

# ABSTRACT

The winemaking industry faces currently an increased demand for novel *Saccharomyces cerevisiae* strains that are well adapted to different wine styles and that contribute to improved aromatic characteristics. In this reasoning, the Centro de Biologia Molecular e Ambiental (CBMA) at the University of Minho gathered one of the largest bio-databanks of *S. cerevisiae*, obtained from winemaking environments in Portugal and France.

During the harvest time of 2001 to 2009, 604 grape samples were collected in appellations of origin in Portugal (Vinho Verde, Dão, Douro, Bairrada, Estremadura, Palmela, Ribatejo, Açores) and France (Languedoc). The grape samples belonged to the varieties Alvarinho, Aragonez, Arinto, Avesso, Baga, Bical, Castelão, Carignan, Loureiro, Maria Gomes, Terrantez, Touriga Nacional and Verdelho. Yeast populations, in particular *S. cerevisiae*, were isolated after spontaneous fermentation of the extracted grape juice. From the final stage of 258 fermentations, 7740 yeast isolates were obtained, belonging mainly (5496 isolates) to the species *S. cerevisiae*. An initial genetic screen, based on mitochondrial DNA restriction fragment length polymorphism (mtDNA RFLP), electrophoretic karyotyping or interdelta sequence analysis, was followed by microsatellite analysis of 6 polymorphic microsatellite loci. Isolates were assigned to 752 different strains, based on their microsatellite allelic distribution. The collection of 752 autochthonous *S. cerevisiae* strains is a valuable resource for the selection of winemaking strains, biodiversity preservation and equitable sharing of genetic data. Within ongoing research, novel strains are selected to enhance aromatic profiles of specific wines. Detailed information about each strain can be accessed at the site of the *Saccharomyces cerevisiae* Wine Strain Collection: <http://scwsc.bio.uminho.pt/>.

# A *Saccharomyces cerevisiae* bio-databank for winemaking strain selection

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## INTRODUCTION

It is preferential to conduct fermentations with *Saccharomyces cerevisiae* strains that were isolated from the same wine regions because such strains:

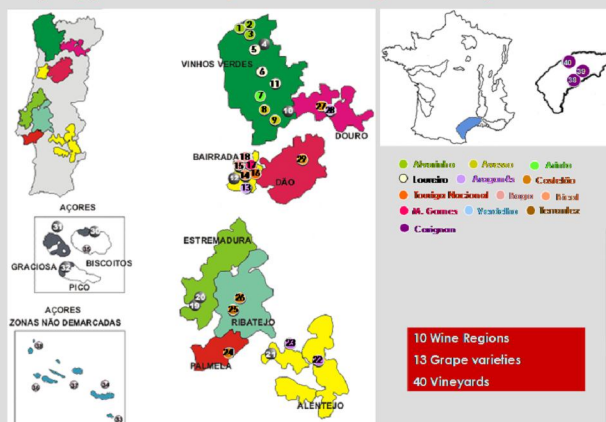
- ❖ Might have a better ability to dominate the non desirable microbial flora;
- ❖ Can influence the typical profile of wines from each region in a positive way;
- ❖ Are better adapted to the micro-ecosystem and climate of each region.
- ❖ Their use ensures the production of wines with consistent and uniform quality in consecutive years.

Among the about 200 *S. cerevisiae* active dry yeast that are commercially available, only three strains were isolated in Portugal (Vinho Verde, Dão and Bairrada wine regions).

From the biological materials that were collected in several research projects during the last years [1], [2], [3], [4], a strain collection of autochthonous *S. cerevisiae* strains was constituted and characterized. A data set for each strain is now available at the site: <http://scwsc.bio.uminho.pt/>.

## METHODS

### Sampling points in the appellations of origin in Portugal and France

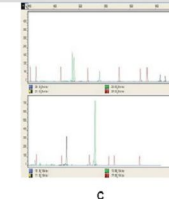
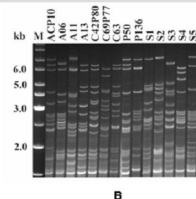
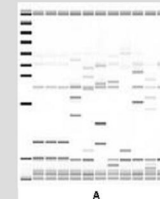


### Yeasts isolation

In each vineyard, 2 kg of grapes were aseptically collected in six sampling points. The extracted grape juice was fermented, and the fermentation progress was monitored by daily weight determinations. In the final fermentation stage, 30 isolates were obtained and stored (-80°C) for latter DNA isolation and molecular identification.

### *S. cerevisiae* strains characterization

The yeasts isolates were characterized by interdelta sequence analysis (A) or mitochondrial DNA restriction analysis (B). When isolates from different samples showed identical pattern, one representative strain of the group of strains was further studied by the allelic combination of 6 polymorphic microsatellites (C).



## RESULTS

### Number of *Saccharomyces cerevisiae* strains obtained

	Nº of Samples	Spontaneous Fermentations	Nº of isolates	Nº of <i>Saccharomyces cerevisiae</i> strains
Vinhos Verdes	282	115	3450	516
Bairrada	126	22	660	137
Açores	88	49	1470	169
Languedoc	108	72	2160	103
<b>Total</b>	<b>604</b>	<b>258</b>	<b>7740</b>	<b>752</b>

### *Saccharomyces cerevisiae* wine strain collection

The gathering of all data led to the creation of a bio-databank of autochthonous *S. cerevisiae* strains (*Saccharomyces cerevisiae* wine strain collection), that is available at the site <http://scwsc.bio.uminho.pt/>. This database contains all microsatellite data and allows different searches among the *S. cerevisiae* strains, according to the wine region, vineyard, grape variety and year of isolation.



## CONCLUSIONS

The collection of *S. cerevisiae* strains is an important resource for:

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

Ongoing studies aim to assess the oenological characteristics of the collection to select the strains that are most suitable for the fermentation of wines from the corresponding winemaking regions, in an approach that we call "yeast à la Carte."

## References

- [1] Schuller, D., et al. FEMS Microbiology Ecology, **2005**, 51: 167-177
- [2] Schuller, D., M. Casal, Antonie Van Leeuwenhoek, **2007**, 91(2): 137-150
- [3] Valero, E., et al, FEMS Yeast Research, **2007**, 7(2): 317-329
- [4] Schuller, D., et al., Yeast, **2007**, 24: 635 – 636

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