Monitoring the spreading of industrial yeast populations in the winery environment

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Introduction

Nowadays, about 50% of the European wine production is based on the use of active dried wine yeast. These strains were selected due to their good fermentation performance and to their capacity to produce a wine with desirable organoleptical characteristics. From an ecological point of view, they are non-indigenous, mostly *S. cerevisiae* strains that are annually introduced in the ecosystem surrounding the winery. The fate of those yeasts in the natural environment in different geographical localizations is totally unknown. The present study aims to evaluate the industrial starter yeasts' ability to survive and spread in nature, and become part of the natural microflora of musts.

Materials and Methods

Samples

The wineries chosen were in close proximity to the vine, and the same industrial yeast strains have been used continuously for the last 5 years. From 6 sampling site, before and after the harvest, grapes were collected to perform small-scale fermentations (0,25-0,5 l). Must samples were plated when 70g/l of CO_2 were released, and 30 randomly selected colonies were analysed.

Molecular identification

Zymaflore VL1, Laffort Oenologie (Vineyard 1 and 2)

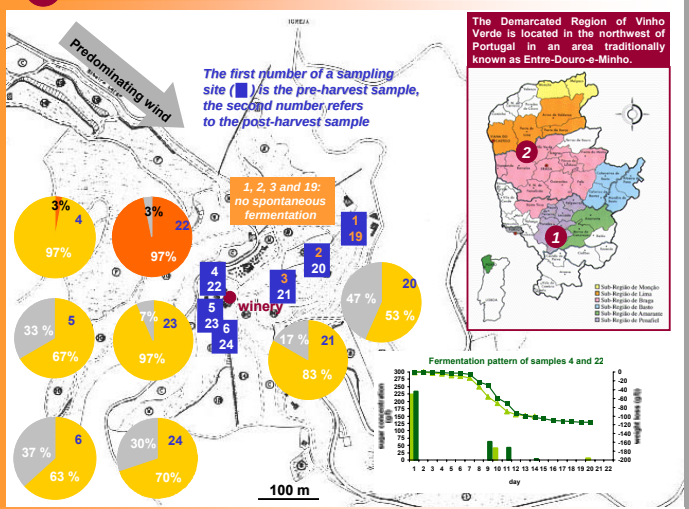
In a first approach, the *S. cerevisiae* strains isolated from vineyard 1 and 2 were analysed by PCR amplification patterns of ∂-sequences [1, 2.] The strains with an identical pattern to the one obtained for VL1 were then further analysed by comparison of their mitochondrial DNA restriction patterns [3].

K1, K34, D254, QA23, D47 (Vineyard 3) and K1, D254, Uvaline BL, BM45, AWRI2, D80 (Vineyard 4)

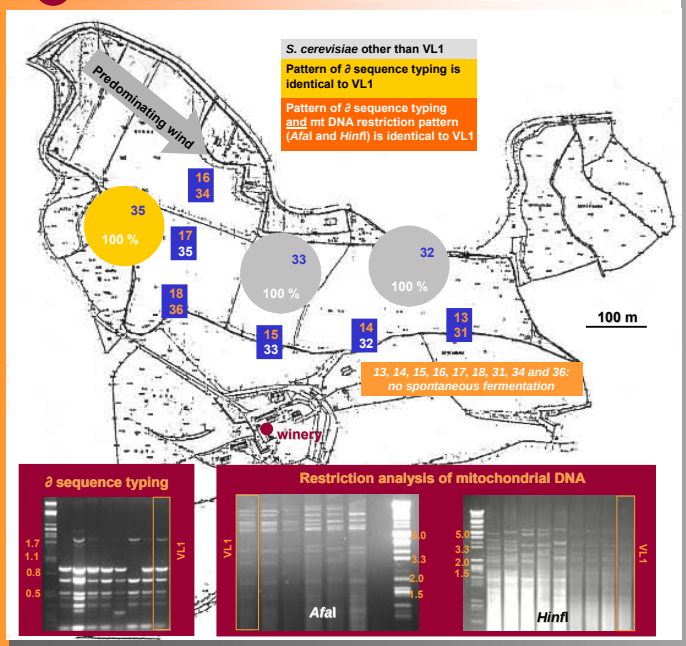
In a first screen the strains isolated from vineyard 3 and 4 unable to use lysine as sole nitrogen source and unable to growth on YPD + cycloheximide (250 mg/) were selected. These strains were analyzed by Pulse Field Gel Electrophoresis (PFGE) karyotyping using the TAFE system [4].

Results

1 Quinta da Covela - S. Tomé de Covela - Baião

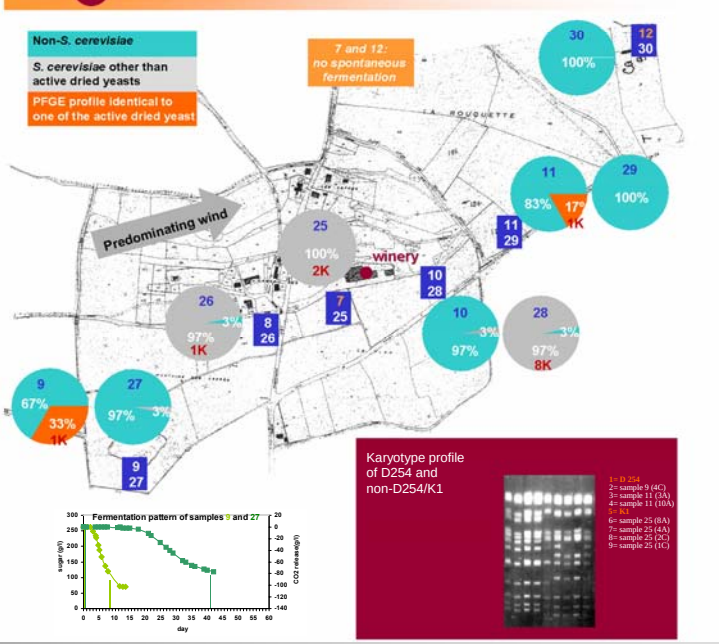


2 Quinta do Ameal - Ponte de Lima



3 Saint-Saturnin de Lucian

4 Montaud



Conclusions

Vineyard 1 and 2:

- 11 spontaneous fermentation occurred in a total of 24 samples.
- 330 strains were analysed by ∂ sequence typing and about 20 distinct patterns were observed
- The pattern identical to VL1 was found in 218 strains (66%)
- Additionally, the 218 strains were analysed by mtDNA restriction pattern and 30 strains revealed an identical pattern to VL1
- The 30 VL1-pattern strains were only found in the sampling site at the closest proximity to the winery (ca. 20 m)

Vineyard 3 and 4:

- 13 spontaneous fermentation occurred in a total of 24 samples
- 390 strains were analysed by their ability to growth on lysine as sole nitrogen source, and to growth on high concentration of cycloheximide
- 5 fermentations contained no *S. cerevisiae*
- Among the 161 *S. cerevisiae* strains analysed by PFGE, 16 distinct karyotype profiles were observed. In all fermentations except 2, only one pattern was found.
- 15 strains from 2 pre-harvest samples showed a profile identical to D254 (4%)
- No immediate release of the starter yeast was observed

References

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