A collection of indigenous Saccharomyces cerevisiae strains from appellations of origin in Portugal and France


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The model organism *Saccharomyces cerevisiae* stands today at the forefront of molecular biology, functional analysis and genomics.

This yeast is predominantly found in association with human activities, particularly the production of alcoholic beverages such as wine.

In winemaking environments, a huge phenotypic diversity of *S. cerevisiae* strains can be encountered, and this diversity has been explored for several decades to select strains with the best oenological properties.
Previous studies:

- Great biodiversity of *S. cerevisiae* strains in Vinhos Verdes and Languedoc wine region,

- each vineyard contains differentiated *S. cerevisiae* populations, hypothesizing the occurrence of specific native strains that can be associated with a *terroir* (Schuller *et al.*, 2005; Schuller *et al.*, 2007)

- Microsatellite analysis showed that genetic differences among *S. cerevisiae* populations derived from both “diagnostic” vineyard-, specific alleles and the accumulation of small allele-frequency differences across ten microsatellite loci (Schuller and Casal, 2007)

- Dissemination of commercial yeasts in the vineyard is restricted to short distances and limited periods of time (Valero *et al.* 2007).

From the biological materials that were collected under the mentioned research projects, in winemaking environments in Portugal and France, we constituted one of the largest bio-databanks of *S. cerevisiae* strains.
Sampling sites

Since 2001, 604 grape samples were collected

10 Wine Regions
13 Grape varieties
40 Vineyards
Materials and Methods

Yeast isolation

Spontaneous fermentation

Isolation

- 0 g/L
- -2 g/L
- -35 g/L
- -70 g/L

Molecular characterization and Identification of isolates
**Materials and Methods**

**Molecular typing**

A previous survey performed with 23 commercial yeasts strains showed the same discrimination power of the methods.

(Schuller et al, 2004)

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### Microsatellite analysis

<table>
<thead>
<tr>
<th>Microsatellite</th>
<th>Chromosome</th>
<th>Position/ORF</th>
<th>Repeat</th>
</tr>
</thead>
<tbody>
<tr>
<td>ScAAT 1</td>
<td>XIII</td>
<td>86 901 – 87 129</td>
<td>ATT</td>
</tr>
<tr>
<td>ScAAT 2</td>
<td>II</td>
<td>YBL084c</td>
<td>ATT</td>
</tr>
<tr>
<td>ScAAT 3</td>
<td>IV</td>
<td>YDR160w</td>
<td>ATT</td>
</tr>
<tr>
<td>ScAAT 4</td>
<td>VII</td>
<td>431 334 – 431 637</td>
<td>ATT</td>
</tr>
<tr>
<td>ScAAT 5</td>
<td>XVI</td>
<td>897 028 - 897 259</td>
<td>TAA</td>
</tr>
<tr>
<td>ScAAT 6</td>
<td>IX</td>
<td>105 661 – 105 926</td>
<td>TAA</td>
</tr>
<tr>
<td>YPL009</td>
<td>XV</td>
<td>YOR156c</td>
<td>TAA</td>
</tr>
<tr>
<td>ScYOR267C</td>
<td>XV</td>
<td>YOR267c</td>
<td>TGT</td>
</tr>
<tr>
<td>C4</td>
<td>XV</td>
<td>110 701-110 935</td>
<td>TAA+TAG</td>
</tr>
<tr>
<td>C5</td>
<td>VI</td>
<td>210 250-210 414</td>
<td>GT</td>
</tr>
</tbody>
</table>

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**Mitochondrial DNA restriction analysis (mtDNA RFLP)**

**Interdelta sequence analysis**
## Results

### Number of *S. cerevisiae* strains obtained from the wine regions

<table>
<thead>
<tr>
<th>Region</th>
<th>Nº of grape samples</th>
<th>Nº of spontaneous fermentations</th>
<th>Nº of isolates</th>
<th>Nº of <em>Saccharomyces cerevisiae</em> strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vinhos Verdes</td>
<td>282</td>
<td>115</td>
<td>3450</td>
<td>516</td>
</tr>
<tr>
<td>Bairrada</td>
<td>126</td>
<td>22</td>
<td>660</td>
<td>137</td>
</tr>
<tr>
<td>Açores</td>
<td>88</td>
<td>49</td>
<td>1470</td>
<td>169</td>
</tr>
<tr>
<td>Languedoc</td>
<td>108</td>
<td>72</td>
<td>2160</td>
<td>103</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>604</strong></td>
<td><strong>258</strong></td>
<td><strong>7740</strong></td>
<td><strong>752</strong></td>
</tr>
</tbody>
</table>
Results

Fermentation kinetics

**S. cerevisiae**

Nº of strains

![Graph showing fermentation kinetics of S. cerevisiae](image)

**Non-Saccharomyces species**

Nº of strains

![Graph showing fermentation kinetics of non-saccharomyces species](image)
**Results**

**S. cerevisiae strains involved in spontaneous fermentations**

![Bar chart showing the frequency of different strains involved in spontaneous fermentations for different years and grape varieties.](chart.png)

**Vinhos Verdes**

- No spontaneous fermentation
- Ongoing analysis
- Samples collected in the same vineyard
S. cerevisiae strains involved in spontaneous fermentations

Results

Baga | Bical | M. Gomes | Aragonês | T. Nacional


0 1 2 3 4 5 6

Spontaneous fermentations

Bairrada

No spontaneous fermentation

Ongoing analysis

Non-Saccharomyces

Samples collected in the same vineyard

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S. cerevisiae strains involved in spontaneous fermentations

Açores - 2009

Hybrid grape varieties “Uva americana”

No spontaneous fermentation  ●  Ongoing analysis

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The site encloses information about:

- Grape varieties
- Wine Regions
- Molecular methods
- Genetic data
- Publications
The database allows different searches among the *S. cerevisiae* strains:

- Collection number
- Wine region
- Vineyard
- Grape variety

Each strain contains complete information about all microsatellite data, origin, and isolation.
The collection of *S. cerevisiae* strains is an important resource for:

- ecological studies and biodiversity conservation;
- sustainable development of genetic resources;
- equitable sharing of genotypic and phenotypic data;
- selection of winemaking strains that could be used to produce wines with characteristic aromas.
Acknowledgements

To all producers that provided samples...

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