MOLECULAR METHODS EVALUATION FOR THE TYPING OF *SACCHAROMYCES CEREVISIAE* WINE STRAINS

D. Schuller [1], E. Valero [2], S. Dequin [2] and M. Casal [1]

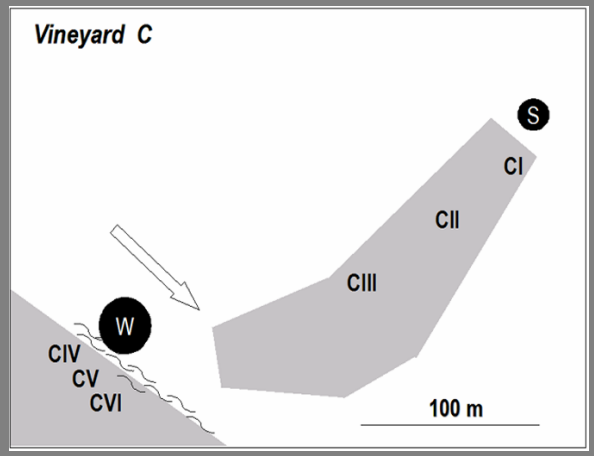
[1] Centro de Biologia
Universidade do Minho
Portugal

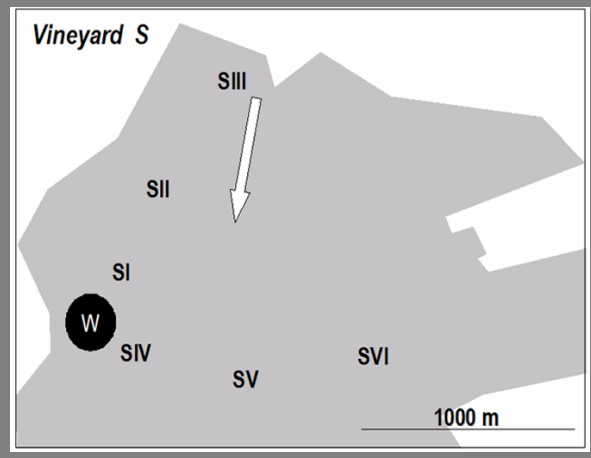
[2] Institut Nacional de la Recherche Agronomique
UMR Sciences pour l'Oenologie
France.

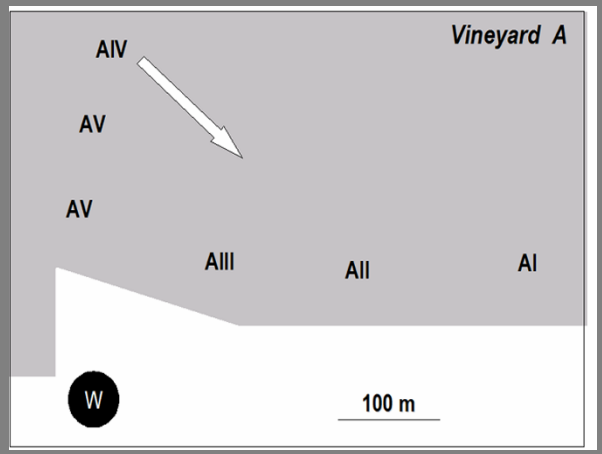
XII Jornadas van Uden

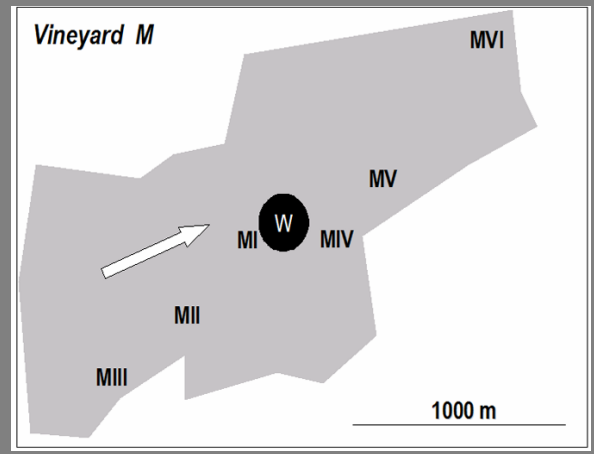
Geographic localization of the vineyards belonging to the Languedoc (S, M, O) and Vinho Verde (A, C, P) Wine Regions and indication of the sampling sites in each of the six vineyards.

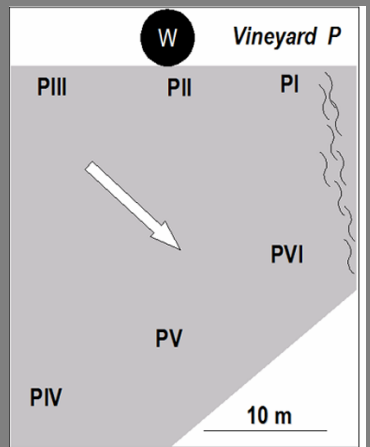
The factors that may have influence in the dissemination of commercial yeast strains are indicated in the Figure.









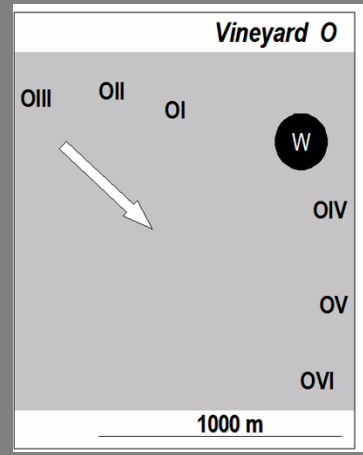


W Winery

S Storage site for harvest equipment

Water rill

Predominating wind direction



Commercial Saccharomyces cerevisiae *strains*

- ***Wine production by the use of selected Saccharomyces cerevisiae strains, is an enological practice extensively applied nowadays***

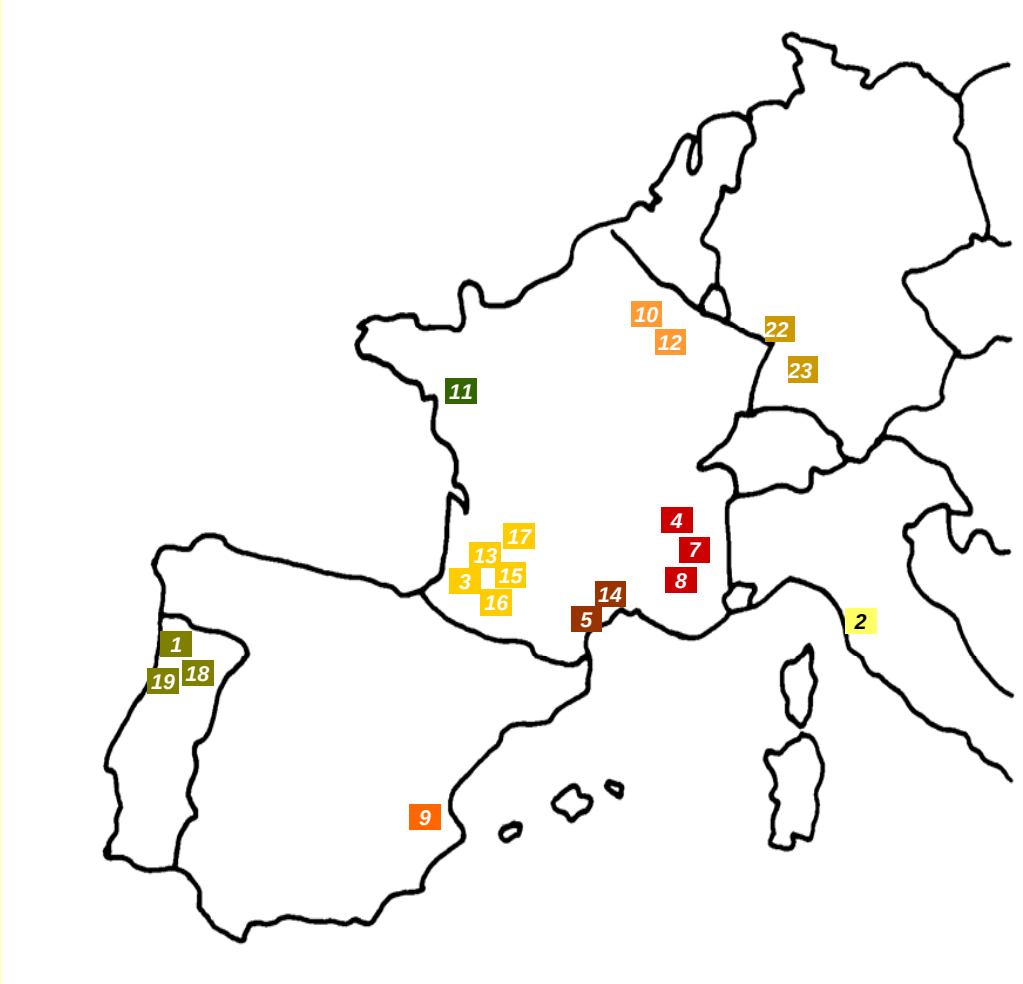
- ***Isolation of wild yeasts from the natural environment associated with the winemaking area of interest***

- ***Enological aptitude of the isolates***

  - ***Ability to dominate the fermentation process***
  - ***Enhance the sensorial characteristics of wines originating from different grapewine cultivars***
  - ***Owing other biotechnological properties that are important in winemaking***

Materials and Methods

Strain	Origin
1	P - Minho
2	I - Tuscany
3	F - Bordeaux
4	F - Côtes du Rhône
5	F - Midi
6	SA - Stellenbosh
7	F - Côtes du Rhône
8	F - Côtes du Rhône
9	E - Valencia
10	F - Champagne
11	F - Loire
12	F - Champagne
13	F - Bordeaux
14	F - Midi
15	F - Bordeaux
16	F - Bordeaux
17	F - Bordeaux
18	P - Dão
19	P - Bairrada
20	D – not known
21	Not known
22	D - Pfalz
23	D - Baden-Würtenberg



Materials and Methods

PCR-based interdelta-analysis

Location transactivating region (LTR) flanking retrotransposons TY1 and TY2

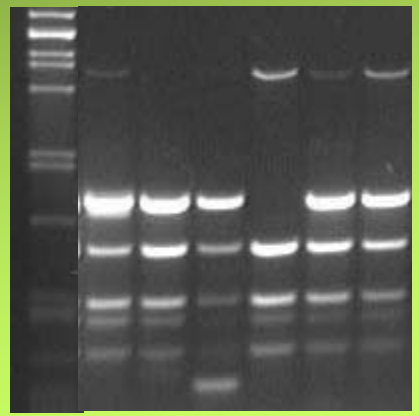
Separate elements dispersed throughout the genome

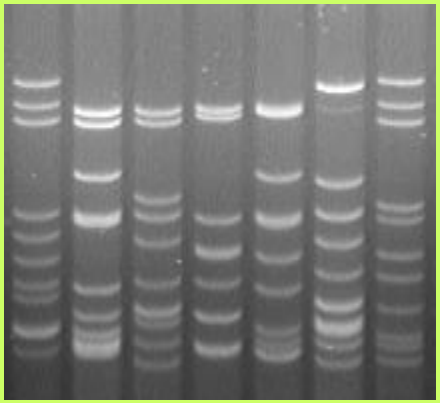
300 delta elements in strain S288C

Good targets for identification of polymorphisms

primer pair δ1- δ2 *(Ness et al., 1993)*

primer pair δ12- δ2 *(Legras et al., 2003)*





Mitochondrial DNA restriction analysis (mtDNA RFLP)

(Querol et al., 1992; Lopez et al., 2001)

Mitochondrial DNA of S. cerevisiae: ~70 kb

Enological yeast strains have a large mtDNA diversity

Digestion with HinfI or RsaI

Materials and Methods

Karyotype analysis

(Carle and Olson., 1995;
Blondin and Vezinhet, 1988)

Differentiation of strains according to the size distribution of their chromosomes by Pulse-field electrophoresis

Microsatelite analysis – 6 loci

(Pérez et al., 2001)

SSR (Simple Sequence Repeats)
short (1-10 nucleotides) DNA tandem repeats
dispersed throughout the genome
high degree of variability

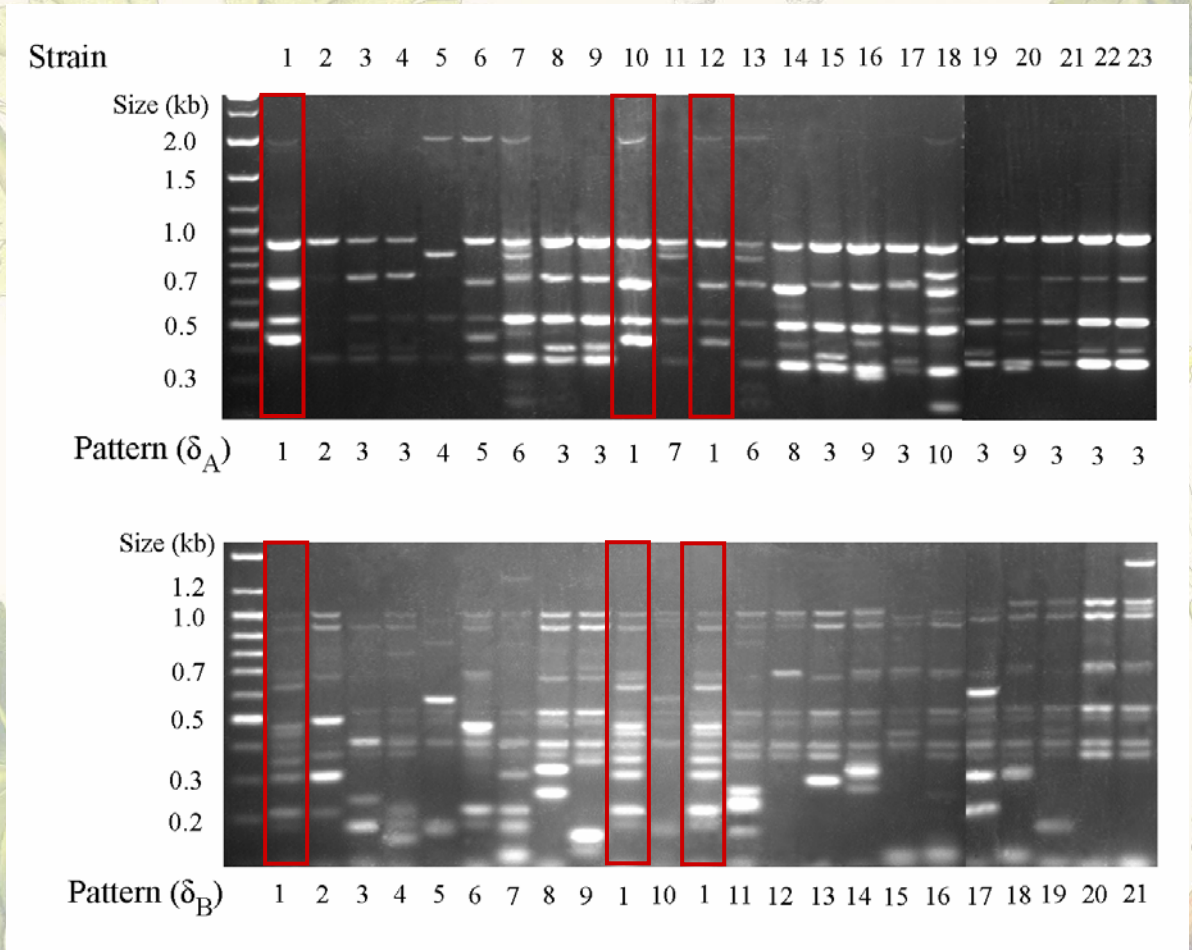
AAT1	*FAM*	*145-246 pb*
AAT4	*TET*	*278-335 pb*
AAT6	*HEX*	*249-267 pb*
AAT2	*HEX*	*370-406 pb*
AAT3	*FAM*	*247-445 pb*
AAT5	*TET*	*216-225 pb*

2 multiplex reactions

R E S U L T S

Interdelta analysis

δ1 - δ2



δ12 - δ2

Number of distinct patterns:

10

21

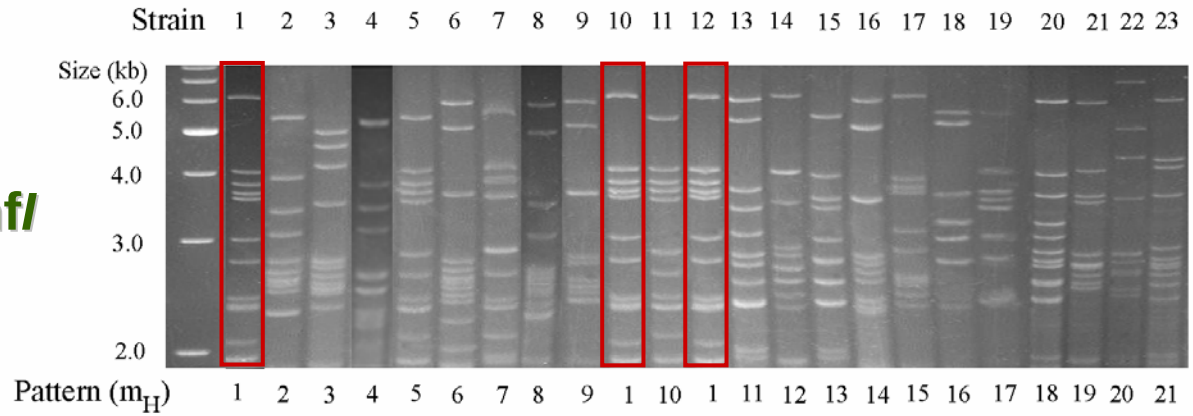
Substitution of primer δ1 by primer δ12: 2-fold increase in the number of patterns

R E S U L T S

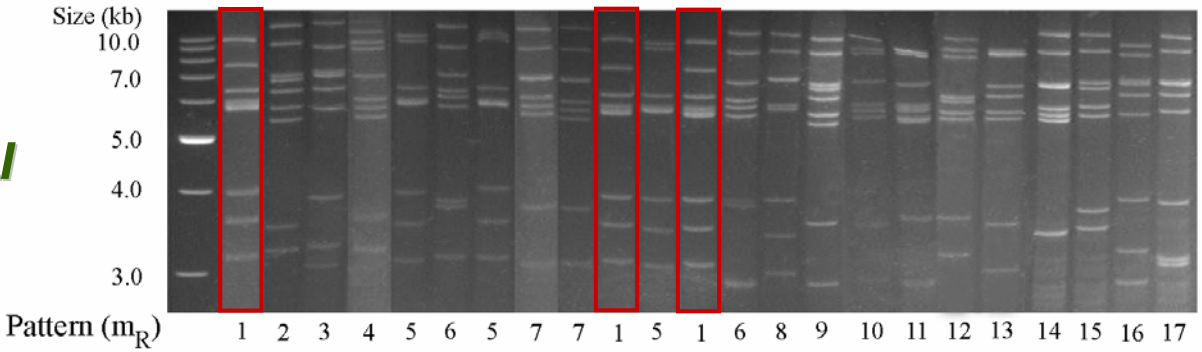
mtDNA RFLP

Number of distinct patterns:

HinfI — *21*

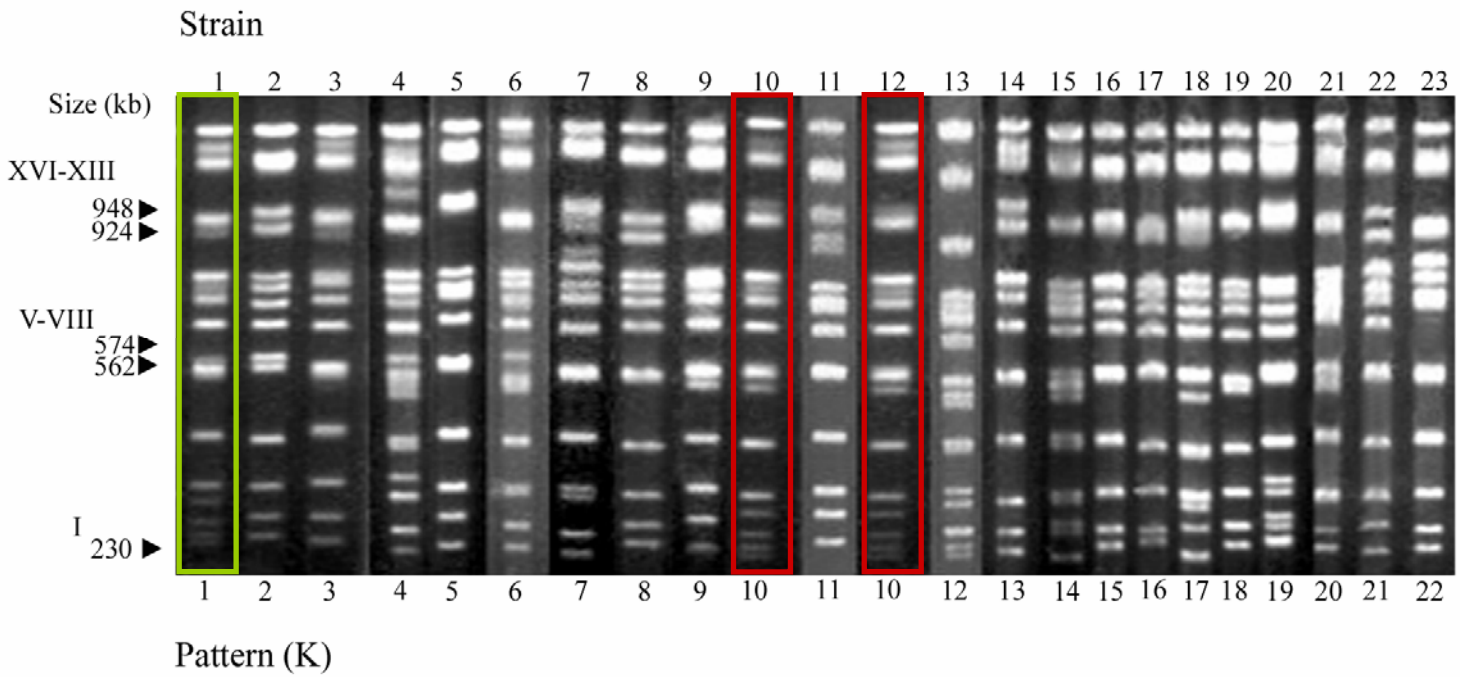


RsaI — *17*



R E S U L T S

Karyotype analysis



Number of distinct patterns: **22**

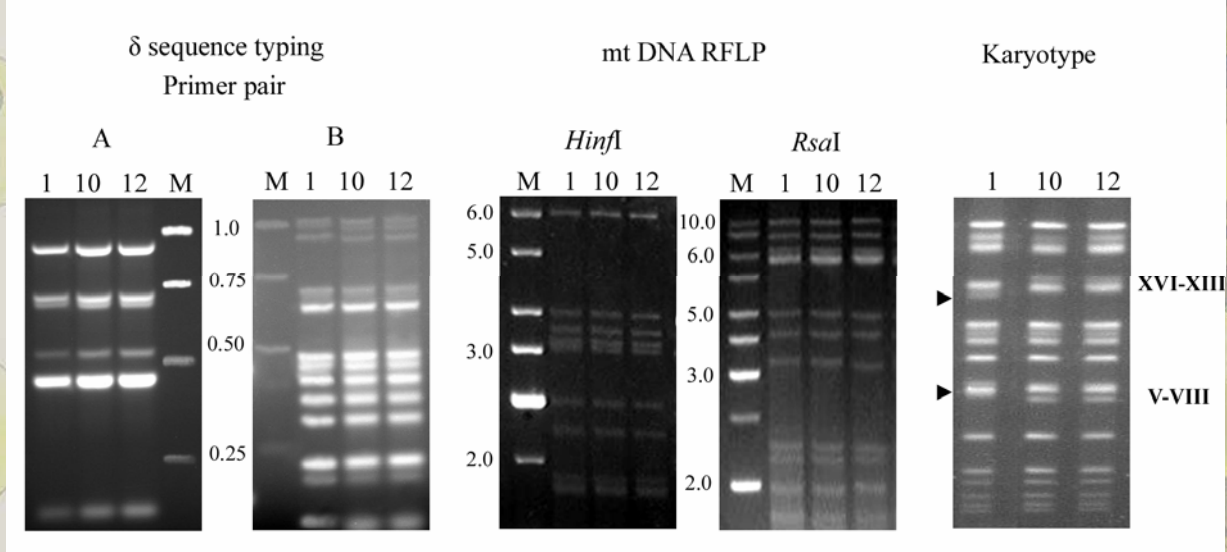
R E S U L T S

Microsatellite analysis

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
N° alleles	15	7	11	6	3	3
N° genotypes	18	11	14	8	6	4

R E S U L T S

COMPARISON OF STRAINS 1, 10 AND 12



reciprocal translocation between chromosomes VIII and XVI, generating two new chromosomes $VIII^{XVI}$ and XVI^{VIII}

rearrangement in wine yeast strains involved in their adaptative evolution, since the translocation results in higher expression of SSU1

Conclusions

- ***Microsatellite typing (loci ScAAT1-6),***
  Optimized interdelta sequence analysis
  mtDNA RFLP (*HinfI*)

  same discriminatory power:

  23 commercial yeast strains → 21 distinct patterns

- ***Karyotype analysis originated 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

R E S U L T S

Summarizing …

depending on the technique used, distinct levels of discrimination were obtained, varying from 10 to 22 different patterns

Strain	Pattern					
	δ sequence		mt DNA RFLP		Microsatellite SCAAT 1-6	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