Dissemination and survival of commercial wine yeast in the vineyard: a large-scale, three years study

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Abstract

The use of commercial wine yeast strains as starters has been extensively generalised over the past two decades. In this study, a large-scale sampling plan was devised over a period of three years in six different vineyards to evaluate the dynamics and survival of industrial yeast strains in the vineyard. A total of 198 grape samples were collected at various distances from the wineries, before and after harvest, and yeast strains isolated after spontaneous fermentation were subsequently identified by molecular methods. Among 3780 yeast strains identified, 296 isolates had a genetic profile identical to that of commercial yeast strains. For a large majority (94%), these strains were recovered at very close proximity to the winery (10-200m). Commercial strains were mostly found in the post harvest samples, reflecting immediate dissemination. Analysis of population variations from year to year indicated that permanent implantation of commercial strains in the vineyard did not occur, but instead that these strains were subject to natural fluctuations of periodical appearance/disappearance like autochthonous strains. Our data show that dissemination of commercial yeast in the vineyard is restricted to short distances and limited periods of times and is largely favoured by the presence of water runoff.

Introduction

Today, the majority of wine production is based on the use of active dried yeast, which ensures rapid and reliable fermentations, and reduces the risk of sluggish or stuck fermentations and of microbial contaminations. Most commercial wine yeast strains available today have been selected in the vineyard for enological traits such as fermentation performance, ethanol tolerance, absence of off-flavours and production of desirable metabolites. These and other technological developments have contributed to an improvement in wine quality, and enhanced the ability of winemakers to control fermentation processes and achieve specific outcomes.

Recombinant DNA technologies have been successfully applied to wine yeast, generating specialized wine strains, engineered for specific traits such as improved fermentation performance and process efficiency, wine sensory quality and health benefits for consumers [1-7].

From the perspective of a future possible use of genetically modified wine yeasts, a sound evaluation of the potential environmental impact of genetically modified wine yeast is absolutely required. In this context, industrial yeasts used as commercial fermentation starters are a good study model to evaluate the competition and the influence of inoculated strains on the fermentations of the following years, especially those performed according to traditional practices which rely on spontaneous fermentations. Commercial yeasts are classically used in winemaking without any special containment and are annually released in large quantities, together with liquid and solid wine-making residues, in the environment around the winery. The behaviour of these yeasts in the ecosystem of the vineyard is totally unknown as is their potential impact on the natural microflora. In particular, it is not known if commercial strains are able to survive in nature and to become members of the vineyard microflora.

The present large-scale study, which was carried out in different geographical localizations of France and Portugal, aims to evaluate the industrial starter yeasts' ability to spread and survive in nature.

Materials and Methods

Sampling plan / wineries selection

Grapes were harvested during three consecutive years (2001-2003) in six vineyards, three in the south of France (Languedoc) and three in northwest Portugal (Vinho Verde Region). The commercial strains K1M ICV-INRA and VL1 (Lallemand) were predominantly used during at least the last 5 years by the French and Portuguese wineries, respectively. In each vineyard, six sampling points were defined according to the predominating wind direction at a distance between 20 and 1000 m from the winery. In order to evaluate the permanence of commercial yeast over years, a first sampling campaign was performed before the winery started wine production with the use of commercial yeast strains (pre-harvest samples). 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.

Fermentation

The yeast flora from fermenting grape juice was analysed when the must weight was reduced by 70 g/l. Must samples were diluted and spread on YPD plates.

Thirty randomly selected colonies were collected from each spontaneous fermentation and subjected to further analysis.

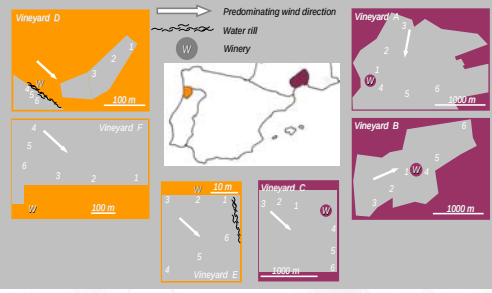
Selection of Saccharomyces sp.

All isolates that were not able to grow on the YNB medium with L-lysine as the sole nitrogen source were considered as Saccharomyces sp. and selected for molecular identification.

Molecular identification

DNA was extracted from yeast cells cultivated in 1 ml YPD medium as previously described [9] with a modified cell lysis procedure, using 25 U of Zymolase (SIGMA, USA). Cell lysis was dependent on the strain and lasted between 20 minutes and 1 hour (37°C).

Several molecular identification methods (mitochondrial DNA restriction profiles, microsatellite analysis and chromosomal profiles were used as described [9-11] and their equivalence has also been demonstrated [12].

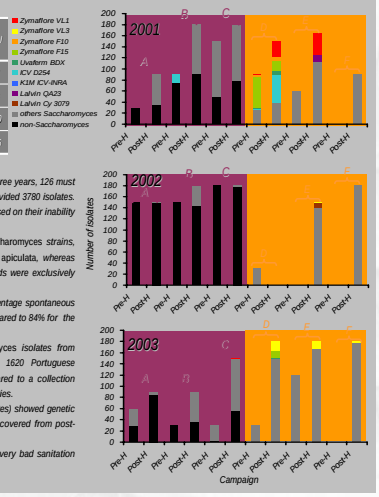


Geographic localization of the vineyards belonging to the Languedoc (A, B, C) and Vinho Verde (D, E, F) wine regions with indication of the sampling sites. In each site, 2 samples (pre- and post-harvest campaign) were collected. Factors that may influence the dissemination of yeasts are also indicated.

RESULTS

Distribution of the total fermentative yeast communities from wineries in France (F) and Portugal (P) during three years in pre- and post-harvest campaigns (pre-H and post-H)

	2001		2002		2003		Total
	F	P	F	P	F	P	
Number of samples	36	36	36	18	36	36	198
Spontaneous fermentations	24	19	33	12	15	23	126
Number of isolates	720	570	990	360	450	690	3780
Saccharomyces strains	406	270	139	60	208	592	2355



- From a total of 198 grape samples collected during three years, 126 must samples started a spontaneous fermentation and provided 3780 isolates. 2355 of them belong to the genus Saccharomyces based on their inability to grow on YNB medium containing L-lysine [8].
- In France, about 2/3 of the isolates were non-Saccharomyces strains, predominantly belonging to the species Kloeckera apiculata, whereas fermentations with grapes from Portuguese vineyards were exclusively carried out by Saccharomyces strains.
- In vineyards from both geographic regions the percentage spontaneous fermentations from pre-harvest samples is 42% compared to 84% for the post-harvest samples.
- Chromosomal pattern analysis of 735 Saccharomyces isolates from France and mtDNA RFLP (HinfI) patterns from 1620 Portuguese Saccharomyces isolates was performed and compared to a collection including all commercial strains used by the six wineries.
- 7.8% of the fermentative yeast community (296 isolates) showed genetic patterns of commercial yeasts, in majority (5.8%) recovered from post-harvest campaigns.
- 18 grape samples could not be collected due to a very bad sanitation state of the grapes after heavy rainfalls.

Distribution of recovered commercial yeast strains

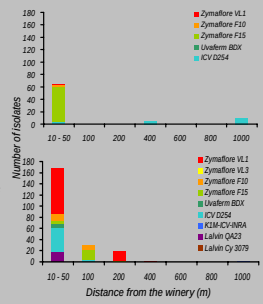
	Vineyard A	B	C	D	E	F	Total
Number of spontaneous fermentations	18	24	29	15	23	15	126
Number of spontaneous fermentations with ≥ 1 commercial yeast strains	0	2	1	11	9	2	25
Number of isolates	570	720	870	480	690	450	3780
Number of commercial yeasts strains	0	15*	1	208	54*+18*	2	296
Commercial yeast / number of isolates (%)	0	2	0.1	43	10	0.5	7.8

*Commercial yeasts initially isolated in the same region

- In the vineyards where sampling sites were placed at a greater distance from the winery (A, B, C and F), the occurrence of commercial yeast was very low. Of the extensively used strains K1M ICV-INRA and VL1 only 1 (vineyard C) and 2 (vineyard F) isolates were found, respectively. It is noteworthy that these strains, that have been used extensively for a considerable length of time, correspond to merely 0.1% of the fermentative flora. Their presence was incidental and may be attributable to factors such as insects or wind; they never dominated the microflora of any of these four vineyards.
- The results were very different in the Portuguese vineyards D and E, where sample sites were situated in close proximity to the winery (see Materials and Methods). In 20 spontaneous fermentations, commercial strains represented 43 and 10% of the fermentative yeast community from vineyards D and E respectively. The vast majority (94%) of commercial strains isolated within were recovered from these two vineyards only, and 70% solely from vineyard D. The presence of water runoff in these sites indicates that dissemination is probably largely favored by water runoff flowing from the winery to the vine.
- Strains ICV D254 and QA23 were initially selected from the Languedoc and the Vinho Verde Region. The appearance of strain D254 in vineyard B and QA23 in vineyard E may therefore not be the result of dissemination.

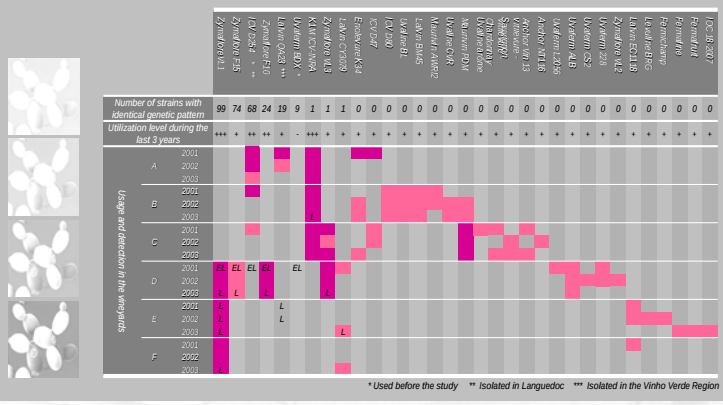
pre-harvest sampling campaigns
27% commercial strains

post-harvest sampling campaigns
73% commercial strains



- In pre-harvest campaigns commercial strains were, with a few exceptions, only collected in sites very close to winery D (10-50 m) and the strain found in the greatest quantity (87%) was Zymaflore F15.
- The majority of commercial strains were collected in post-harvest campaigns (73%) indicating immediate dissemination. Strain VL1 represented the majority (49%) of commercial strains found at 10-20 m from the winery and was recovered from sites close to the winery, water runoff or dumping sites for macerated grape skin. The occurrence of several isolates found at 200 m can be attributed to the proximity of a small building for storage of harvest transport equipment. A lower contribution of other predominantly (Zymaflore F10 and F15) or sporadically (Uvaferm BDX and ICV D254) used strains was apparent at sites between 10-100 m from the wineries.
- Independently of the sampling campaign, in a radius of around 10-200 m from the winery 94 % of commercial strains were found, whereas the large majority (78%) were recovered from sites at very close proximity (10-50 m) to the wineries of vineyards D and E.

The dissemination of commercial yeast strains as a function of their utilization



* Used before the study ** Isolated in Languedoc *** Isolated in the Vinho Verde Region

- 296 strains collected had an identical genetic profile to only 9 of the 34 commercial strains used in the six wineries.
- With a few exceptions, strains with a commercial strain profile were recovered from a vineyard where the respective commercial yeast was used:
  - The most commonly used industrial yeasts VL1 and F10 were usually collected in great abundance in the vineyard.
  - Contrarily, only one isolate of the widely used strains K1M ICV-INRA and Zymaflore VL3, respectively, was recovered.
  - Strain Zymaflore F15, although frequently collected, was used to a lesser extent.
- There is no strict correlation between the utilization level and the frequency of dissemination.

Strains predominantly used during the 5 years preceding the study

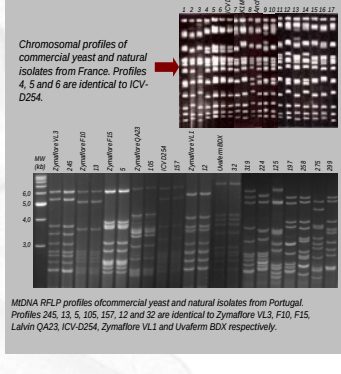
Strains sporadically used during the 5 years preceding the study

E Early sampling campaign - strains recovered from samples collected before the winery started wine production with the use of commercial yeast strains (pre-harvest samples).

L Late sampling campaign - strains recovered from samples collected after the winery started wine production with the use of commercial yeast strains (post-harvest samples).

Examples of molecular fingerprinting of commercial yeast and natural isolates (indicated by numbers)

Chromosomal profiles of commercial yeast and natural isolates from France. Profiles 4, 5 and 6 are identical to ICV-D254.



MtDNA RFLP profiles ofcommercial yeast and natural isolates from Portugal. Profiles 245, 13, 5, 105, 157, 12 and 32 are identical to Zymaflore VL3, F10, F15, Lalvin QA23, ICV-D254, Zymaflore VL1 and Uvaferm BDX respectively.

Conclusions

This systematic study has provided new insights in the impact of commercial yeasts on the communities of fermentative yeasts that inhabit areas surrounding vineyards. The methodology used, based on analysis of the yeast community after spontaneous fermentation, permitted the isolation of a very large number of Saccharomyces wine yeasts, which are poorly found on the grapes. A significant number of non-Saccharomyces strains was also found in the French samples but not from the Portugese grape musts. Climatic factors and differences in phytosanitary treatment may be the reason for these differences.

Dissemination of commercial yeasts in the vineyard is restricted to short distances and limited periods of time. 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. Dispersal of commercial strains occurs mainly after the onset of wine production in the winery, seems to be mainly mediated by water runoff, but also from macerated grape skin dumping sites. This situation was observed during the habitual functioning of a winery, where commercial strains are used without any containment. Avoiding grape-skin deposition and canalisation of water-runoff are low-cost measures, able to reduce significantly the number of commercial yeast strains close to the winery.

Our results clearly show that the presence of the most widely used commercial yeast for the last 5-10 years in French wineries was incidental (K1M ICV-INRA) and occur predominantly in sites close to the wineries in Portuguese vineyards (i.e. Zymaflora VL1). Considering commercial yeasts as non-indigenous strains that are are classically used in winemaking without any special containment and are annually released in large quantities in the environment around the winery, together with liquid and solid wine-making residues, they neither settle in the vineyard nore dominate the vineyard's microflora. Rather, they show natural fluctuations of periodical appearance and disappearance just like autochthonous strains do.

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.

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Acknowledgements:

Financially supported by the programs POCI 2010 (FEDER/FCT, POCTI/AGR/56102/2004) and AGRO (ENOSAFE, Nº 762) and the grant nº 657 C2 from the cooperation agreement between the Portuguese Institute for International Scientific and Technological Cooperation (ICCTI) and the French Embassy in Lisbon. E.V. was a recipient of a Marie Curie Fellowship (European Community programme of Quality of Life (Contract QLK4-CT-2001-51873).

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