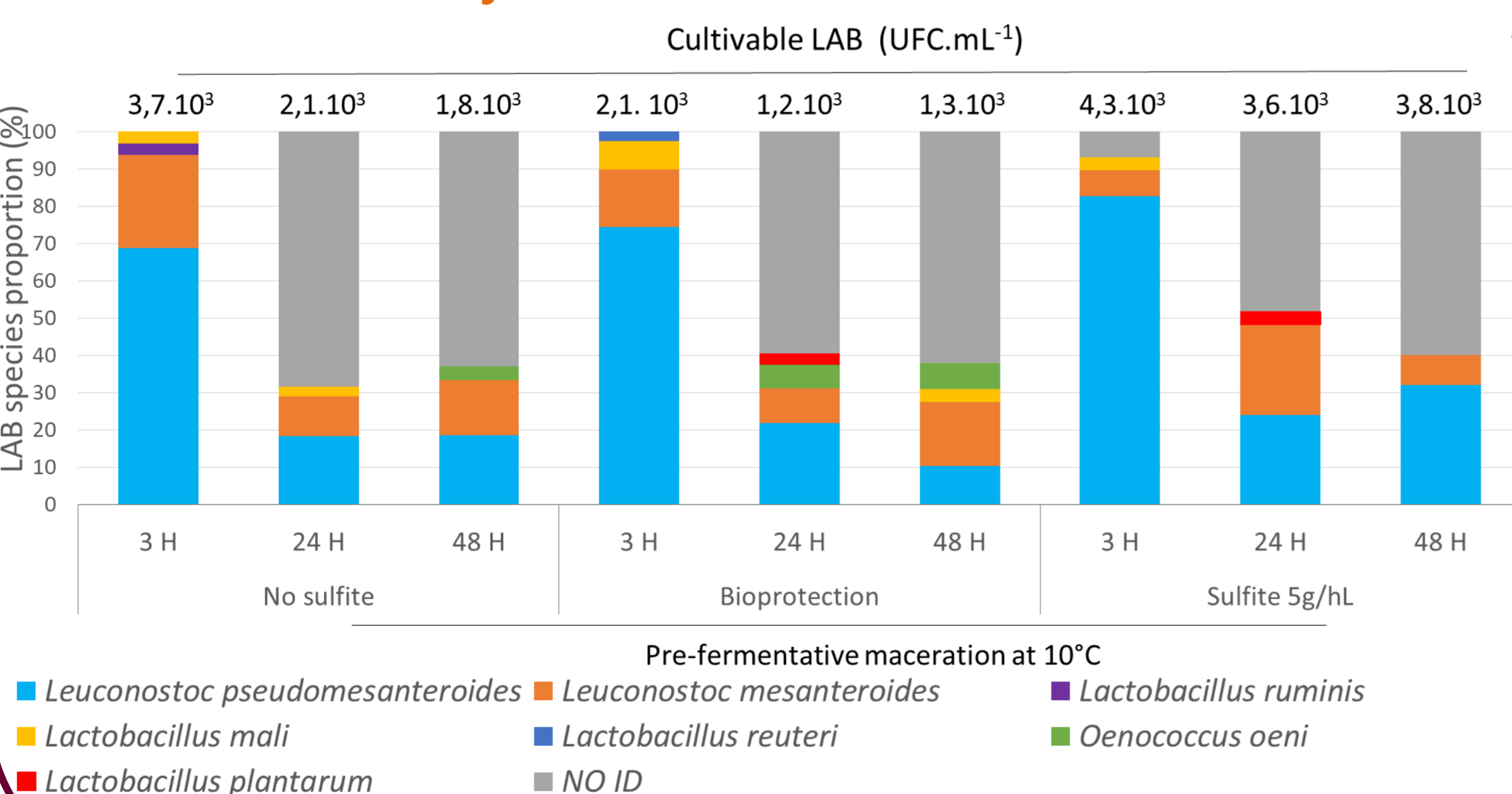**More than 50 strains present on the Oenological Database**

Microbial community identification

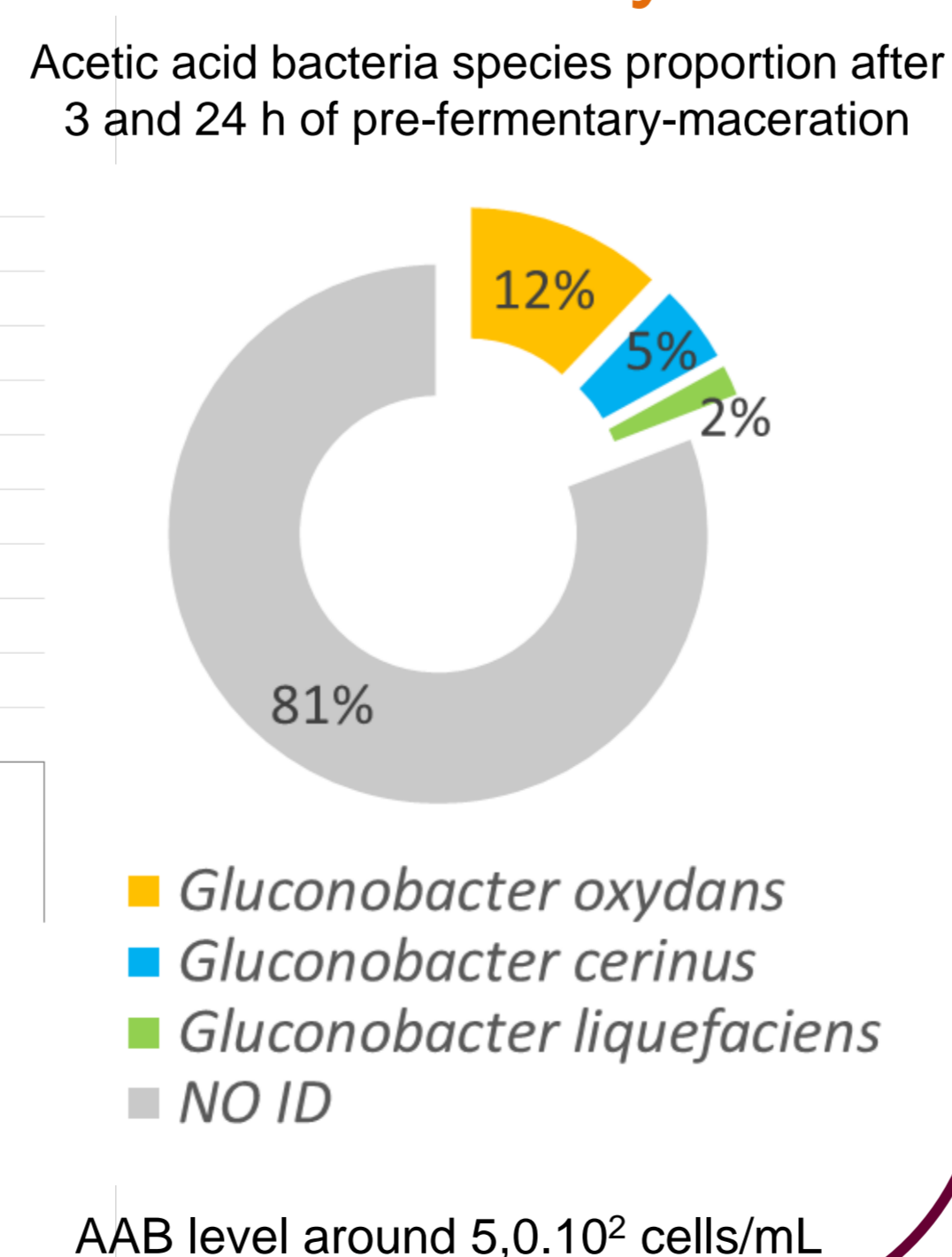### Identification of yeasts and bacteria isolated from must in prefermentative maceration (Merlot, 2018)

• Yeasts : 503 clones analysed

- Whickerhamomyces anomalus 16,15%
- No ID 4,91%
- Torulaspora delbruekii 27,11%
- Lachancea thermotolerans 0,40%
- Aureobasidium pullulans 0,20%
- Cryptococcus flavescens 0,20%
- Candida guillermondii 0,20%
- Metschnikowia pulcherrima 15,75%
- Saccharomyces cerevisiae 4,58%
- Kluveromyces lactis 7,77%
- Hanseniaspora uvarum 22,72%

Cultivable Total yeast (UFC.mL-1)

Legend:
- Whickerhamomyces anomalus
- Hanseniaspora uvarum
- Kluveromyces lactis
- Saccharomyces cerevisiae
- Metschnikowia pulcherrima
- Candida guillermondii
- Cryptococcus flavescens
- Aureobasidium pullulans
- Lachancea thermotolerans
- Torulaspora delbruekii
- Pichia manshurica
- No ID

Pre-fermentative maceration at 10°C (No sulfite / Bioprotection / Sulfite 5g/hL — 3 H, 24 H, 48 H)

95% of correct identification validated by sequencing

Microbial community (bacteria)

• Lactic Acid Bacteria : 280 clones analysed

Cultivable LAB (UFC.mL-1): 3,7.10³, 2,1.10³, 1,8.10³, 2,1.10³, 1,2.10³, 1,3.10³, 4,3.10³, 3,6.10³, 3,8.10³

Pre-fermentative maceration at 10°C (No sulfite / Bioprotection / Sulfite 5g/hL — 3 H, 24 H, 48 H)

- Leuconostoc pseudomesanteroides
- Leuconostoc mesanteroides
- Lactobacillus ruminis
- Lactobacillus mali
- Lactobacillus reuteri
- Oenococcus oeni
- Lactobacillus plantarum
- NO ID

• Acetic Acid Bacteria : 141 clones analysed

Acetic acid bacteria species proportion after 3 and 24 h of pre-fermentary-maceration

- 12%
- 5%
- 2%
- 81%

- Gluconobacter oxydans
- Gluconobacter cerinus
- Gluconobacter liquefaciens
- NO ID

AAB level around 5,0.10² cells/mL

Discussion and perspectives

MALDI-TOF/MS is validated on more than 60 distinct species of yeasts and bacteria isolated from oenological samples (>3000 clones anlaysed)

This study validate the MALDI-TOF/MS method to successfully identify wine microorganisms.
Protocols have been adapted to identify wine yeasts and bacteria, using wine specifics culture and media conditions which are different from existing database.
Moreover, some wine specific microorganism such as O. oeni, B. bruxellensis, C. cantarelli and S. bacillaris (C. zemplinina) and most of acetic acid bacteria are absent or poorly represented in existing database. It was necessary to create the first specific database of MALDI-TOF/MS spectra of microorganisms isolated from wine related environment provided by BRCOeno.

Although these first results from vintage 2018 are very encouraging for yeasts, the implementation of the "oenological database", today composed of more than 50 oenological strains, should be continued, particularly for oenological bacteria.

1- Wieme A. D, Spitaels F., Aerts M., De Bruyne K., Van Landschoot A., Vandamme P., 2014.Identification of beer-spoilage bacteria using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Int J Food Microbiol. 18; 185:41-50. 29.
2- Turvey M.E., Weiland F., Mesneses J., Sterenberg N.,Hoffman P. 2016. Identification of beer spoilage microorganisms using the MALDI Biotyper platform. Appl Microbiol Biotechnol. Mar;100(6):2761-73.
3- Gutiérrez C., Gómez-Flechoso M.A., Beldal., Ruiz J., Kayali N., Polo L. and Santos A., 2017. Wine yeasts identification by MALDI-TOF MS: Optimization of the preanalytical steps and development of an extensible opensource platform for processing and analysis of an in-house MS database. Int J Food Microbiol. 2017 August 254:1-10.

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