Phenotypic Differentiation and Genetic Diversity of Saccharomyces cerevisiae Strains from Vineyards of the Azores Archipelago

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INTRODUCTION

This work aims to evaluate the diversity of Saccharomyces cerevisiae strains from vineyards of the Azores Archipelago and to estimate the degree of phenotypic and genetic differentiation among geographically isolated islands. The Azorean terroirs, correspond to very particular ecosystems whose fermentative flora has not yet been characterized.

MATERIAL AND METHODS

Sampling

During the harvests of 2009 and 2010, 163 grape samples were collected from 36 locations on eight islands of the archipelago. The grape samples belonged to traditional (TV) and hybrid (HV) varieties, including vineyards in appellations of origin, abandoned vineyards (AV) and non-abandoned vineyards (NAV).

Yeast Isolation

From each sample (2 kg grapes), 500 ml ofmust was obtained and fermented. The evolution of fermentation was followed daily and when the weight loss was about 65-70 g/L, aliquots of serial dilutions (10⁻¹ - 10⁻⁰) were spread on plates containing YPD medium. After two days of incubation at 30 °C, 30 colonies were collected from one of the five dilutions containing between 30 and 300 colonies.

Molecular Identification

The S. cerevisiae strains were differentiated by the comparison of polymorphic patterns that were obtained from interdelta sequences amplification by PCR [1, 2].

Phenotypic Characterization

From each strain a dense cell suspension was prepared (A₆₀₀=1.0) and 1 μL was inoculated in duplicate in plates containing different culture media as outlined below. Cell growth was evaluated after incubation at 25 °C – 27 °C for 2 - 3 days.

Phenotypic test

A. Killer phenotype
B. Ethanol resistance
C. Combined ethanol and SO₂ resistance
D. SO₂ resistance
E. β-glucosidase activity
F. H₂S production

CULTURE MEDIUM

A. Multi Extract Agar (MEA) supplemented with 0.03% (v/v) methanol; inoculums layer on a sensitive (DCT 1415) or killer (DCT 2182) reference strain.
B. MEA supplemented with 8% (v/v) ethanol
C. MEA supplemented with 8% (v/v) ethanol and 100 ppm SO₂
D. Estimated from comparison of tests B and C
E. Exculin/Spirit Agar (EGA) medium [3]
F. BioG Milieu Medium (Diotec)

Genetic Diversity

The highest numbers of S. cerevisiae strains per sample were found in Santa Maria and Graciosa islands.

Conclusions

A total of 271 S. cerevisiae strains was obtained from seven islands of the Azores Archipelago. The highest genetic diversity was found in samples from the islands S. Maria (7.8 strains/sample) and Graciosa (6.4 strains/sample).

Phenotypic characterization of the isolates showed that some characteristics might be under selection, depending on environmental conditions such as the extension of cultivated vineyards, strains diversity, type of vineyard (abandoned or non-abandoned), grape variety and enological practices.

The percentage of strains showing strong β-glucosidase activity ranged between 0% (S. Jorge) and 20% (Terceira). No differences were observed between the regular SO₂ usage in wineries that are often located close to non-abandoned vineyards (NAV).

The highest percentage of ethanol resistant strains was found on Terceira Island (82%), whereas this value ranged between 48% and 70% in Pico and S. Jorge, respectively. Global analysis showed that strains from hybrid varieties (HV) tend to be more ethanol resistant (67% to 70%), in comparison with strains from traditional varieties (TV) (58%). This characteristic was not correlated with the sugar content of the samples (172 g/L and 162 g/L in grapes from traditional and hybrid varieties, respectively).

S. cerevisiae strains from Graciosa island were the highest H₂S producers. This might be associated with the predominance of the killer phenotype on this island. Further investigation is underway. The strains from abandoned vineyards (AV) showed a higher H₂S production than the ones from non-abandoned vineyards (NAV).

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