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Microflora variability among grape musts in Languedoc: the case of two grape varieties during two years

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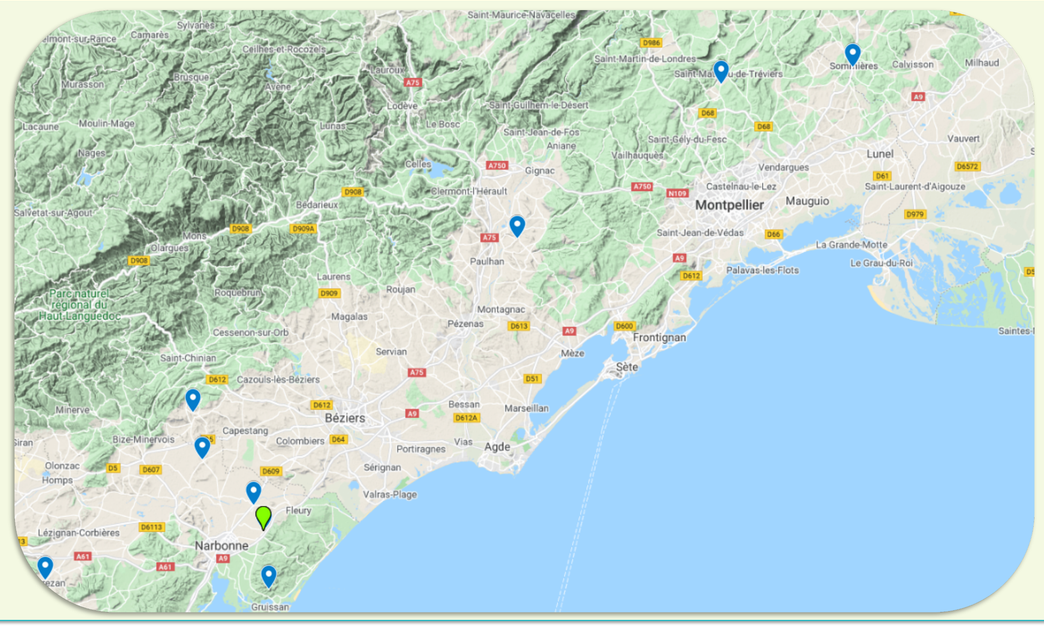
Introduction and objectives

The wine fermentation ecosystem is very likely one of the most explored microbial ecosystem since the pioneer work of Pasteur. Microbiology has considerably evolved since then, and recent metabarcoding based studies have renewed its vision. However, very few studies combine metabarcoding analyses to culture-based making as well as these microbial genetic resources available. In addition, many recent works suggested that the non-*Saccharomyces* component of grape must microflora impacts wine quality. In this context, we undertook the characterization of two grape must varieties by combining metabarcoding analyses and cultural approach on two consecutive years.

Experimental Design

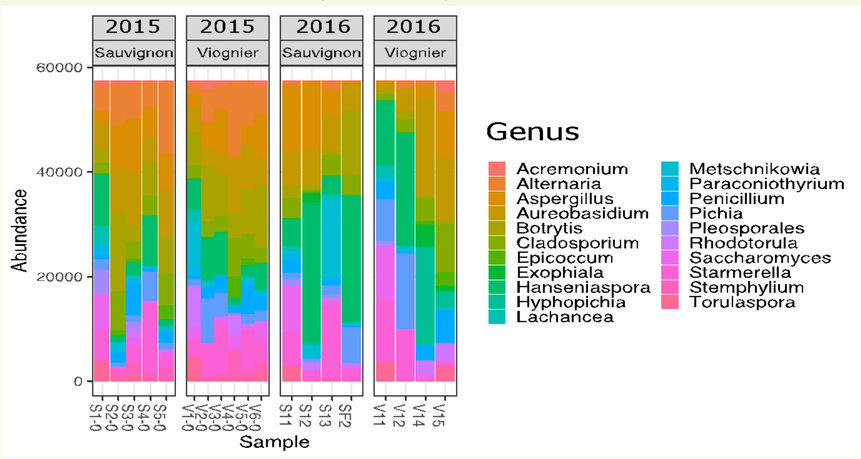
- 20 Samples of grape musts of Viognier and Sauvignon grape varieties collected in 2015 and 2016 on 8 sampling sites.
- Bacterial and fungal component of the microbiota characterized using classical culture approaches (18) and metabarcoding (19) using the V4 region of the 16S rRNA gene for bacteria and the ITS1 region for fungi.
- DNA was extracted using a Qiagen Plant Dneasy kit and amplified targets were sequenced using 2x250 MiSeq v2 sequencing (Illumina). After filtering for quality, reads were analysed using FROGS pipeline (frogs.toulouse.inra.fr).



Diversity encountered in the grape must microbiote

Yeast

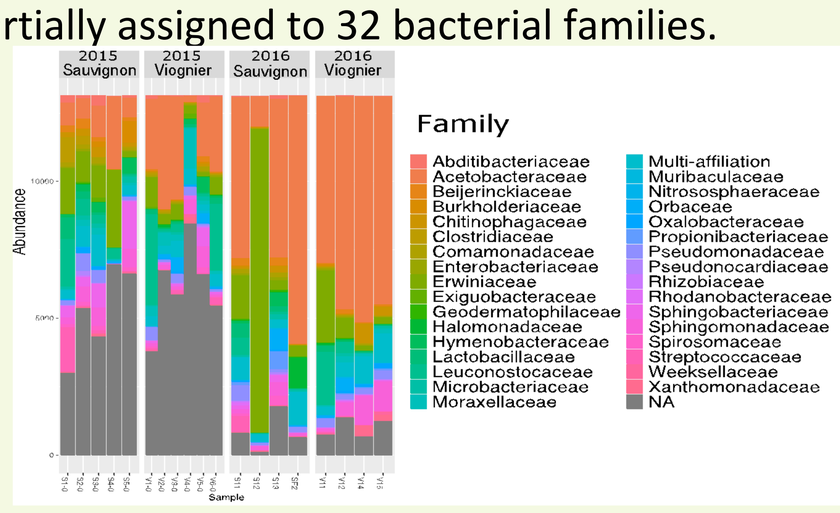
32 fungal species were detected at a frequency higher than 1%, and contain the main yeast species encountered in grape must



Collection and characterization of 994 yeast isolates
 29 yeast species, mainly of the Ascomycetes division.
Hanseniaspora, *Pichia*, *Starmerella and Saccharomyces cerevisiae* were detected in more than 50 % of the samples

Bacteria

107 bacterial OTU were detected at a frequency higher than 2%, and partially assigned to 32 bacterial families.



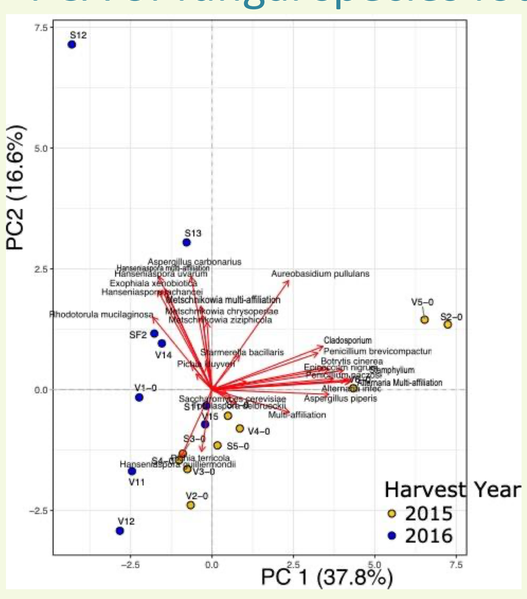
Collection of 1036 isolates, among which 569 were selected for identification by DNA 16S sequencing.
 88 different species were identified belonging to 36 genera and 3 distinct phyla: Firmicutes (48%), Proteobacteria (39%) and Actinobacteria 14%.
Among lactic acid bacteria, ***Lactiplantibacillus plantarum*** is the most represented species and was found in 86% of samples.

Evaluation of different factors on Grape must Microbial diversity

The evaluation of these factors has been done from the metabarcoding data (Cultural methods did not reveal clear differences)

	Bacteria			Yeast / Fungi		
Factor	Observed	Shannon Index	Simpson Index	Observed	Shannon Index	Simpson Index
Grape Variety	0.936	0.529	0.682	0.272	0.900	0.684
Harvest year	0.321	**0.002**	**0.002**	0.053	**0.008**	**0.006**
Departement	0.671	0.541	0.296	0.918	0.844	0.453
Locality	0.259	**0.014**	0.112	0.169	**0.032**	0.075

PCA of fungal species found for two harvest years



=> PCA reveals clear differences in fungal species (i.e. *B cinerea*) with harvest year suggesting differences in sanitary conditions.

Harvest year and locality are the two main factors that drive grape microbiota diversity

Conclusion

This sampling enabled us to show the effect of the "Millesime" on the grape microbiota, very likely through its climate, and of the locality. We could not detect regional differences, very likely because of insufficient power.
Bacterial and yeast strains collected were integrated to both INRAE BRC: CIRM-BIA (https://collection-cirmbia.fr/) and CIRM-Levures (https://cirm-levures.bio-aware.com/) where they are distributed for research and development purpose.

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