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The genetic characterization of Saccharomyces cerevisiae commercial enological strains: a survey of molecular typing techniques

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Abstract

Wine production by the addition of active dry wine yeast is today widely accepted, being about 50% of the wine produced in Europe in this way. This enological practice required the development of techniques that were able to distinguish the inoculated strain from the rest of the wild yeast strains present in the must. The aim of this study is to validate the usefulness of different typing methods (karyotype analysis [1], ∂ sequence typing [2][5], mtDNA restriction analysis [3] and microsatellite genotyping [4]) by studying the degree of polymorphism generated by each of them in 23 commercially available winery yeasts from 5 companies.

The amplification of delta sequence interspersed DNA regions generated 8 patterns for primer pair A [2] and 21 for primer pair B [5] respectively. The discriminative power of karyotype analysis and mtDNA RFLP (using the restriction enzyme HinfI) was very similar, and generated 21 patterns for the 23 strains. The results obtained by both methods indicated the occurrence of one strain that is commercialized by 3 different active dry yeast producers. Microsatellite typing unequivocally confirmed the results obtained by karyotyping and by mtDNA RFLP. The results show that microsatellite analysis is a very precise and fast method for the typing of S. cerevisiae strains.

Introduction

Wine production by the use of selected *Saccharomyces cerevisiae* strains, commercially available as active dry yeast, is widely accepted, being an enological practice extensively applied nowadays. The use of techniques that enable to distinguish the inoculated strain from the remaining yeast flora present in the grape must is regarded with great practical interest. In the last years, several methodologies of typing based on DNA polymorphisms have been developed which allowed the discrimination among closely related yeast strains.The aim of this study is to validate the usefulness of each typing method (karyotype analysis by pulse field gel electrophoresis [1], ∂ sequence typing [2][5], mitochondrial DNA (mtDNA) restriction analysis [3], and microsatellite genotyping [4]) by studying the degree of polymorphism generated by each of them in 23 commercial winery yeasts.

Commercial yeast strains

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-Würtenberg, Germany

Materials and Methods

DNA isolation

Yeast cells were cultivated in 5 ml of YPD medium (24 h, 28ºC, 160 rpm) and DNA isolation was performed using the method described by López et al. [6].

Delta sequence typing

Amplification reactions were performed on a BioRad iCycler thermal cycler, using the primers ∂1 (5'-CAAAATTCACCTATATCT-3') and ∂2 (5'-GTGGATTTTTATTCCAAC-3') (primer pair A) [2] or ∂12 (5'-TCAACAATGGAATCCCAAC-3') and ∂2 (primer pair B) [5]. 15µl reaction mixture was prepared with 60 ng of DNA, 0.5 U Taq polymerase , 10 x Taq buffer (both from MBI Fermentas) 25 pmoles 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 30s, 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 % agarose gel containing ethidium bromide, visualized and photographed.

Restriction Fragment Length Polymorphism of mitochondrial DNA (mtDNA RFLP)

The reactions were preformed with the restriction enzymes RsaI and HinfI using the method described by López et al. [6].

Pulse field gel electrophoresis (PFGE)

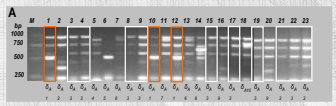
Yeast chromosomal DNA was prepared in plugs as previously described [1], and washed one time in TE buffer (1 mM EDTA, 10 mMTris-HCl, pH 8.0) at 50°C for 30 min and then three times in the same buffer at room temperature for 30 min. The plugs were loaded in a 1 % (w/v) agarose (Seakem® Gold) gel and electrophoresis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

Microsatellite amplification

The six trinucleotide microsatellite loci described as ScAAT1, ScAAT2, ScAAT3, ScAAT4, ScAAT5 and ScAAT6 [4] were amplified in two multiplex reactions using 20 ng of template DNA, fluorescently labeled primers, 0,5 U Taq polymerase (MBI Fermentas), the corresponding Taq buffer, 0.2 mM of each dNTP and 2 mM $MgCl_2$. Multiplex reaction A contained primer pairs for detection of loci ScAAT1 (0.05 pmol), ScAAT6 (0.05 pmol) and ScAAT4 (0.03 pmol), containing the respective forward and reverse primers. The multiplex reaction B was composed of primer pairs for ScAAT2 (0.05 pmol), ScAAT3 (0.1 pmol) and ScAAT5 (0.075 pmol) loci. In both cases, the total reaction volume was 6 µl, and cycling was performed as described [4]. Samples were analysed by capillary electrophoresis (15 KV, 60ºC, 30 min in an ABI Prism 310 DNA sequencer (Applied Biosystems) and by using the corresponding GENESCAN software.

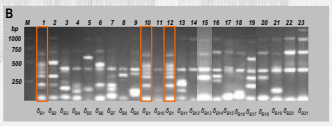
RESULTS

Delta sequence typing



Primer pair A

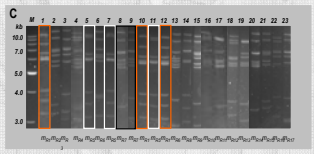
- *Most of the patterns share several common bands (500, 750 and 970 bp.*
- *Pattern δ_{A3} was found in 10 of the 23 strains analyzed.*
- *Strains 1, 10 and 12 show an identical and characteristic pattern (δ_{A2}).*
- *Assignment of 10 different patterns.*



Primer pair B

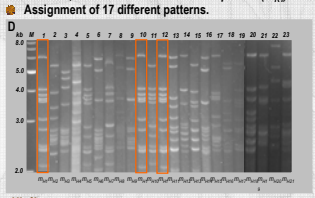
- *Very high polymorphism due to the presence of many intense bands of different sizes.*
- *Almost all patterns appear to have only one band in common (400) bp*
- *Strains 1, 10 and 12 show an identical pattern (δ_{B1}), with four bands sized between 300 and 500 bp.*
- *Assignment of 21 different patterns.*

RFLP of mitochondrial DNA



RsaI

- *Strains 5, 7 and 11 shared pattern m_{R9} while pattern m_{R7} was shared by strains 8 and 9.*
- *Average size of fragments: 8 – 10 kb.*
- *Strains 1, 10 and 12 show an identical pattern (m_{R1}).*
- *Assignment of 17 different patterns.*



HinfI

- *Average size of fragments: 3 – 6 kb.*
- *Unique patterns with exception of strains 1, 10 and 12 that show the pattern m_{H1}.*
- *Assignment of 21 different patterns.*

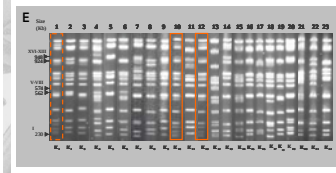
Microsatellite amplification

Allelic diversity (bp) of 6 microsatellite loci

Strain	Microsatellite					
	SCAAT1	SCAAT2	SCAAT3	SCAAT4	SCAAT5	SCAAT6
1	189, 237	375	250, 348	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, 284	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, 348	302	219, 222	250, 256
11	195	375	256	329	222	256, 259
12	189, 237	375	250, 348	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
N° alleles	17	11	15	8	6	3
N° genotypes	18	7	11	6	3	3

- *Unique patterns were found for 21 strains, while an identical pattern was found for strains 1, 10 and 12.*
- *The number of alleles found for each locus varies between 3 and 17, being the loci AAT1 and AAT3 characterized by the highest polymorphism.*
- *The number of genotypes varies between 3 and 18 for each locus separately analyzed.*

Pulse field gel electrophoresis

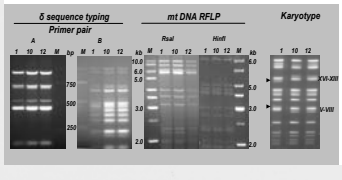


- *In the range below 600 Kb, where the resolution is best, the greatest variability was found, both in the position and in the number of bands, which varied from five to ten.*
- *The patterns of the strains 10 and 12 (K10) were again identical whereas in strain 1 small differences in the zones of about 600 Kb (chromosomes XVI-XIII) and 900 Kb (chromosomes V-VIII) were observed.*
- *Except for 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.*
- *Assignment of 22 different chromosomal patterns.*

Summary of the results obtained by all molecular typing methods. Results are summarized by assigning (for each typing method) a different number to a pattern when it is different from the others. Depending on the technique used, distinct levels of discrimination were obtained, varying from 13 to 22 different patterns.

Strain	Pattern					
	δ sequence		mt DNA RFLP		Microsatellite ScAAT 1-6	Karyotype
	δ_{A2}	δ_{B2}	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	4	4	4	4	4	4
5	5	5	5	5	5	5
6	6	6	6	6	6	6
7	7	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

Interdelta sequence typing, mtDNA RFLP and microsatellite analysis: identical genetic fingerprints of strains 1, 10 and 12; Karyotype analysis by pulse field gel electrophoresis: Pattern of strain 1 is slightly different from the identical patterns of strains 10 and 12.



CONCLUSIONS

Our results show that microsatellite typing and the optimized interdelta analysis have a similar discriminatory power like the mtDNA restriction analysis and karyotyping. The discriminatory power among all the methodologies gave a good correlation. Among the 23 commercial yeast strains used in this study, 22 distinct patterns were obtained. None of the typing methods was able to discriminate among two S. cerevisiae commercial strains (10 and 12).

Karyotyping by Pulse Field Gel Electrophoresis is very efficient to discriminate between strains that are genetically closely related.

The discriminating power obtained by combining the allele sizes from the six microsatellite loci was very high. The combination of the results from loci AAT1 and AAT3 that generated the highest polymorphism (18 and 11 genotypes) is sufficient for the unequivocal characterization of the present population of 23 strains. For studies aiming at the characterization of strains that are genetically closer related, it may be necessary to include data obtained for the other loci.

Due to the verified equivalence of the results, any of these methods can be applied at the industrial scale, e.g. in the monitorization of fermentative processes. The improved PCR amplification of delta sequences described by Legras et al. (2003) is a very convenient method that does not require high equipment investment.

References

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Acknowledgements

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