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Comparison of RAPDs, AFLPs and SSR markers for the genetic analysis of yeast strains of *Saccharomyces cerevisiae*

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Abstract

We evaluated the usefulness of different molecular techniques for the genetic analysis of *Saccharomyces cerevisiae* strains. Three commonly used PCR-derived genetic methods, random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphism (AFLPs) and simple sequence repeats (SSRs; microsatellites), were used to characterize 27 wine yeast strains of *S. cerevisiae* from the "Denominación de Origen Vinos de Madrid" (Spain). Using these methods, we were able to overcome certain limitations associated with classical taxonomic methods. Based on the presence or absence of amplified fragments for each genotype, AFLPs and SSRs showed a similar discriminatory power superior to that of the RAPDs. Genetic relationships between strains were also estimated using the three methods. In general, very poor correlations were found, reflecting the different genomic regions for which the methods are screened. Results are discussed in terms of which molecular technique is most appropriate for use with a particular aspect of genetic evaluation.

Keywords: RAPDs; AFLPs; SSRs; Molecular markers; Genetic analysis; *Saccharomyces cerevisiae*

1. Introduction

The use of molecular markers has provided important advances in the characterization and genetic identification of *Saccharomyces cerevisiae* strains, the species that plays the major role in the fermentation of alcoholic beverages. The usefulness of these molecular methods for the identification of *S. cerevisiae* at the strain level is of particular interest in enology, where they can be used to investigate the ecology and genetic diversity of a species that predominates during spontaneous fermentation of the must. Moreover, they can be used for typing, monitoring and controlling commercially selected strains of *S. cerevisiae*.

PCR-based techniques, including interdelta analysis (Ness et al., 1993; Legras and Karst, 2003) and the use of intro splice site primers (deBarros Lopes et al., 1996), randomly amplified polymorphic DNA (RAPDs) (Quesada and Cenís, 1995; Van der Westhuizen et al., 1999), simple sequence repeats (SSRs) (Gallego et al., 1998; González Techera et al., 2001; Hennequin et al., 2001; Pérez et al., 2001), and amplified fragment length polymorphism (AFLPs) (deBarros Lopes et al., 1999), have previously allowed the discrimination as well as estimation of genetic variation in *S. cerevisiae* strains. Differences exist, however, among the methods used with respect to complexity, cost, speed of use, and resolution power. This paper compares the usefulness of three PCR-based methods—RAPDs, AFLPs and SSRs—for the genetic analysis of *S. cerevisiae* strains.

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2. Materials and methods

2.1. Yeast strains

Must samples undergoing spontaneous fermentation were collected from wineries (A, B and C) representing three viticultural areas (Arganda, Navalcarnero and San Martín de Valdeiglesias) of the "Denominación de Origen Vinos de Madrid" (Spain). The number of tanks sampled in cellars A, B and C were two, four and two, respectively. Fermentation processes were conducted at temperatures between 18 and 20 °C. Populations of viable yeast cells in each step of the fermentation process were of the order of 10^7 cfu/mL. From these populations, a total of 27 *S. cerevisiae* (Kreger Van Rij, 1984; Kurtzman and Fell, 1998) strains were isolated and chosen at random for use in this study. These strains have been included in the culture collection of the IMIA (El Encín, Madrid). The reference code, origin and source of each yeast strain are listed in Table 1.

2.2. DNA extraction

This process was carried out following the technique described by Pérez et al. (2001).

Table 1
S. cerevisiae strains used in this study

Strain (reference code)	Viticultural area	Winery	Isolation sample	Step of fermentation
1a	Arganda	A	20	Middle
2a	Arganda	A	20	Middle
3a	Arganda	A	20	Middle
4a	Arganda	A	20	Middle
5a	Arganda	A	20	End
6a	Arganda	A	21	Middle
7a	Arganda	A	21	Middle
8a	Arganda	A	21	Middle
9a	Arganda	A	21	End
1n	Navalcarnero	B	32	End
2n	Navalcarnero	B	32	Middle
3n	Navalcarnero	B	32	End
4n	Navalcarnero	B	33	Middle
5n	Navalcarnero	B	33	End
6n	Navalcarnero	B	33	End
7n	Navalcarnero	B	33	End
8n	Navalcarnero	B	34	End
9n	Navalcarnero	B	35	Middle
1s	San Martín	C	46	Middle
2s	San Martín	C	46	Middle
3s	San Martín	C	46	End
4s	San Martín	C	46	End
5s	San Martín	C	47	Middle
6s	San Martín	C	47	End
7s	San Martín	C	47	End
8s	San Martín	C	47	End
9s	San Martín	C	47	End

2.3. RAPD analysis

RAPD amplifications were carried out according to the methodology described by Gallego and Martínez (1997) using a DNA Thermal Cycler 9600 (Applied Biosystems) under the following conditions: a preliminary step of 2 min at 94 °C; 10 cycles each of 30 s at 94 °C, a ramp of 1.5 °C s^{-1} to reach annealing temperature, 1 min at 55 °C (decreasing 1 °C per cycle to a final temperature of 46 °C), and a ramp of 1.5 min to reach 72 °C followed by 4.5 min at this temperature; 25 cycles each of 30 s at 94 °C, a ramp of 1.5 °C s^{-1} to reach annealing temperature, 1 min at 45 °C, and a ramp of 1.5 min to reach 72 °C followed by 4.5 min at this temperature; and a final step of 1 min at 72 °C.

Forty primers from sets B and C of Operon Technologies (Alameda, CA) were used in preliminary experiments in order to evaluate their performance. Thirty-two resulted in satisfactory amplifications and were selected for use in the remainder of the assay. Amplification products were separated by electrophoresis in 1.5% (w/v) agarose gels, and visualized under UV light after staining with ethidium bromide.

2.4. AFLP analysis

AFLP markers were developed following the protocol supplied by Applied Biosystems (Foster City, California, USA), based on Vos et al. (1995). DNA digestion was carried out using the restriction enzymes *Eco*RI and *Mse*I. Six selective amplifications were performed using five primers. Two of the primers included the *Mse*I adaptor sequence plus the selective CTT and CAA. The remaining three primers contained the *Eco*RI adaptor sequence plus AC, AT and AAG. The *Eco*RI selective primers were 5′-labeled with one of the following fluorescent dyes: 6-FAM, TET or HEX (Applied Biosystems). PCR products were separated by capillary electrophoresis in an ABI Prism 310 DNA Sequencer (Applied Biosystems). The number of fragments generated by each primer pair was obtained directly from Genescan Analysis software, using the local Southern method to size the fragments.

2.5. SSR analysis

SSR analysis was performed according to the methodology developed by Pérez et al. (2001), using a panel of six effective microsatellite loci as sequence-tagged site markers.

Loci were amplified using two multiple PCR reactions, including ScAAT2, ScAAT3, ScAAT5 in the first reaction (PCR1) and ScAAT1, ScAAT4, and ScAAT6 in the second (PCR2). Each PCR reaction was performed in 25 μL final volume containing 10–400 ng of template DNA and 0.2 mM of each dNTP (Applied