Assessment of environmental impact of commercial wine yeast in vineyard ecosystems

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Introduction

Modern winemaking practices and diversification of wine products involve an increasing quest for specialised wine yeasts. During the last two decades, considerable efforts have been made to improve wine yeast strains through the use of new biotechnologies. Today, about 50% of European wine production is based on the use of active dried yeast. Commercial yeasts are classically used in winemaking without any special containment and are released into the environment in large numbers with various effluents and by-products. The behaviour of these yeasts in natural habitats is practically unknown, as well as their potential impact on the natural microflore. There is very little available data that could contribute to the evaluation of the importance of starter yeast dissemination and permanence in the vineyard (Frezier and Dubourdieu, 1992; Vezinhet *et al.* 1992; Guillamón *et al*., 1996). Recently, a large-scale biogeographical study in South African vineyards was carried out in five areas situated in the Coastal Region vineyards of the Western Cape. Commercial yeasts were recovered in 3 of 13 samples (van der Westhuizen *et al*., 2000a and 2000b).

In the present 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

The sampling plan included 36 sites in 6 vineyards, 3 of which were located in the South of France (A, B and C) and 3 in the North of Portugal (D, E and F). The total

duration of these studies was 3 years (2001-2003). The wineries selected had used one or more commercial yeast strains consecutively in the last 5 years. In each vineyard, six sampling points were defined according to the local conditions (size and orientation of the vineyard, predominating wind direction), and the distance between the winery and the sampling sites varied between 20 to 1000 m. In order to evaluate the remanence of commercial yeast over the years, a first sampling campaign was performed before the winery started wine production with the use of commercial yeast strains (pre-harvest campaigns). In a second post-harvest sampling campaign, the grapes were collected after the onset of wine production, in order to evaluate the immediate commercial yeast dissemination from the winery. With the present experimental design, 72 grape samples were collected each year.

From each sampling point, approximately 2 kg of grapes were aseptically collected, and the extracted grape juice was fermented at 20ºC. Daily weight determinations allowed the monitoring of the fermentation progress. The yeast flora was analysed when the must weight was reduced by 70 g/l, corresponding to the consumption of about 2/3 of the sugar content. Must samples were diluted and spread on plates with YPD medium, and after 2 days of incubation 30 randomly selected colonies were collected from each spontaneous fermentation and subjected to molecular identification.

Results and discussion

A total of 198 samples were collected during three consecutive campaigns (2001-2003), 108 of which were taken in France and 90 in Portugal. Of the 198 samples, 126 musts (64%) produced spontaneous fermentations, 20% and 44% in must from pre-harvest and post-harvest campaigns respectively. The percentages of spontaneous fermentations were similar in both countries. A total of 3780 colonies were isolated, of which 2355 were identified as *Saccharomyces* strains. Molecular characterisation of these led to the identification of 296 strains with a genetic profile similar to that of commercial yeasts (Table 1). These strains represent 7.8% of the fermentative yeast community, the majority of which (5.8%) were recovered in post-harvest campaigns. The global data reflects a very different situation. In 4 out of 6 vineyards (3 French vineyards and vineyard F in Portugal) where the sampling sites were placed at a greater distance from the winery, the occurrence of commercial yeast was very low, representing between 0% and 2% of the fermentative

community, and these strains were isolated from only five samples (Table 1). The results were very different in the Portuguese wineries D and E, for which a high number of commercial strains was isolated representing 43 and 10% of the fermentative yeast community respectively.

An overview of the dissemination of commercial strains in relation to their distance from the winery is shown in Figure 1. Ninety four percent of commercial strains were found in a radius of around 10-200 m from the winery and a large majority (78%) was recovered in sites at very close proximity (10-50 m) to the wineries (vineyards D and E). A major proportion (73%) was collected in post-harvest campaigns, indicating immediate dissemination.

As a whole, the evolution of the fermentative yeast communities over the three years studied showed that the same strains were not found in the same sites from one year to another. This indicates that if some of these strains are able to remain in the ecosystem, they are not capable of dominating the natural yeast community of the vineyard.

Conclusion

This study has provided new insight into the impact of commercial yeasts on the communities of fermentative yeast microflore surrounding vineyards. The data show that dissemination of commercial yeasts in the vineyard is restricted to short distances and limited periods of time and largely favoured by the present of liquid effluents. More than 90% of commercial yeasts were found at a radius between 10 and 200 m from the winery and did not become implanted in the ecosystem in a systematic way. Given that they are used in large quantities, commercial strains tend to out-compete autochthonous strains inside the winery (Beltran et al, 2002). In contrast, they do not become implanted systematically in the ecosystem and are not able to dominate the natural microflore although they are subject to natural fluctuations of periodical appearance and disappearance in the same way as autochthonous strains are. Considering commercial yeast strains as an appropriate model system for genetically modified yeast strains, our data also contribute to the in-depth environmental risk assessment concerning the use of such strains in the wine industry.

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Tables and Figures

Table 1: 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
Isolates	570	720	870	480	690	450	3780
Commercial yeasts strains	0	15*	1	206	54+18*	2	296
% Commercial yeast / nb of isolates	0	2	0.1	43	10	0.5	7.8

*Commercial yeasts initially isolated in the same region

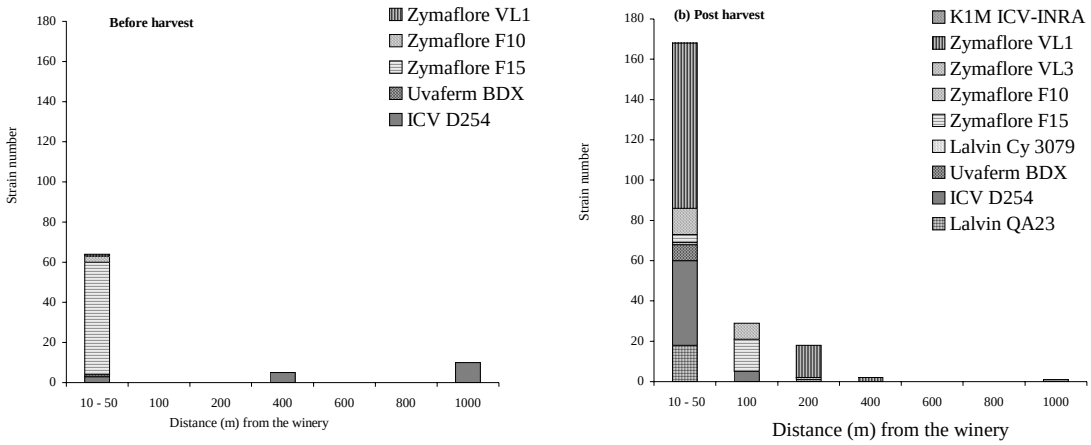

Fig. 1. Overall (three years) distribution of commercial yeast strains according to the distance from the wineries in pre-harvest (a) and in post-harvest (b) campaigns.