

<sup>1</sup>UMR Sciences pour l'Œnologie, Microbiologie et Technologie des fermentations INRA, 2 place Viala, 34060 Montpellier, France.

<sup>2</sup>IMIDRA. Instituto Madrileño de Investigación y Desarrollo Rural Agrario y Alimentario. Madrid. Spain. E-mail: [eva.valero@madrid.org](mailto:eva.valero@madrid.org)

<sup>3</sup>Centro de Ciências do Ambiente, Departamento de Biologia, Universidade do Minho, 4710-057 Braga, Portugal.

\*Contributed equally to this work

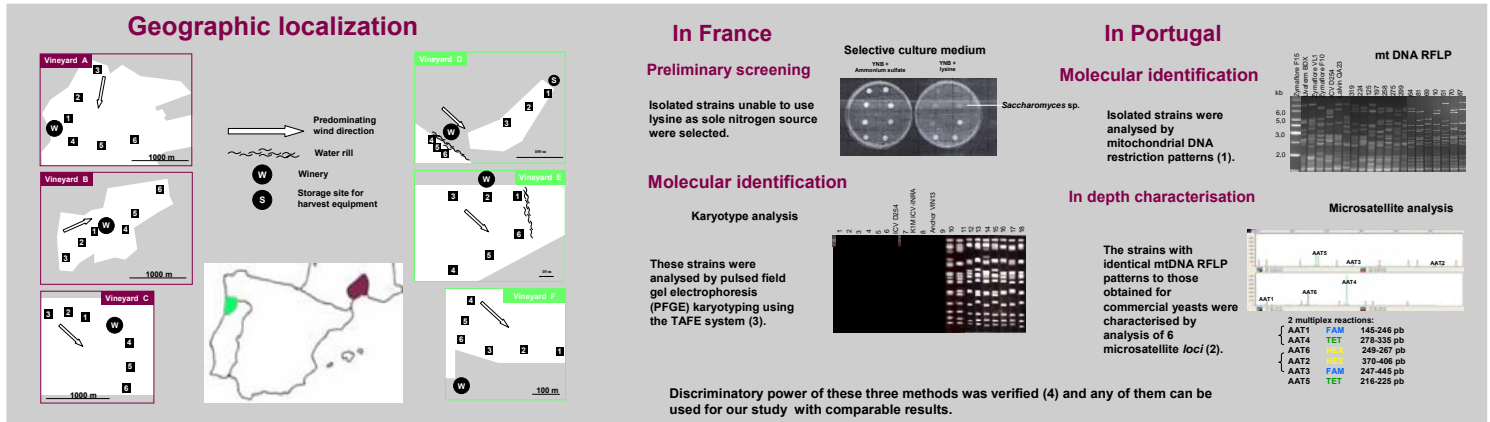
## Introduction

Modern winemaking practices and diversification of wine products involve an increasing quest for specialised wine yeasts. The use of commercial wine yeast strains as starters has been extensively generalised over the past two decades. Wine yeast strains are annually released into the wineries' environment. However, little is known about the fate of these strains in the vineyard. In this study a large-scale sampling plan was established with the aim of evaluating the industrial starter yeasts' ability to spread and survive in nature. This study provides a consistent assessment of potential environmental risks associated with the use of genetically engineered winery yeast strains using commercial wine yeast as a model.

## Material and Methods

**Sampling plan:** This includes 36 sites in 6 vineyards, 3 in France (A, B, C) and 3 in Portugal (D, E, F) that have used industrial starter yeasts for at least 5 years. Samples were taken before harvest (annual remanence) and at late harvest (immediate release), at 3 distances from the winery (20-1000 m) and from opposite directions. The overall duration of these studies was 3 years.

**Yeast isolation:** For each sample about 2 Kg of grapes were collected to perform small-scale fermentations (0.25-0.5 l). Must samples were plated when 70 g/l of CO<sub>2</sub> were released and 30 randomly selected colonies were analysed.



## Results

### Distribution of the global data by country and year

	2001		2002		2003		TOTAL
	France	Portugal	France	Portugal	France	Portugal	
Samples	36	36	36	18	36	36	198
Spontaneous fermentations	24	19	33	12	15	23	126
Isolates	720	570	990	360	450	690	3780
Saccharomyces strains	406	570	120	360	293	690	2355

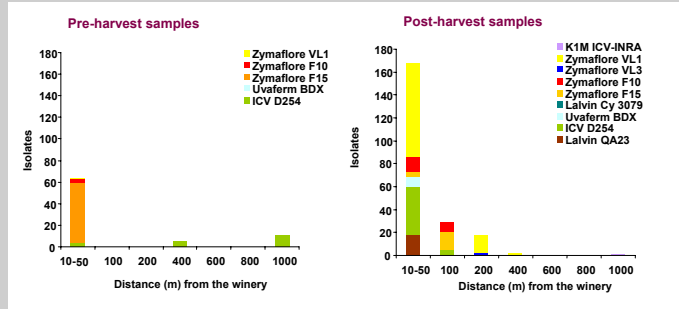
### Commercial yeast strains recovered in each vineyard over the 3 years

Vineyards	A	B	C	D	E	F	TOTAL
Spontaneous fermentations	19	24	29	16	23	15	126
with 21 commercial yeast strains	0	2	1	11	9	2	25
Isolates	570	720	870	480	690	450	3780
Commercial yeast strains	0	15*	1	206	54+18*	2	296
% Commercial yeast strains/nb of isolates	0	2	0.1	42	10	0.5	7.8

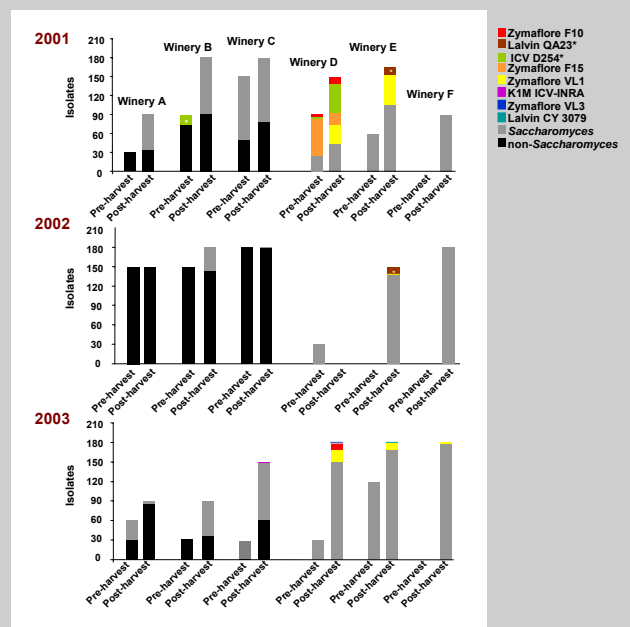
\*strains isolated in the same region

In four vineyards (A, B, C in France and F in Portugal) where the sampling sites were placed at a greater distance from the winery, the occurrence of commercial yeast was very low, between 0-2% of the fermentative yeast community. The large majority (94%) of the commercial strains isolated within the six vineyards were recovered from Portuguese vineyards D and E, and 70% solely from vineyard D.

### Distribution of commercial yeast strains according to the distance from the wineries



### Evolution of the total fermentative yeast communities from each of the wineries during the 3 years



The same strains were not found in the same sites from one year to another. This indicates that if some commercial yeast strains are able to remain in the ecosystem, as suggested by the presence of commercial yeasts in pre-harvest samples taken in 2001 in Portugal, their permanence is limited and they are not capable of dominating the natural yeast community of the vineyard.

## Conclusions

- Commercial yeasts represented 7.8% of the fermentative yeast community, the majority of which (5.8%) were recovered in post-harvest campaigns indicating immediate dissemination.
- The dissemination of commercial yeast strains in the vineyard can be detected mainly in close proximity to the winery (10-50 m).
- Liquid effluents are an important vector for the release of yeasts into the environment.
- Permanent implantation of commercial strains in the vineyard did not occur, instead these strains were subject to natural fluctuations of periodical appearance / disappearance like autochthonous strains.
- Significant genetic diversity is found in the different vineyards, varying from year to year.

These results indicate that the dissemination of commercial yeasts in the vineyard and their impact on the ecosystem is very limited in terms of space and time. The associated risks of contamination of grape must are consequently very weak. Considering commercial yeast strains as an appropriate model system for genetically modified yeast strains, our data can contribute to the in-depth environmental risk assessment concerning the use of such strains in the wine industry.

## References

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