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Survey of molecular methods for the typing of wine yeast strains

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Abstract

A survey of the genetic polymorphisms produced by distinct methods was performed in 23 commercial winery yeast strains. Microsatellite typing, using six different loci, an optimized interdelta sequence analysis and restriction fragment length polymorphism of mitochondrial DNA generated by the enzyme *Hin*fI had the same discriminatory power: among the 23 commercial yeast strains, 21 distinct patterns were obtained. Karyotype analysis gave 22 patterns, thereby allowing the discrimination of one of the three strains that were not distinguished by the other methods. Due to the equivalence of the results obtained in this survey, any of the methods can be applied at the industrial scale.

Keywords: Molecular typing; Karyotyping; Interdelta sequence; Mitochondrial DNA restriction fragment length polymorphism; Microsatellite; Commercial yeast

1. Introduction

Wine production by the use of selected *Saccharomyces cerevisiae* strains, commercially available as active dry yeast, is widely accepted, being an extensively applied enological practice nowadays. The use of techniques that make it possible to distinguish the inoculated strain from the remaining yeast flora present in the grape must is regarded with great practical interest [1]. In recent years, several methodologies of typing based on DNA polymorphisms have been developed which allowed discrimination among closely related yeast strains.

Chromosome separation by pulsed field electrophoresis [2] revealed considerable variability in the chromosomal constitution of commercial yeast strains [3], and turned out to be a useful method for yeast strain identification [4,5]. As chromosome karyotyping may be too complex, laborious and time-consuming for the analysis of numerous yeast isolates, several other molecular methods of typing have been developed for this purpose.

Restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) [5,6] was simplified [7,8] to render it a fast and easy method. Digestion of mtDNA with restriction enzymes like *Hin*fI or *Rsa*I is associated to a high polymorphism, and was also used to study the authenticity of commercial wine yeast strains [9].

The *S. cerevisiae* genome contains repetitive DNA sequences, such as the δ sequences that are frequently associated with the Ty1 transposon [10,11]. The number and the location of these elements have a certain intraspecific variability and were used as genetic fingerprints to identify *S. cerevisiae* strains [11]. Polymerase chain reaction (PCR) profile analysis of δ sequences has a good discriminating power for analyzing commercial strains [12]. On the other hand, it seems to be a minor discriminatory method when used to identify indigenous strains in a given viticultural region [13]. More recently, an extensive BLAST search allowed the optimization of the pair of primers used for interdelta analysis, resulting in highly polymorphic patterns. This improved PCR typing had a similar discriminatory power to pulsed field electrophoresis karyotyping [14].

In the last few years, fingerprinting of microsatellite or simple sequence repeat loci, which are short (1–10 nucleotides) DNA tandem repeats dispersed throughout the genome and with a high degree of variability, has been revealed to be very useful to discriminate *S. cerevisiae*

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strains [15–19]. Searching the genomic DNA database of *S. cerevisiae*, six microsatellite loci were selected that generated 44 genotypes (with a total of 57 alleles) from 51 strains originating from a spontaneous fermentation [20]. This method is fast, allowing multiplex PCR reactions, precise and reproducible, and therefore very powerful.

In the present paper four different genetic fingerprinting techniques (karyotype analysis, δ sequence typing, mtDNA restriction analysis and microsatellite genotyping) were used for the detailed genotyping of 23 commercial wine yeast strains. The analysis of the polymorphisms produced by each of the methods allowed a detailed comparison of the advantages and disadvantages of each method showing the utility and efficiency of these modern approaches for fingerprinting relatively large sets of winery yeast strains.

2. Materials and methods

2.1. Yeast strains

Twenty-three commercially available *S. cerevisiae* wine strains were used in this study. Their geographic origin is indicated in Table 1.

Active dried wine yeast strains were rehydrated and maintained in frozen stocks (glycerol, 30% v/v) at −80°C or, for short-term storage, on YPD agar medium (yeast extract, 1% w/v, peptone, 2% w/v and glucose, 2% w/v).

Table 1
Commercial *S. cerevisiae* strains used in the present study

Strain	Origin
1	Portugal
2	Sangiovese, Italy
3	Bordelais, France
4	Rhône, France
5	Languedoc, France
6	Stellenbosch, South Africa
7	Rhône, France
8	Rhône, France
9	Valencia, Spain
10	Champagne, France
11	Loire, France
12	Champagne, France
13	Gironde, France
14	Languedoc, France
15	Gironde, France
16	Bordelais, France
17	Gironde, France
18	Portugal
19	Portugal
20	Germany
21	Not known
22	Pfalz, Germany
23	Baden-Württemberg, Germany

2.2. DNA isolation

Yeast cells were cultivated in 5 ml of YPD medium (24 h, 28°C, 160 rpm) and DNA isolation was performed using a previously described method [7]. The progress of cell lysis was dependent on the strain and could last between 1 and 3 h. DNA was quantified and used for δ sequence typing, mitochondrial RFLP and microsatellite analysis.

2.3. Delta sequence typing

Amplification reactions were performed on a Bio-Rad iCycler thermal cycler, using the primers δ1 (5′-CAAAATTCACCTATATCT-3′) and δ2 (5′-GTGGATTTTTATTCCAAC-3′) (primer pair A) [7] or δ12 (5′-TCAACAATGGAATCCCAAC-3′) and δ2 (primer pair B) [7]. 15 μl reaction mixture was prepared with 60 ng of DNA, 0.5 U Taq polymerase (MBI Fermentas), Taq buffer (10 mM Tris–HCl, 50 mM KCl, 0.08% Nonidet P-40), 25 pmol of each primer, 0.4 mM of each dNTP and 3 mM $MgCl_2$. After initial denaturation (95°C for 2 min), the reaction mixture was cycled 35 times using the following program: 95°C for 30 s, 43.2°C for 1 min, 72°C for 1 min followed by a final extension at 72°C during 10 min. The amplification products were separated by electrophoresis on a 1.5% (w/v) agarose gel containing ethidium bromide, visualized and photographed.

2.4. Chromosomal polymorphisms

Yeast chromosomal DNA was prepared in plugs as previously described [7], washed in TE buffer (1 mM EDTA, 10 mM Tris–HCl, pH 8.0) at 50°C for 30 min and then washed again three times in the same buffer at room temperature for 30 min. The plugs were loaded in a 1% (w/v) agarose (Seakem® Gold) gel and electrophoresis was performed using a TAFE (transverse alternating field electrophoresis) system (Geneline, Beckman) under the following conditions: constant voltage of 250 V for 6 h run time with 35 s pulse time, followed by 20 h at 275 V with 55 s pulse time at constant temperature (14°C). The electrophoresis buffer consisted of 10 mM Tris base, 0.5 mM EDTA free acid and 4 mM acetic acid. After staining the gel with ethidium bromide, bands were visualized and photographed.

2.5. Mitochondrial DNA restriction patterns

The reactions were performed overnight at 37°C and prepared for a final volume of 20 μl as follows: 17 μl of total DNA (60–120 μg), isolated as described, 0.5 μl of the restriction endonucleases *Hin*fI or *Rsa*I (10 U μl^{-1}, MBI Fermentas), 2 μl of the appropriate 10× buffer and 0.5 μl of RNase (10 mg ml^{-1}) (MBI Fermentas). The DNA fragments were separated on a 1.5% (w/v) agarose gel containing ethidium bromide, visualized and photographed.

2.6. Microsatellite amplification

The six trinucleotide microsatellite loci described as ScAAT1, ScAAT2, ScAAT3, ScAAT4, ScAAT5 and ScAAT6 [20] were amplified in two multiplex reactions using 20 ng of template DNA, 0.5 U Taq polymerase (MBI Fermentas), the corresponding Taq buffer (10 mM Tris–HCl, 50 mM KCl, 0.08% Nonidet P-40), 0.2 mM of each dNTP and 2 mM $MgCl_2$. Multiplex reaction A contained 0.05 pmol of each ScAAT1 and ScAAT6 primer pairs as well as 0.03 pmol of ScAAT4 primer pair. Multiplex reaction B contained 0.05 pmol of ScAAT2, 0.1 pmol of ScAAT3 and 0.075 pmol of ScAAT5 primer pairs. One oligonucleotide of each pair was labeled with fluorescent dye (MWG Biotech). In both cases, the total reaction volume was 6.0 μl, and cycling was performed as described [20] in a Bio-Rad iCycler thermal cycler. PCR reactions were diluted (1:5 for multiplex A and 1:20 for multiplex B), and 2-μl aliquots were mixed with 14 μl of formamide and 0.3 μl of a red DNA size standard (Genescan-500 ROX, Applied Biosystems). Samples were then denatured at 94°C for 5 min and separated by capillary electrophoresis (15 kV, 60°C, 24 min and 27 min for multiplex reactions A and B respectively) in an ABI Prism 310 DNA sequencer (Applied Biosystems) and analyzed using the corresponding Genescan software.

2.7. Reproducibility

All typings were performed at least in duplicate. The reproducibility of the described techniques was also assessed by comparing the results obtained by the analysis of DNA from two independent extractions for five randomly chosen yeast strains.

3. Results

3.1. Delta sequence typing

PCR amplification of δ sequence interspersed regions using primer pair A or B showed a distinct degree of pattern heterogeneity as shown in Fig. 1. For primer pair A, a total of 10 distinct patterns were obtained, and most of them shared three common bands around 500, 750 and 970 bp. These three bands are characteristic for pattern δ_{A3}, the pattern found in 10 of the 23 strains analyzed. Further patterns are characterized by the appearance of an additional band in close proximity to one of the three main bands (e.g., patterns δ_{A1}, δ_{A6}, δ_{A7}), by the absence of some of the three main bands (e.g., patterns δ_{A2}, δ_{A4}), or by the appearance of other extra bands (e.g., pattern δ_{A10}). For primer pair B, almost all patterns appear to have several bands in common of about 400–500 bp, and the presence of many other intense bands of different sizes produced a very high polymorphism compared to primer pair A, allowing the assignment of 21 different patterns among the 23 strains. The group of 10 strains showing the identical pattern δ_{A3} could be distinguished from each other using primer pair B that generated 10

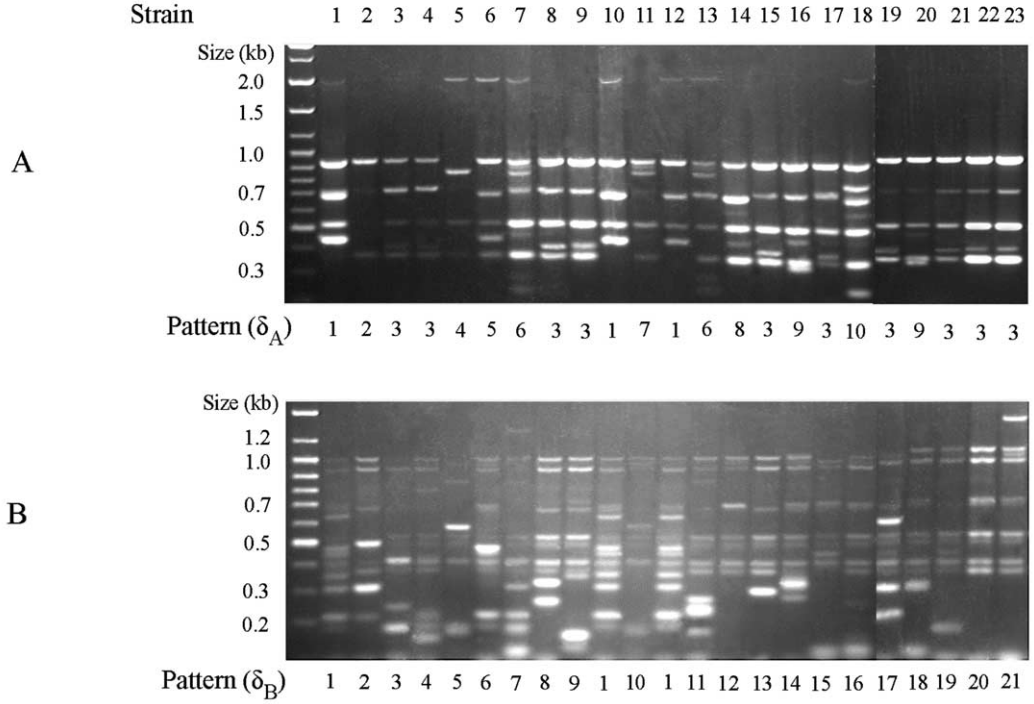

Fig. 1. PCR amplification fragments of δ sequence interspersed regions using primer pair A (A) or B (B). The numbers in the upper part of the figure correspond to the strains used. (δ_A) 1–10 and (δ_B) 1–21 refer to the pattern classification.

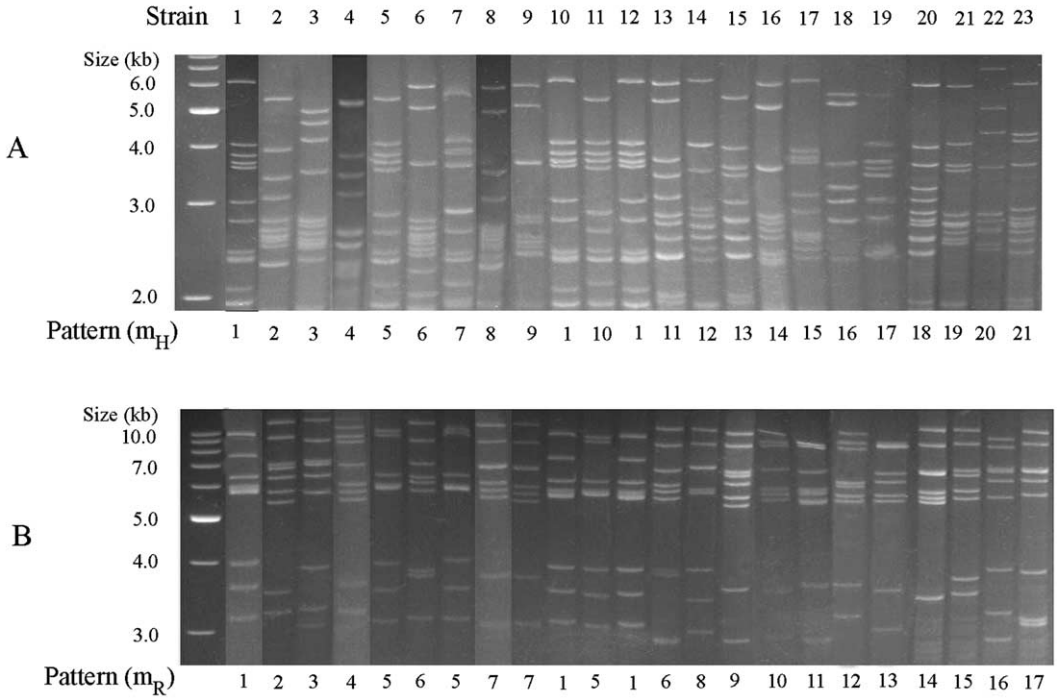

Fig. 2. Mitochondrial DNA restriction patterns of the 23 commercial strains analyzed in this work. The patterns (m_H and m_R) were obtained by digestion with *Hin*fI (A) or *Rsa*I (B). The numbers in the upper part of the figure correspond to the strains used. (m_H) 1–17 and (m_R) 1–21 refer to the pattern classification.

different patterns. Interestingly, strains 1, 10 and 12 show a very characteristic pattern (δ_{B1}), with five bands sized between 300 and 500 bp. These three strains also showed a unique pattern (δ_{A1}) when PCR amplification was performed with primer pair A (Fig. 1), indicating that they are identical or genetically very closely related.

Several faint bands, probably associated with unspecific amplification due to the low annealing temperature and to the high $MgCl_2$ concentration (3.0 mM), were not always amplified in replicates, but they were not decisive for the assignment of a pattern, as sufficient polymorphisms were obtained from the intense bands.

3.2. RFLP of mitochondrial DNA

The analysis of the genetic variability of 23 *S. cerevisiae* wine strains by means of mtDNA restriction analysis showed a very high level of polymorphisms (Fig. 2). Digestion with *Rsa*I was less discriminating than *Hin*fI, generating 17 and 21 distinct patterns, respectively. Strains 5, 7 and 11 shared pattern m_{R5}, while pattern m_{R7} was shared by strains 8 and 9. The average size of fragments obtained by *Hin*fI digestion was between 2.5 and 6 kb, whereas bigger fragments (mainly between 6 and 10 kb) were obtained by *Rsa*I digestion. Again, with the exception of strains 1, 10 and 12, unique patterns were found with the restriction enzyme *Hin*fI. Fig. 4 shows the identical mtDNA restriction patterns of these three strains using *Hin*fI or *Rsa*I.

3.3. Analysis of chromosomal patterns

As shown in Fig. 3, the pulsed field electrophoretic karyotypes of the 23 strains analyzed showed 22 different chromosomal patterns. In the range below 600 kb, where the resolution is better, the greatest variability was found, both in the position and in the number of bands, which varied from five to 10. There was also considerable variability in the region of approximately 900 kb, where for most strains one or two bands were observed in different positions.

The patterns of strains 10 and 12 (K10) were again identical whereas in strain 1 differences in the zones of about 600 kb (chromosomes XVI–XIII) and 900 kb (chromosomes V–VIII) were observed. A lower weak band was lost and another higher weak band appeared in the zone of around 600 kb. In addition, a band of smaller size in strain 1 replaced a weak band present in the region about 900 kb. Except for these two bands, the pattern of strain 1 is identical to that of strains 10 and 12, indicating that these strains are genetically very closely related.

3.4. Microsatellite analysis

The results obtained for the analysis of the six microsatellite loci ScAAT1–ScAAT6 are summarized in Table 2. Unique patterns were found for 20 strains, while an identical pattern was found for strains 1, 10 and 12. The number of alleles found for each locus varies between three

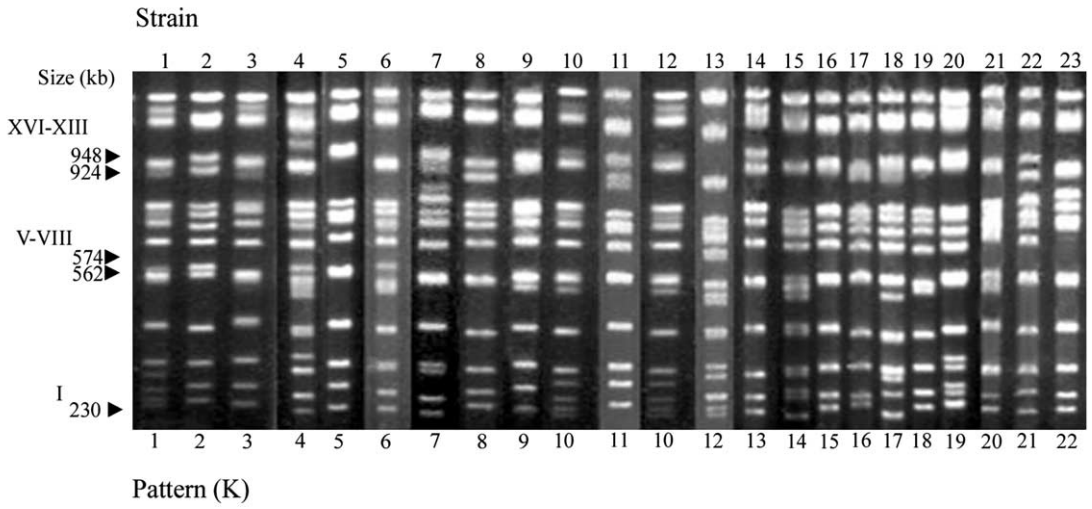

Fig. 3. Electrophoretic karyotype patterns of the 23 commercial strains analyzed. The numbers in the upper part of the figure correspond to the strains used. (K) 1–22 refers to the pattern classification. Numbers on the left give the sizes of chromosomes XVI–XIII, V–VIII and I of the reference strain S288C.

and 15, the loci ScAAT1 and ScAAT3 being characterized by the highest polymorphism. The number of genotypes varied between four and eighteen for each locus separately analyzed (Table 2).

4. Discussion

In the present study, different methods have been applied to genetically differentiate 23 commercial wine starter yeast strains. As summarized in Table 3, depending on the technique used, distinct levels of discrimination were obtained, varying from 10 to 22 different patterns.

The power of discrimination of *S. cerevisiae* strains by PCR-based interdelta typing depended on the primer pairs used. Amplification with the initially described [11] primer pair δ1-δ2 (primer pair A) resulted in 10 different patterns, whereas the substitution of primer δ1 by primer δ12 (primer pair B) resulted in a two-fold increase in the number of patterns obtained (Table 3). The optimized primer pair B, found by an extensive BLAST search, raised the detection of polymorphisms and allowed the unequivocal differentiation of 53 industrial, laboratory and wild-type yeast strains [14]. Delta sequence typing with the standard primer (pair A) has been reported to be very useful and easy to perform for the typing of commercial strains. However, for the delimitation of genetically closely related indigenous yeast strains, this method has a low discrimination power and therefore should be combined with other typing methods like mtDNA or karyotype analysis [13,21]. In the present study, the interdelta typing of the 23 industrial strains with optimized primer pair B had almost the

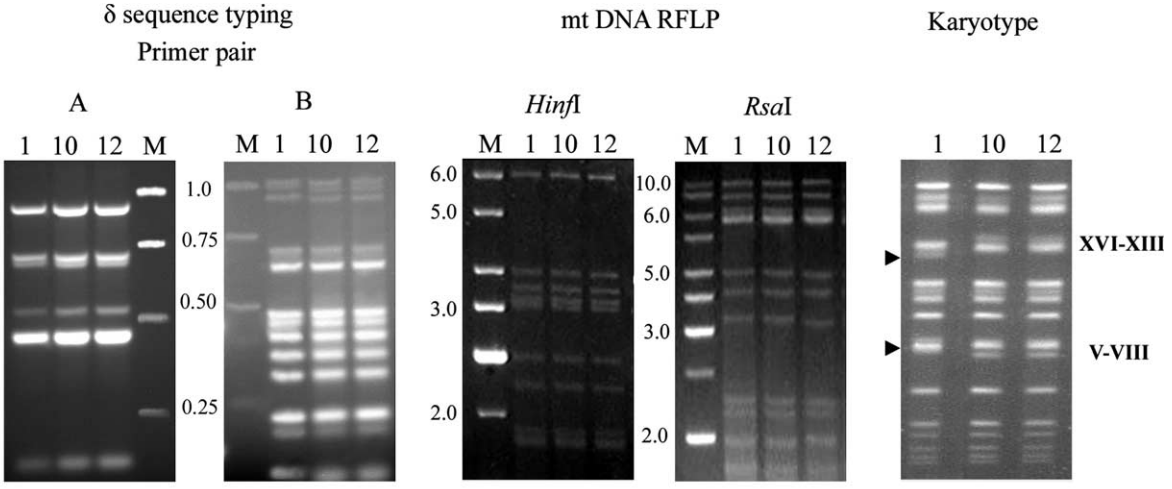

Fig. 4. Analysis by δ sequence typing, mtDNA RFLP and pulsed field electrophoresis of strains number 1, 10 and 12. The three strains present identical patterns, except the slight differences in strain 1 indicated by arrows.

Table 2
Allelic diversity of the 23 *S. cerevisiae* commercial starter strains

Strain	Microsatellite					
	ScAAT1	ScAAT2	ScAAT3	ScAAT4	ScAAT5	ScAAT6
1	189, 237	375	250, 346	302	219, 222	250, 256
2	201	378	247	329	216	256
3	204, 222	372, 378	259, 265	317, 329	216, 219	256, 259
4	165	384	262, 304	302, 329	216, 219	256, 259
5	246	378	262	329	216	259
6	189, 228	375, 378	250, 262	302, 329	216, 222	256
7	222	369, 384	247	302, 329	216	256
8	195	378	241	332	219	256
9	195, 216	375, 381	256	329	216	256
10	189, 237	375	250, 346	302	219, 222	250, 256
11	195	375	256	329	222	256, 259
12	189, 237	375	250, 346	302	219, 222	250, 256
13	216, 219	372, 378	247, 265	329	216, 219	256, 259
14	174	387	247	338	222	259
15	204, 219	372, 381	265	329	219, 222	256, 259
16	195	378	265	329	222	256
17	201	378	247	329	222	256
18	171, 201	375, 378	259, 268	329	219	256
19	204	369	259, 271	329	219	259
20	192	378	247, 271	329	216	256, 259
21	207	378	262	329, 332	216	256
22	219	381	259	329	219	256
23	189	381	247	290	219	256
Number of alleles	15	7	11	6	3	3
Number of genotypes	18	11	14	8	6	4

same level of discrimination as pulsed field karyotyping. These results are consistent with the ones previously described [14].

As shown in Table 3, the 21 patterns generated by mitochondrial DNA restriction with *Hin*fI match exactly the patterns obtained by PCR typing using primer pair B, microsatellite typing, as well as pulsed field karyotyping (with the exception of strain 1). Additionally, in the present study, digestion with *Hin*fI allowed a much better resolution than with *Rsa*I.

Both mtDNA restriction analysis and electrophoretic karyotyping have been used in numerous studies related to the yeast ecology of spontaneous fermentations, biogeography and biodiversity [22–27]. It was shown that both methods had a very similar resolving power at the strain level. Nevertheless, the results obtained using the improved interdelta typing method are very promising, indicating its equivalence to mtDNA RFLP, karyotyping and microsatellite analysis.

Using interdelta amplification, mtDNA RFLP and microsatellite typing, strains 1, 10 and 12 generated the same patterns (Table 3). The chromosomal patterns of strains 10 and 12 are identical, and were very similar to that of strain 1. Strain 1 differs from the other two strains due to changes in the position of two weak bands in the zones of about 600 and 900 kb. Two pairs of chromosomes, XVI/XIII and VIII/V, very close in size, are found in these regions. Interestingly, a reciprocal translocation between chromosomes VIII and XVI, generating two new chromosomes $VIII^{XVI}$ and XVI^{VIII}, has been described as occurring frequently in wine yeast strains [28]. This rearrangement, found in wine yeast strains, is involved in their adaptive evolution, since the translocation results in higher expression of *SSU1*, thus enabling the cells to resist higher sulfite concentrations [29]. Indeed, wine yeast strains exhibit either normal chromosome VIII (of about 560 kb), chromosome $VIII^{XVI}$ (of about 920 kb), or both [29,30]. Both are actually present in strains 10 and 12 (results not shown) and the opposite variations in the size of bands in strain 1 may indicate different rearrangement events related to these two chromosomes. All these lines of evidence strongly suggest strains 10 and 12 are genetically related to strain 1.

Strain 'families' having the same mtDNA restriction profile and δ sequence PCR product patterns, differing only by faint variations of chromosomal band position or the presence of doublets, have been described [23]. Differently sized chromosomes can be explained by structural reorganizations, leading to structural heterozygosis [30]. Such chromosomal rearrangements have been described in wine yeast genomes during vegetative growth [31] or during wine fermentation [32].

The discrimination obtained by combining the allele sizes from the six microsatellite loci was very high. The combination of the results from loci ScAAT1 and ScAAT3 generated the highest polymorphism (18 and 14 geno-

Table 3
Summary of the results obtained by all typing methods used

Strain	Pattern					
	δ sequence		mtDNA RFLP		Microsatellite ScAAT1–6	Karyotype
	δ_A	δ_B	m_R	m_H		
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
4	3	4	4	4	4	4
5	4	5	5	5	5	5
6	5	6	6	6	6	6
7	6	7	5	7	7	7
8	3	8	7	8	8	8
9	3	9	7	9	9	9
10	1	1	1	1	1	10
11	7	10	5	10	10	11
12	1	1	1	1	1	10
13	6	11	6	11	11	12
14	8	12	8	12	12	13
15	3	13	9	13	13	14
16	9	14	10	14	14	15
17	3	15	11	15	15	16
18	10	16	12	16	16	17
19	3	17	13	17	17	18
20	9	18	14	18	18	19
21	3	19	15	19	19	20
22	3	20	16	20	20	21
23	3	21	17	21	21	22

For each method a different number was assigned to distinct patterns.

types), and was sufficient for the unequivocal characterization of the present population of 23 strains. However, for studies aiming at the characterization of strains that are genetically more closely related, it may be necessary to include data obtained for the other four loci.

In summary, our results show that microsatellite typing and the optimized interdelta analysis have similar discriminatory power compared with both mtDNA restriction analysis and karyotyping. None of the typing methods was able to discriminate between two *S. cerevisiae* commercial strains (10 and 12). At least two hypotheses can be raised to explain this result: the strains are identical, although having different commercial designations, or the techniques used are not sufficiently accurate to discriminate between them. Concerning the first hypothesis, there are references reporting equivalent situations in commercial yeast strains [9]. The common geographical origin of these two strains supports this hypothesis.

The improved PCR amplification of δ sequences described by Legras et al. [14] is a very convenient method that does not require high equipment investment and can replace other methods advantageously. However, some critical aspects of δ sequence typing have to be mentioned, as the PCR banding patterns depend on the quantity of template DNA [9]. Occasionally, we also found weakly amplified bands that can make the interpretation of the results difficult (not shown).

Mitochondrial DNA restriction analysis could be a good technique to differentiate yeast strains from the same ecosystem. This technique is also easy to use once the conditions have been carefully standardized and the reproducibility is better than that of δ sequences analysis.

Karyotyping was shown to be very efficient in discriminating between strains genetically closely related as we confirmed in this study, and is still the method of choice for the detection of chromosome rearrangements. Nevertheless, this technique is time-consuming and complicated for use in industry.

The detection of microsatellite polymorphisms is a promising and powerful tool, providing accurate and unequivocal results expressed as base pair number (or as a number of repeats). This technique is the most appropriate for large-scale studies like determination of genetic proximity (phylogenetic studies) and biogeographical distribution of indigenous *Saccharomyces* strains and/or species by means of numerical analysis. It requires higher equipment investment and skilled human resources which can be seen as the only disadvantages of this technique.

In conclusion, due to the verified equivalence of the results, any of these methods could be applied for industrial applications, such as quality assurance during dry yeast production, implantation studies or tracing of contamination routes. For standard control during the fermentation process PCR amplification of δ sequences and mtDNA restriction analysis are the most appropriate methods. The choice of the most convenient technique should depend on the resources available and the objective of the work.

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References

[1] Degré, R., Thomas, D.Y., Ash, J., Mailhiot, K., Morin, A. and Dubord, C. (1989) Wine yeast strain identification. Am. J. Enol. Vitic. 40, 309–315.
[2] Carle, G.F. and Olson, M.W. (1985) An electrophoretic karyotype for yeast. Proc. Natl. Acad. Sci. USA 81, 2965–2969.
[3] Blondin, B. and Vezinhet, F. (1988) Identification de souches de levures oenologiques par leurs caryotypes obtenus en électrophorèse en champs pulsée. Rev. Fr. Oenol. 28, 7–11.
[4] Guillamon, J.M., Barrio, E. and Querol, A. (1996) Characterization of wine strains of the *Saccharomyces* genus on the basis of molecular markers: relationship between genetic distance and geographic or ecological distance. Syst. Appl. Microbiol. 19, 122–132.
[5] Vezinhet, F., Blondin, B. and Hallet, J.-N. (1990) Chromosomal DNA patterns and mitochondrial DNA polymorphisms as tools for identification of enological strains of *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 32, 568–571.
[6] Dubourdieu, D., Sokol, A., Zucca, J., Thalouarn, P., Datte, A. and Aigle, M. (1984) Identification des souches de levures isolées de vins par l'analyse de leur ADN mitochondrial. Conn. Vigne Vin 21, 267–278.
[7] Lopez, V., Querol, A., Ramon, D. and Fernandez-Espinar, M.T. (2001) A simplified procedure to analyse mitochondrial DNA from industrial yeasts. Int. J. Food Microbiol. 68, 75–81.
[8] Querol, A., Barrio, E., Huerta, T. and Ramon, D. (1992) Molecular monitoring of wine fermentations conducted by active dry yeast strains. Appl. Environ. Microbiol. 58, 2948–2953.
[9] Fernandez-Espinar, M.T., Lopez, V., Ramon, D., Bartra, E. and Querol, A. (2001) Study of the authenticity of commercial wine yeast strains by molecular techniques. Int. J. Food Microbiol. 70, 1–10.
[10] Cameron, J.R., Loh, E.Y. and Davis, R.W. (1979) Evidence for transposition of dispersed repetitive DNA families in yeast. Cell 16, 739–751.
[11] Ness, F., Lavallée, F., Dubourdieu, D., Aigle, M. and Dulau, L. (1993) Identification of yeast strains using the polymerase chain reaction. J. Sci. Food Agric. 62, 89–94.
[12] Lavallée, F., Salvas, Y., Lamy, S., Thomas, D.Y., Degre, R. and Dulau, L. (1994) PCR and DNA-fingerprinting used as quality-control in the production of wine yeast strains. Am. J. Enol. Vitic. 45, 86–91.
[13] Masneuf, I. and Dubourdieu, D. (1994) Comparaison de deux techniques d'identification des souches de levures de vinification basées sur le polymorphisme de l'ADN génomique: Réaction de polymerisation en chaine (PCR) et analyse des caryotypes (electrophorèse en champ pulsée). J. Int. Sci. Vigne Vin. 28, 153–160.
[14] Legras, J.L. and Karst, F. (2003) Optimisation of interdelta analysis for *Saccharomyces cerevisiae* strain characterisation. FEMS Microbiol. Lett. 221, 249–255.
[15] Baleiras Couto, M.M., Eijsma, B., Hofstra, H., HuisintVeld, J.H.J. and van der Vossen, J. (1996) Evaluation of molecular typing techniques to assign genetic diversity among *Saccharomyces cerevisiae* strains. Appl. Environ. Microbiol. 62, 41–46.
[16] Techera, A.G., Jubany, S., Carrau, F.M. and Gaggero, C. (2001) Differentiation of industrial wine yeast strains using microsatellite markers. Lett. Appl. Microbiol. 33, 71–75.
[17] Gallego, F.J., Perez, M.A., Martinez, I. and Hidalgo, P. (1998) Microsatellites obtained from database sequences are useful to characterize *Saccharomyces cerevisiae* strains. Am. J. Enol. Vitic. 49, 350–351.
[18] Hennequin, C., Thierry, A., Richard, G.F., Lecointre, G., Nguyen, H.V., Gaillardin, C. and Dujon, B. (2001) Microsatellite typing as a new tool for identification of *Saccharomyces cerevisiae* strains. J. Clin. Microbiol. 39, 551–559.
[19] Perez, M.A., Gallego, F.J. and Hidalgo, P. (2001) Evaluation of molecular techniques for the genetic characterization of *Saccharomyces cerevisiae* strains. FEMS Microbiol. Lett. 205, 375–378.
[20] Perez, M.A., Gallego, F.J., Martinez, I. and Hidalgo, P. (2001) Detection, distribution and selection of microsatellites (SSRs) in the genome of the yeast *Saccharomyces cerevisiae* as molecular markers. Lett. Appl. Microbiol. 33, 461–466.
[21] Egli, C.M., Edinger, W.D., Mitrakul, C.M. and Henick-Kling, T. (1998) Dynamics of indigenous and inoculated yeast populations and their effect on the sensory character of Riesling and Chardonnay wines. J. Appl. Microbiol. 85, 779–789.
[22] Frezier, V. and Dubourdieu, D. (1992) Ecology of yeast strain *Saccharomyces cerevisiae* during spontaneous fermentation in a Bordeaux winery. Am. J. Enol. Vitic. 43, 375–380.
[23] Versavaud, A., Courcoux, P., Roulland, C., Dulau, L. and Hallet, J.-N. (1995) Genetic diversity and geographical distribution of wild *Saccharomyces cerevisiae* strains from the wine-producing area of Charentes, France. Appl. Environ. Microbiol. 61, 3521–3529.
[24] Torija, M.J., Rozes, N., Poblet, M., Guillamon, J.M. and Mas, A. (2001) Yeast population dynamics in spontaneous fermentations: Comparison between two different wine-producing areas over a period of three years. Antonie Van Leeuwenhoek 79, 345–352.
[25] Comi, G., Maifreni, M., Manzano, M., Lagazio, C. and Cocolin, L. (2000) Mitochondrial DNA restriction enzyme analysis and evaluation of the enological characteristics of *Saccharomyces cerevisiae* strains isolated from grapes of the wine-producing area of Collio (Italy). Int. J. Food Microbiol. 58, 117–121.
[26] Sabate, J., Cano, J., Esteve-Zarzoso, B. and Guillamon, J.M. (2002) Isolation and identification of yeasts associated with vineyard and winery by RFLP analysis of ribosomal genes and mitochondrial DNA. Microbiol. Res. 157, 267–274.
[27] Beltran, G., Torija, M.J., Novo, M., Ferrer, N., Poblet, M., Guillamon, J.M., Rozes, N. and Mas, A. (2002) Analysis of yeast populations during alcoholic fermentation: A six year follow-up study. Syst. Appl. Microbiol. 25, 287–293.
[28] Perez-Ortin, J.E., Querol, A., Puig, S. and Barrio, E. (2002) Molecular characterization of a chromosomal rearrangement involved in the adaptive evolution of yeast strains. Genome Res. 12, 1533–1539.
[29] Goto-Yamamoto, N., Kitano, K., Shiki, K., Yoshida, Y., Suzuki, T., Iwata, T., Yamane, Y. and Hara, S. (1998) SSU1-R, a sulfite resistance gene of wine yeast, is an allele of SSU1 with a different upstream sequence. J. Ferment. Bioeng. 86, 427–433.
[30] Bidenne, C., Blondin, B., Dequin, S. and Vezinhet, F. (1992) Analysis of the chromosomal DNA polymorphism of wine strains of *Saccharomyces cerevisiae*. Curr. Genet. 22, 1–7.
[31] Longo, E. and Vezinhet, F. (1993) Chromosomal rearrangements during vegetative growth of a wild strain of *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 59, 322–326.
[32] Puig, S., Querol, A., Barrio, E. and Perez-Ortin, J.E. (2000) Mitotic recombination and genetic changes in *Saccharomyces cerevisiae* during wine fermentation. Appl. Environ. Microbiol. 66, 2057–2061.