Genotypic and pheno-metabolomic characterization of a *Saccharomyces cerevisiae* strain collection

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5 – Department of Chemistry & QOPNA, Aveiro

XXXIV WORLD CONGRESS OF VINE AND WINE
"The Wine Construction"
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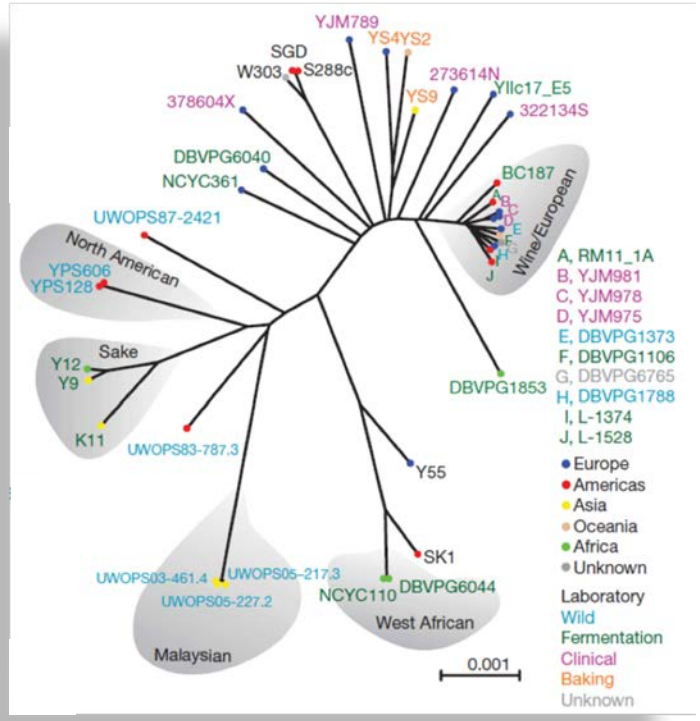
INTRODUCTION

Saccharomyces cerevisiae:

- World's premier commercial microorganism for biotechnological applications
- Most of European wine producers use commercial starter yeasts to guarantee the reproducibility and the predictability of wine quality
- Winemaker has control over the microbiology of a fermentation containing starter cultures, and in these fermentations the inoculated yeast strain predominates and suppresses the indigenous flora

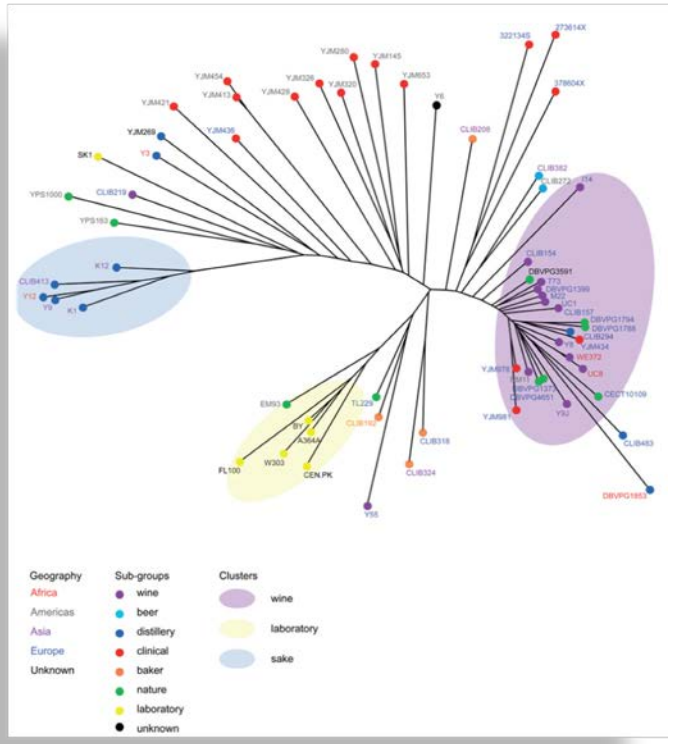
INTRODUCTION

The population structure of *S. cerevisiae*



Liti et al., Nature, 2009

235,127 SNPs
14,051 nucleotide insertions or deletions



Schacherer et al., Nature, 2009

1.89 x 10^6 SNP (30,097 SNPs per strain)
3,985 deletions (200 bp length)

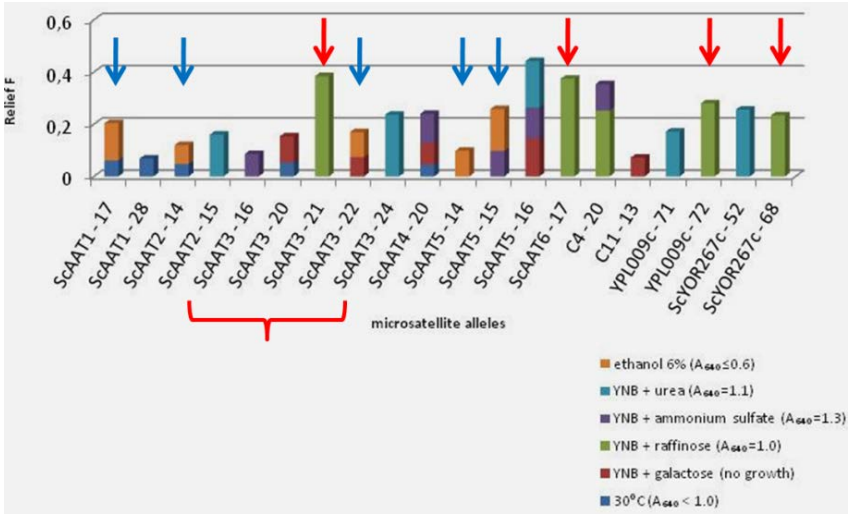
few well-defined, geographically isolated lineages
many different mosaics of these lineages

INTRODUCTION

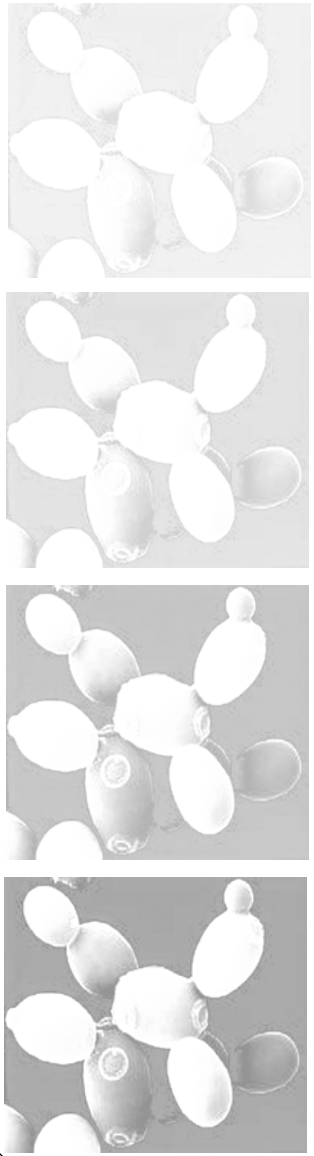
Franco-Duarte, *et al.*, 2009

- Evaluation of phenotypic and genetic variability of *S. cerevisiae* strains from the *Vinho Verde* region using computational approaches

Strains / phenotypic tests	4 °C	4 °C	18°C	30°C	30°C	45°C	45°C	YNB + galactose	YNB + galactose	YNB + maltose	YNB + maltose	YNB + raffinose	YNB + raffinose	YNB + ammonium sulphate	YNB + urea	YNB + urea	ethanol 6%	H_2S production
	A_{640}=0.1	A_{640}=0.1	A_{640}=1.4	A_{640} < 1.0	A_{640} ≥ 1.0	A_{640} ≤ 0.2	A_{640} = 0.1	A_{640} = 0.1	A_{640} = 1.1	A_{640} = 0.8	A_{640} ≥ 1.2	$0.5 \leq A_{640} \geq 0.6$	A_{640} = 1.0	A_{640} = 1.3	A_{640} = 1.1	A_{640} = 1.4	A_{640} ≤ 0.6	classes 1, 2 and 3
Modelling technique	linear	linear	kNN	tree	kNN	linear	linear	linear	linear	linear	tree	kNN	linear	linear	linear	linear	linear	tree
AUC	0.83	0.77	0.80	0.77	0.76	0.77	0.75	0.77	0.76	0.90	0.77	0.75	0.77	0.85	0.80	0.79	0.77	0.83
Percentage (%)	8.2	8.2	30.0	**100**	22.3	6.9	7.3	**100**	30.0	50.0	61.8	23.1	**100**	**90.9**	**100**	79,3	**100**	21.4



OBJECTIVES



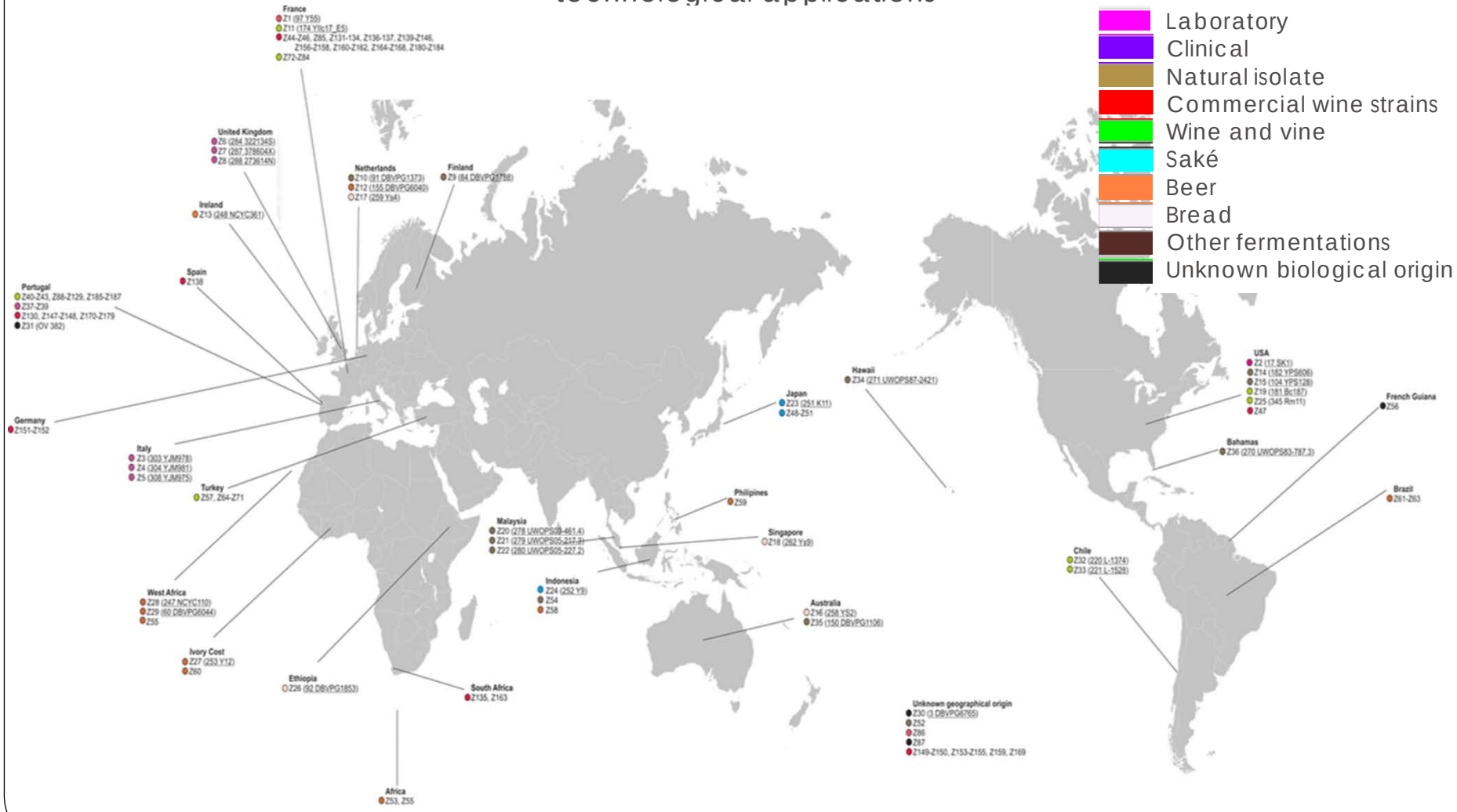
- To gain a deeper understanding of the phenotypic and genetic diversity of a *S. cerevisiae* strain collection

- To use high-throughput quantitative and qualitative methods in combination with bioanalytical data

MATERIAL & METHODS

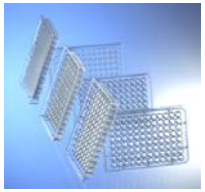
Strain collection

- *S. cerevisiae* collection comprising 187 strains with different geographical origins and technological applications



MATERIAL & METHODS

Phenotypic characterization



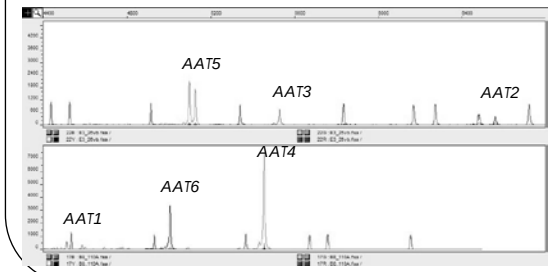
- Wine must + compound
- 30 ºC
- 200 rpm
- quadruplicate

Phenotypes tested:

- Temperatures (18, 30 and 40ºC)
- Tolerance to stress
  - pH values (2 and 8)
  - Osmotic/saline stress (KCl and NaCl)
  - Growth in finished wines supplemented with glucose
- Growth in the presence of
  - Potassium bisulfite
  - Copper sulfate
  - Sodium dodecyl sulphate
  - Iprodion
  - Procymidon
  - Cycloheximide

Genetic characterization

Microsatellite analysis



Microsatellite	Chromosome	Position/Gene	Repeat	Nº of alleles	Ref.
ScAAT1	XIII	86 901 – 87 129	ATT	29	Perez and Gallego, 2001 / Legras *et al.*, 2005
ScAAT2	II	CDC27	ATT	18	
ScAAT3	IV	SSY1	ATT	19	
ScAAT4	VII	431 334 – 431 637	ATT	17	
ScAAT5	XVI	897 028 - 897 259	TAA	6	
ScAAT6	IX	105 661 – 105 926	TAA	10	
YPL009	XV	NFI1	TAA	13	
SCYOR267C	XV	HRK1	TGT	12	
C4	XV	110 701-110 935	TAA+TAG	9	
C5	VI	210250-210414	GT	19	
C11	X	518870-519072	GT	18	

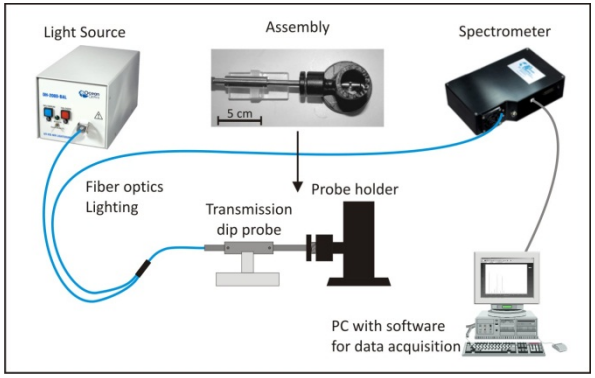
MATERIAL & METHODS

Must fermentations

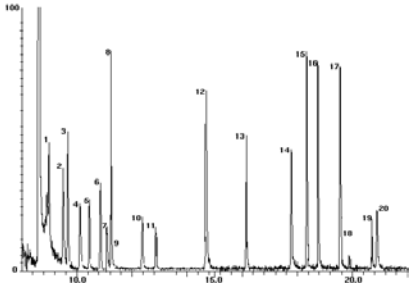
- Wine must
- 18 ºC
- Weight loss determination due to CO_2 liberation
- Samples were withdrawn and frozen when a constant weight was recorded

Fiber optics spectroscopy

- Transmittance fiber optics UV-VIS-SWNIR spectroscopy (200 – 1200 nm)
- Silva *et al.*, 2008
- Poster #



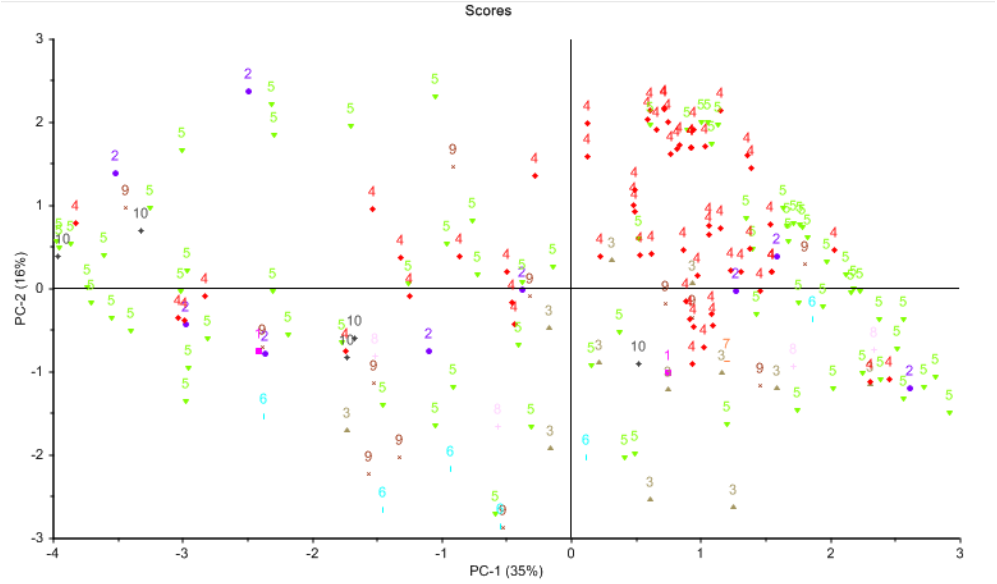
Bioanalytical analysis



- **HPLC-RI** to quantify fructose, glucose, ethanol, glycerol and organic acids (tartaric, malic, acetic, and succinic)
- **GC-MS** analysis performed by solid phase microextraction (SPME), and using 3-octanol as internal standard (Silva Ferreira *et al.*, 2003)

RESULTS

Phenotypic characterization



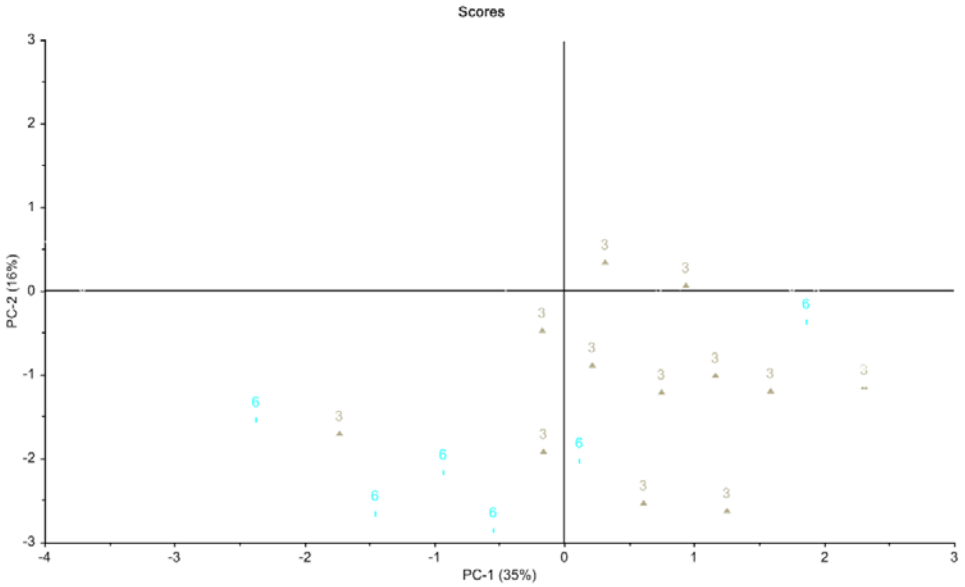
1 – Laboratory
2 – Clinical
3 – Natural isolate (soil woodland, plants, insects)
4 – Commercial wine strains
5 – Wine and vine
6 – Saké
7 – Beer
8 – Bread
9 – Other fermentations
10 – Unknown biological origin

PCA analysis:

- segregation of strains in the first two components

RESULTS

Phenotypic characterization



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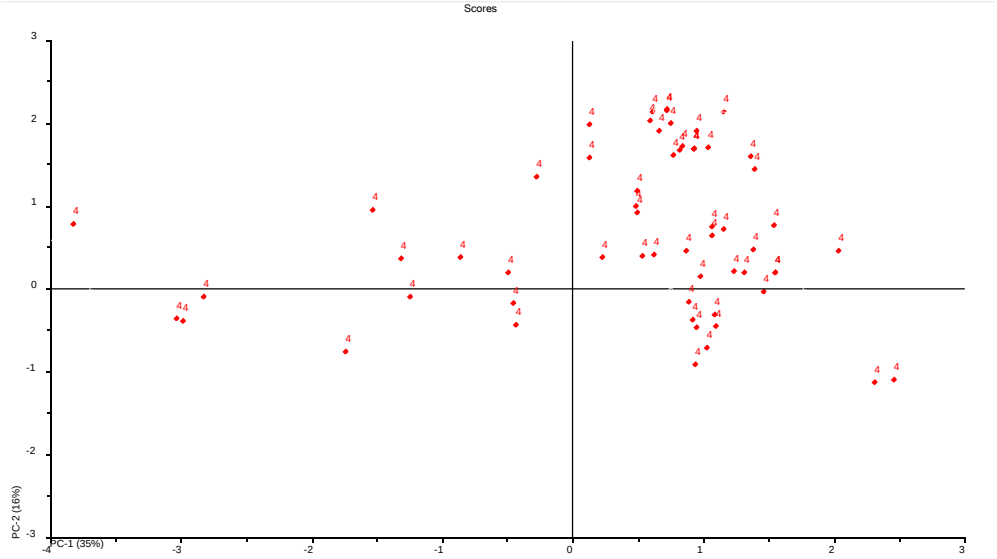
10 – Unknown biological origin

PCA analysis:

- natural isolates (3)
- saké (6)

RESULTS

Phenotypic characterization



- **1** – Laboratory
- **2** – Clinical
- **3** – Natural isolate (soil woodland, plants, insects)
- **4** – Commercial wine strains
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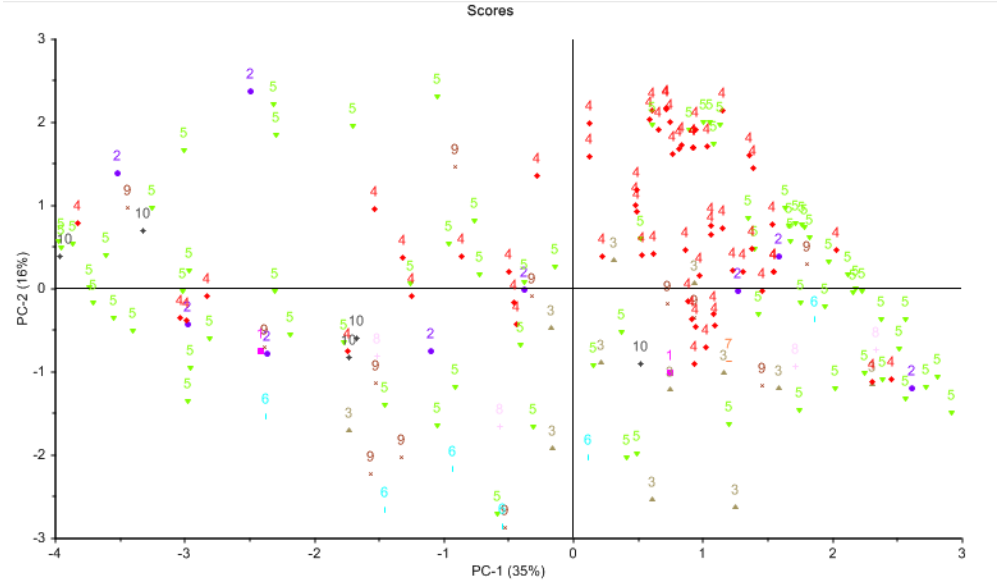
- **8** – Bread
- **9** – Other fermentations
- **10** – Unknown biological origin

PCA analysis:

- commercial strains (4)

RESULTS

Phenotypic characterization



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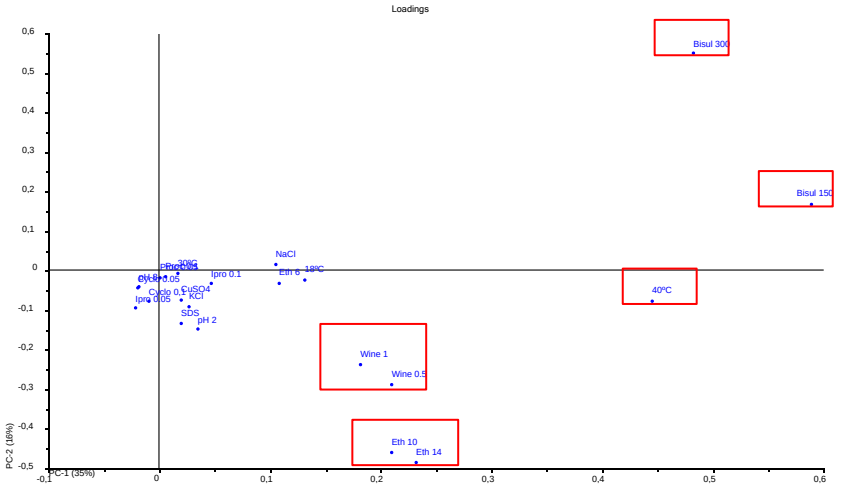
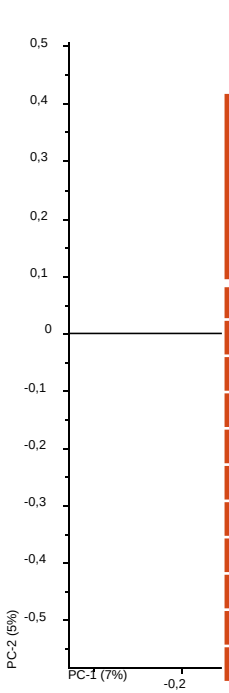
- Potassium bisulfite
- 40 ºC
- Wine supplemented with glucose
- Ethanol



RESULTS

Genetic characterization

11 microsatellites ⟶ 236 alleles



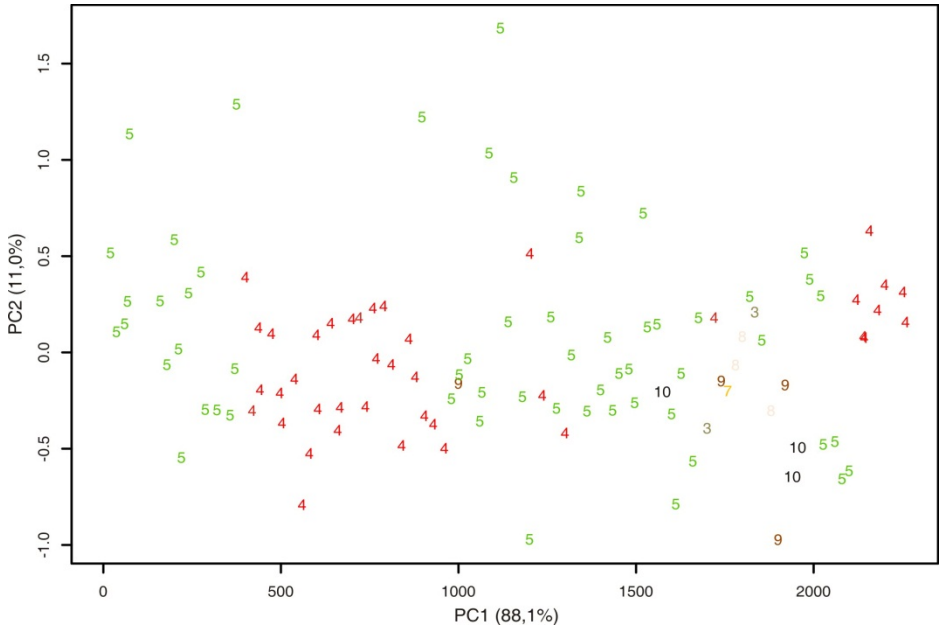
Microsatellite	Total number of alleles	Most variable alleles (bp) identified by PCA analysis	Percentage of most variable alleles among the total number of alleles
C5	44	115, 211	4.55
YPL009c	40	256, 298, 301	7.50
YOR267c	40	278, 290	5.00
C11	32	189, 191, 209, 211, 213	15.63
ScAAT1	31	171, 174, 201, 204, 219	16.13
ScAAT3	24	232, 241, 247, 262, 265	20.83
C4	23	245, 260	8.70
ScAAT4	21	302, 332	9.52
ScAAT2	18	372, 375, 378, 381	22.22
ScAAT6	15	256, 259	13.33
ScAAT5	13	219, 222	15.38

PCA analysis:

- **34 alleles**
  - significant variable between strains

RESULTS

Fiber optics spectroscopy



3 – Natural isolate (soil woodland, plants, insects)
4 – Commercial wine strains
5 – Wine and vine
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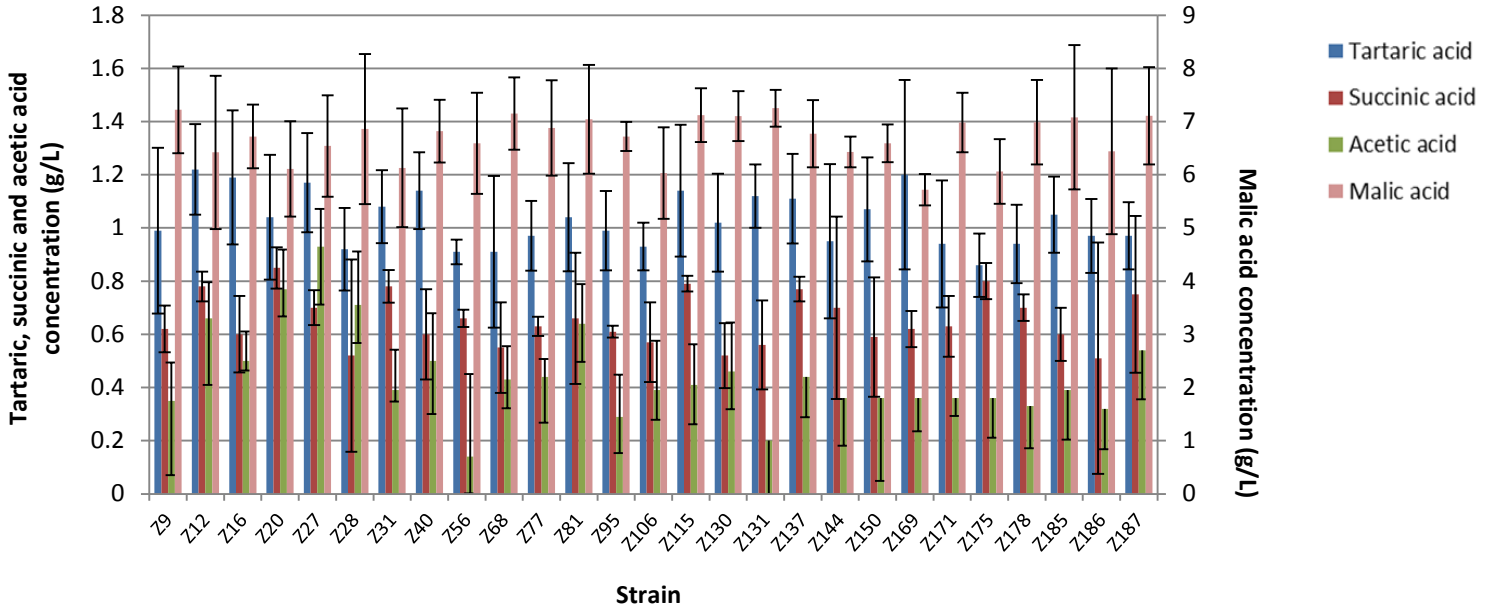
187 strains → 28 most heterogenous strains

→ fermentations (3 replicates) → HPLC, GC-MS

RESULTS

HPLC analysis

- Strain-dependent differences were observed



Tartaric acid concentrations: 0.5 – 1.4 g/L

Succinic acid concentrations: 0.3 – 1.3 g/L

Acetic acid concentrations: 0 – 1.2 g/L

Malic acid concentrations: 4.7 – 8.2 g/L

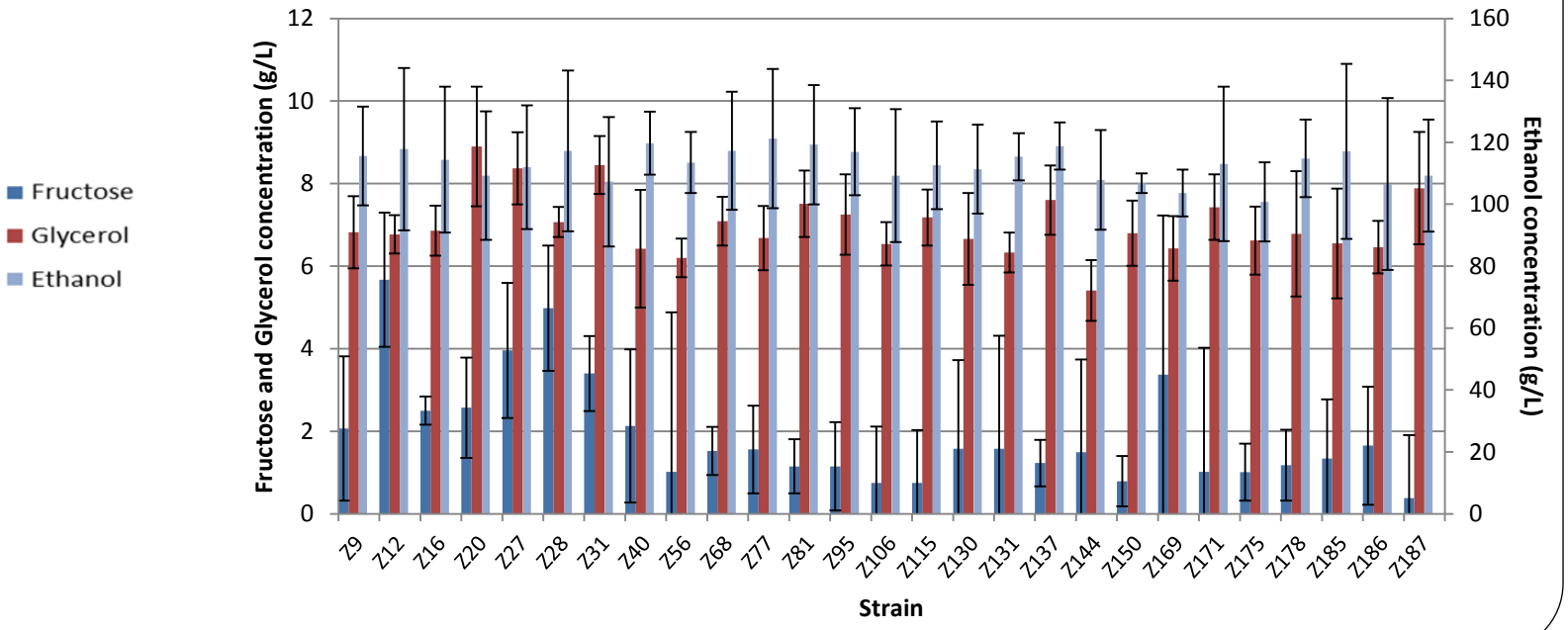
RESULTS

HPLC analysis

Fructose concentrations: 0 – 9 g/L

Glycerol concentrations: 5 – 9.75 g/L

Ethanol concentrations: 80 - 138 g/L



RESULTS

GC-MS analysis

Concentration of the quantified aromatic compounds

Compounds	Ethyl acetate	Isobutyl acetate	Ethyl butanoate	2-methyl-1-propanol	Isoamyl acetate	2-Methy-1-butanol	Ethyl hexanoate	Hexyl acetate	Ethyl lactate	1-Hexanol	cis-hex-3-en-1-ol	2-Phenylethyl acetate	2-Phenyl ethanol
Sensorial threshold µg L^{-1} [a] mg L^{-1} [b]	12.3 [b]	1.6 [b]	20 [a]	75 [b]	30 [a]	30 [b]	14 [a]	1 [b]	14 [b]	5.2 [b]	400 [a]	650 [a]	14 [b]
Odor description	Solvent, fruity, nail polish	Banana, sweet, fruity	Papaya, butter, sweet, apple, fragrant, fruity	alchool	Banana, apple, solvent	alcohol, banana, sweet, aromatic, cheese	apple, fruity, sweet, aniseed-flavored	sweet, aromatic, fragrant	Strawberry, raspberry	coconut, green leefs	green leafs, banana, sweet, herb	roses, honey, apple, sweet, floral	Roses, sweet, fragrant, flowery, honey-like
Z9	3970.4	1.8	38.9	666.1	260.9	128.9	0.5	14.9	nd	227.1	55.8	6.5	2820.1
Z12	3213.3	2.6	38.2	575.0	266.6	nd	0.6	16.2	nd	188.4	nd	nd	735.0
Z16	4390.1	1.7	41.2	473.9	339.9	nd	nd	11.9	nd	153.8	nd	nd	446.3
Z20	2600.4	nd	16.8	1754.7	65.0	nd	0.6	5.0	nd	181.7	34.4	1.6	965.7
Z27	714.0	nd	13.1	1178.0	27.0	nd	nd	3.4	nd	125.5	nd	6.7	799.2
Z28	2785.5	nd	15.5	337.4	102.9	nd	0.6	10.9	nd	181.4	36.2	1.9	614.9
Z31	3112.6	0.4	24.0	476.0	98.1	nd	0.6	6.8	735.9	135.8	27.4	0.9	810.8
Z40	2614.4	0.8	16.0	789.3	67.4	nd	0.5	10.6	nd	177.1	37.9	nd	606.7
Z63	2430.9	nd	16.6	840.9	24.9	nd	0.7	5.9	nd	209.4	nd	nd	414.6
Z77	3699.9	2.2	43.3	920.9	306.3	nd	0.6	13.0	889.0	105.4	29.2	3.6	555.6
Z81	1583.1	nd	7.4	1086.1	13.2	nd	0.5	2.5	952.0	111.3	34.1	nd	nd
Z89	2008.7	0.9	10.8	284.1	54.9	nd	0.5	10.0	nd	143.0	30.6	1.2	194.9
Z95	2534.8	0.7	23.5	218.9	44.3	96.3	0.5	8.8	nd	150.8	33.7	nd	560.1
Z103	2231.2	nd	29.1	354.7	152.5	nd	0.5	18.3	nd	154.0	33.7	5.2	773.4
Z115	3565.2	2.5	32.3	401.8	242.2	nd	0.6	21.2	nd	192.0	37.1	2.4	454.2
Z127	2949.4	nd	26.4	329.2	154.9	nd	nd	10.3	nd	177.4	34.0	nd	605.1
Z131	nd	2.3	34.7	1790.4	256.9	nd	0.6	15.4	nd	163.8	nd	2.3	897.9
Z137	1462.6	nd	9.7	744.4	44.8	nd	0.6	8.8	1395.0	146.9	36.2	1.7	850.8
Z169	6381.6	1.0	26.6	526.2	105.9	nd	0.7	11.6	1919.6	185.7	36.7	1.1	650.0
Z171	732.3	0.2	6.6	549.3	12.7	nd	0.6	3.5	nd	123.3	31.2	nd	595.8
Z178	1747.6	nd	14.3	467.9	102.9	nd	0.6	12.6	1353.2	127.4	31.8	1.8	909.2
Z185	1097.5	1.2	11.4	609.8	107.8	nd	0.7	12.4	196.9	186.3	37.2	1.5	668.3
Z186	646.5	0.6	9.1	121.0	80.3	nd	0.6	12.9	401.3	151.4	30.8	1.0	364.9
Z187	1821.1	nd	11.5	214.8	106.0	nd	nd	15.5	nd	171.6	nd	nd	502.4
non-fermented must	nd	nd	nd	nd	nd	48.6	0.6	nd	nd	97.8	43.0	nd	nd

nd – bellow detection limits

RESULTS

GC-MS analysis

- Compounds above sensorial threshold

- ***Ethyl butanoate*** ***(24.0 – 43.3 µg/L)***
- ***Isoamyl acetate*** ***(44.3 – 339.9 µg/L)***

Compounds			Ethyl butanoate		Isoamyl acetate								
Sensorial threshold µg L^{-1} [a] mg L^{-1} [b]	12.3 [b] $	1.6 [b] $	20 [a]	75 [b] $	30 [a]	30 [b] $	14 [a] $	1 [b] $	14 [b] $	5.2 [b] $	400 [a] $	650 [b] $	14 [b] $
Odor description	Solvent, fruity, nail polish [1,2,3]	Banana, sweet, fruity [1,2]	Papaya, butter, sweet, apple, fragrant, fruity	alchool [1,8]	Banana, apple, solvent	alcohol, banana, sweet, aromatic, cheese [1,5,7]	apple, fruity, sweet, aniseed-flavored [1,5]	sweet, aromatic, fragrant [1,8]	Strawberry, raspberry [1,2,9]	coconut, green leefs [1,9,10]	green leafs, banana, sweet, herb [1,4,5,11]	roses, honey, apple, sweet, floral [1,5,9]	Roses, sweet, fragrant, flowery, honey-like [1,2,4,5,6,12]
Z9	3970.4	1.8	38.9	666.1	260.9	128.9	0.5	14.9	nd	227.1	56.8	6.5	2620.1
Z12	3213.3	2.6	38.2	575.0	266.6	nd	0.6	16.2	nd	188.4	nd	nd	735.0
Z16	4390.1	1.7	41.2	473.9	339.9	nd	nd	11.9	nd	153.8	nd	nd	446.3
Z20	2600.4	nd	16.8	1754.7	65.0	nd	0.6	5.0	nd	181.7	34.4	1.6	965.7
Z27	714.0	nd	13.1	1178.9	27.0	nd	nd	3.4	nd	125.5	nd	6.7	799.2
Z28	2785.5	nd	15.5	337.4	102.9	nd	0.6	10.9	nd	181.4	36.2	1.9	614.9
Z31	3112.6	0.4	24.0	476.6	98.1	nd	0.6	6.6	735.9	135.8	27.4	0.9	910.8
Z40	2614.4	0.8	16.0	789.3	67.4	nd	0.5	10.6	nd	177.1	37.9	nd	606.7
Z63	2430.9	nd	16.6	840.9	24.9	nd	0.7	5.9	nd	209.4	nd	nd	414.6
Z77	3699.9	2.2	43.3	920.9	306.3	nd	0.6	13.0	889.0	105.4	29.2	3.6	555.6
Z81	1589.1	nd	7.4	1086.1	13.2	nd	0.5	2.5	952.0	111.3	34.1	nd	nd
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Z185	1097.5	1.2	11.4	609.8	107.8	nd	0.7	12.4	196.9	186.3	37.2	1.5	668.3
Z186	646.5	0.6	9.1	121.0	80.3	nd	0.6	12.9	401.3	151.4	30.8	1.0	364.9
Z187	1821.1	nd	11.5	214.8	106.0	nd	nd	15.5	nd	171.6	nd	nd	502.4
non-fermented must	nd	nd	nd	nd	nd	48.6	0.6	nd	nd	97.8	43.0	nd	nd

nd – bellow detection limits

RESULTS

GC-MS analysis

- Large variance between strains

- ***Isobutyl acetate (nd – 2.5 mg/L)***
- ***2-methyl-1-propanol (284.1 – 1790.4 mg/L)***
- ***Ethyl lactate (nd – 1919.6 mg/L)***
- ***2-phenylethyl acetate (nd – 6.7 µg/L)***

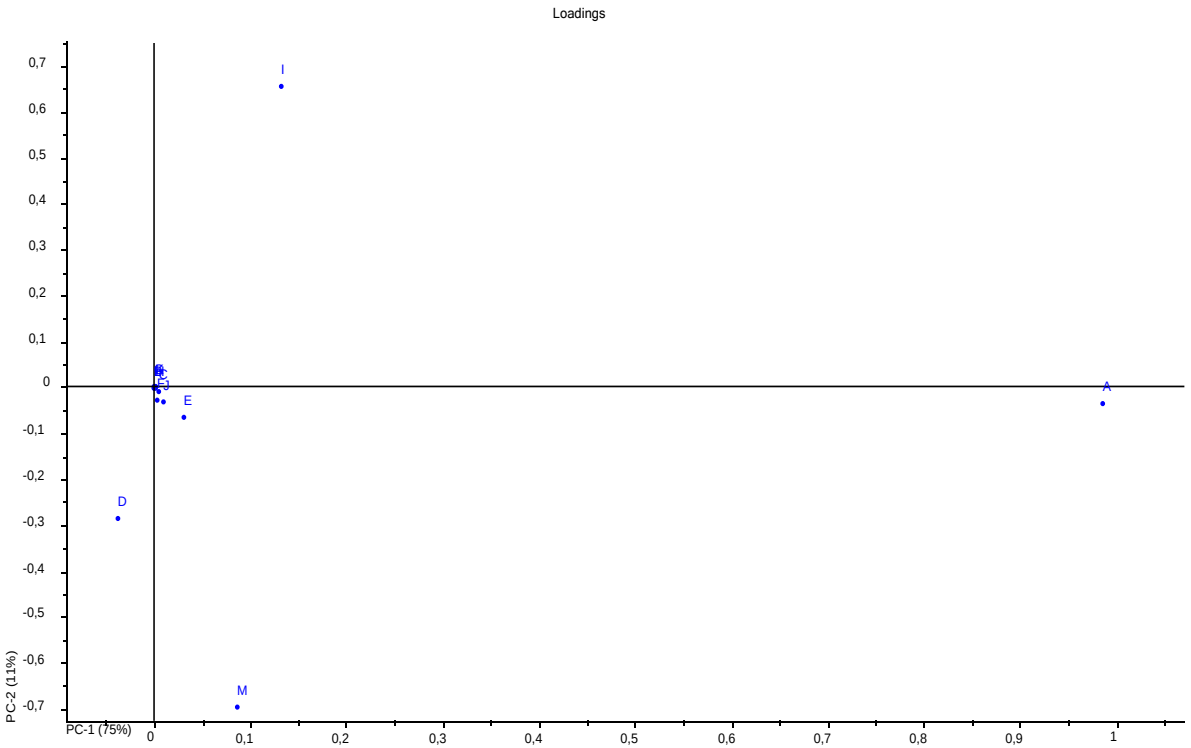
Compounds		Isobutyl acetate		2-methyl-1-propanol					Ethyl lactate			2-Phenylethyl acetate	
Sensorial threshold µg L^{-1} (a) mg L^{-1} (b)	12.3 (b) &	1.6 (b) &	20 (a) $	75 (b) $	30 (a) $	30 (b) $	14 (a) &	1 (b) $	14 (b) $	5.2 (a) $	400 (a) $	650 (a) $	14 (b) &
Odor description	Solvent, fruity, nail polish [1,2,3]	Banana, sweet, fruity	Papaya, butter, sweet, apple, fragrant, fruity [1,4,5,2,6]	alchool	Banana, apple, solvent [1,2,4,5]	alcohol, banana, sweet, aromatic, cheese [1,5,7]	apple, fruity, sweet, aniseed-flavored [1,5]	sweet, aromatic, fragrant [1,8]	Strawberry, raspberry	coconut, green leefs [1,9,10]	green leafs, banana, sweet, herb [1,4,5,11]	roses, honey, apple, sweet, floral	Roses, sweet, fragrant, flowery, honey-like [1,2,4,5,6,12]
Z9	3970.4	1.8	38.9	666.1	260.9	128.9	0.5	14.9	nd	227.1	55.8	6.5	2520.1
Z12	3213.3	2.6	38.2	575.0	266.6	nd	0.6	16.2	nd	188.4	nd	nd	735.0
Z16	4390.1	1.7	41.2	473.9	339.9	nd	nd	11.9	nd	153.8	nd	nd	446.3
Z20	2600.4	nd	16.8	1754.7	65.0	nd	0.6	5.0	nd	181.7	34.4	1.6	965.7
Z27	714.0	nd	13.1	1178.0	27.0	nd	nd	3.4	nd	125.5	nd	6.7	709.2
Z28	2785.5	nd	15.5	337.4	102.9	nd	0.6	10.9	nd	181.4	36.2	1.9	614.9
Z31	3112.6	0.4	24.0	476.0	98.1	nd	0.6	6.8	735.9	135.8	27.4	0.9	810.4
Z40	2614.4	0.8	16.0	789.3	67.4	nd	0.5	10.6	nd	177.1	37.9	nd	606.7
Z63	2430.9	nd	16.6	840.9	24.9	nd	0.7	5.9	nd	269.4	nd	nd	414.6
Z77	3699.9	2.2	43.3	920.9	306.3	nd	0.6	13.0	889.0	195.4	29.2	3.6	555.6
Z81	1583.1	nd	7.4	1086.1	13.2	nd	0.5	2.5	952.0	111.3	34.1	nd	nd
Z89	2008.7	0.9	10.8	284.1	54.9	nd	0.5	10.0	nd	143.0	30.6	1.2	194.9
Z95	2534.8	0.7	23.5	218.9	44.3	96.3	0.5	8.9	nd	150.8	33.7	nd	560.1
Z103	2231.2	nd	29.1	354.7	152.5	nd	0.5	18.3	nd	154.0	33.7	5.2	773.4
Z115	3565.2	2.5	32.3	401.8	242.2	nd	0.6	21.2	nd	192.0	37.4	2.4	454.2
Z127	2949.4	nd	26.4	329.2	154.9	nd	nd	10.3	nd	177.4	34.0	nd	605.1
Z131	nd	2.3	34.7	1790.4	256.9	nd	0.6	15.4	nd	193.8	nd	2.3	897.9
Z137	1462.6	nd	9.7	744.4	44.8	nd	0.6	8.8	1395.0	146.9	36.2	1.7	850.8
Z169	6381.6	1.0	26.6	526.2	105.9	nd	0.7	11.6	1919.6	185.7	36.7	1.1	650.0
Z171	732.3	0.2	6.6	549.3	12.7	nd	0.6	3.5	nd	123.3	31.2	nd	595.8
Z178	1747.6	nd	14.3	467.9	102.9	nd	0.6	12.6	1353.2	127.4	31.8	1.8	909.2
Z185	1097.5	1.2	11.4	609.8	107.8	nd	0.7	12.4	196.9	186.3	37.2	1.5	668.3
Z186	646.5	0.6	9.1	121.0	80.3	nd	0.6	12.9	401.3	151.4	30.8	1.0	364.9
Z187	1821.1	nd	11.5	214.8	106.0	nd	nd	15.5	nd	171.6	nd	nd	502.4
non-fermented must	nd	nd	nd	nd	nd	48.6	0.6	nd	nd	97.8	43.0	nd	nd

nd – bellow detection limits

RESULTS

GC-MS analysis

PCA analysis

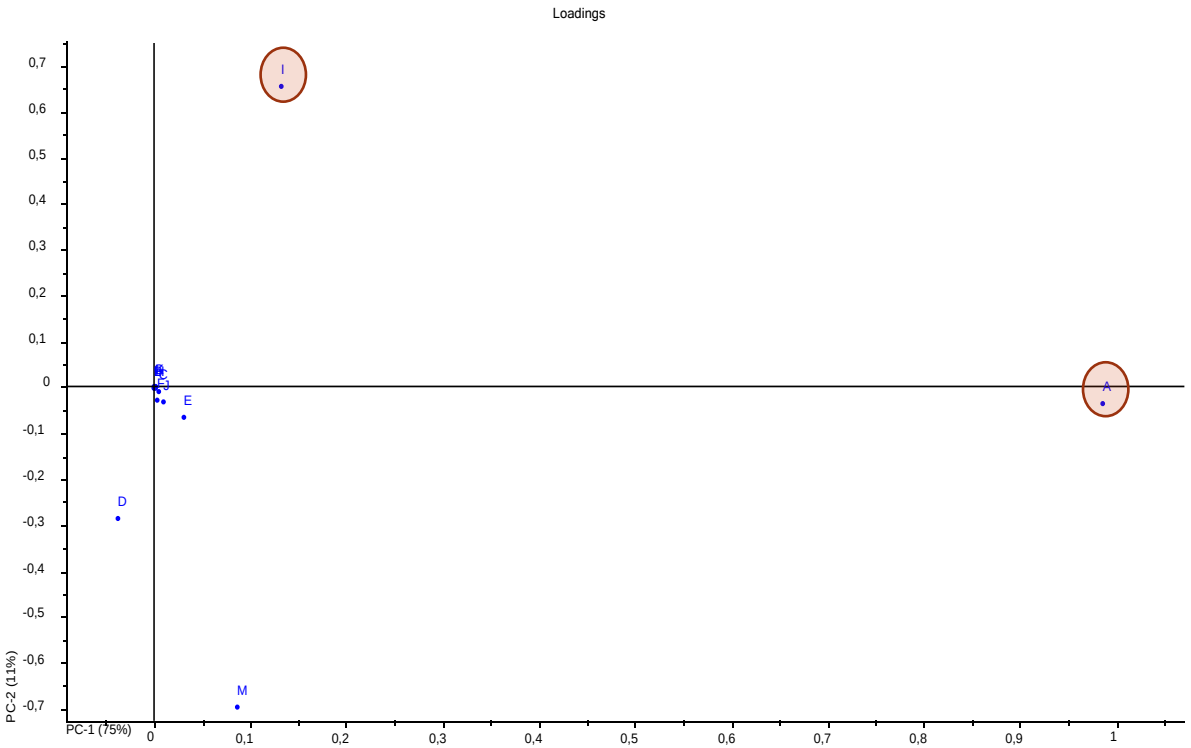


A - ethyl acetate
B – isobutyl acetate
C – ethyl butanoate
D - 2-methyl-1-propanol
E – isoamyl acetate
F - 2-methy-1-butanol
G - ethyl hexanoate
H - hexyl acetate
I - ethyl lactate
J - 1-hexanol
K - cis-hex-3-en-1-ol
L - 2-phenylethyl acetate
M – 2-phenylethanol

RESULTS

GC-MS analysis

PCA analysis



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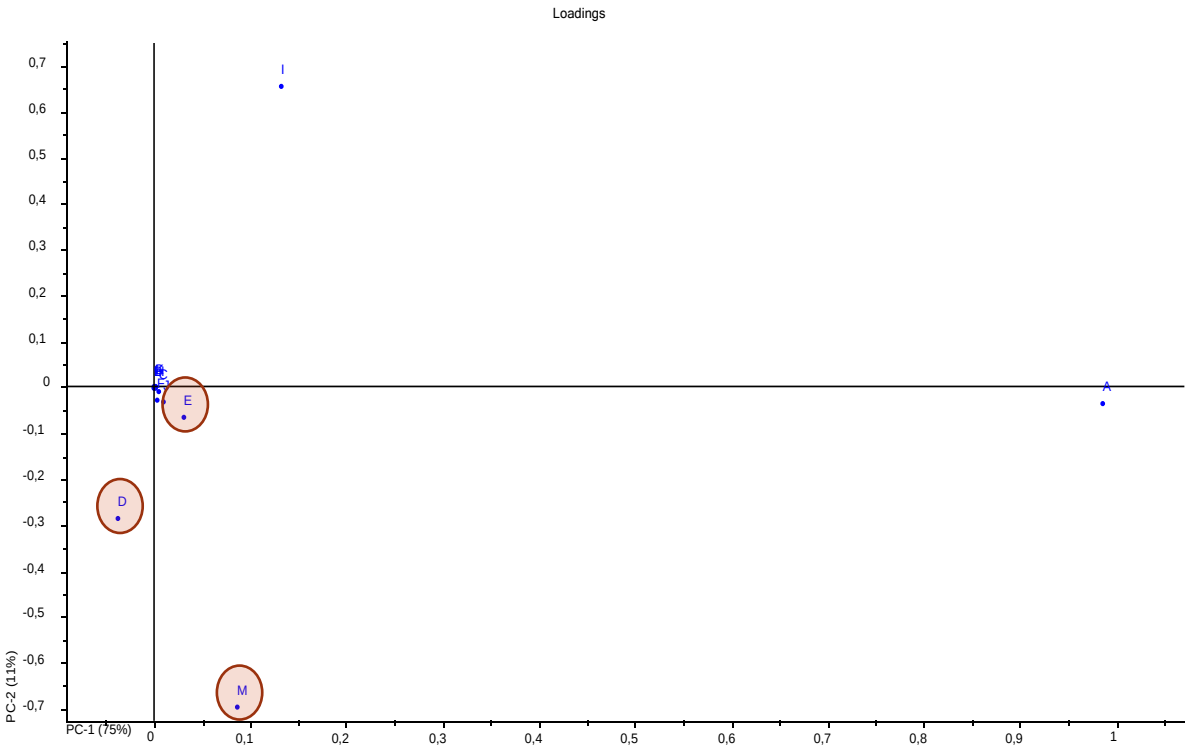

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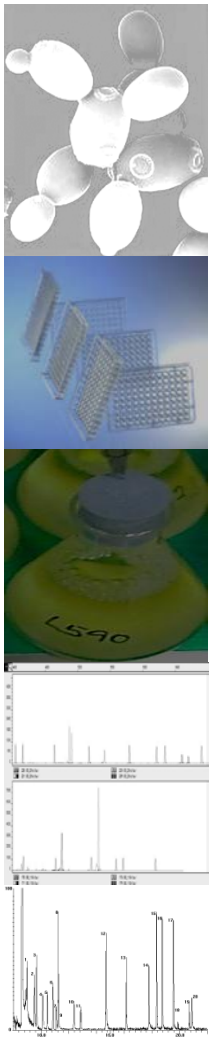
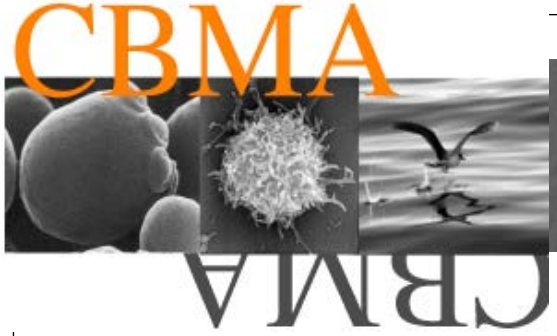
Summary and Conclusions



- ✓ The present work contributes to a better understanding of intra-strain differences regarding the pheno-metabolomic characterization of *S. cerevisiae* isolates
- ✓ A set of 11 microsatellite markers and 22 phenotypic characteristics explained large part of strain diversity
- ✓ Fiber-optics spectroscopy analysis separated strains according to their geographical origins and technological use
- ✓ Aromatic profiles of final fermentations obtained by GC-MS analysis, and quantification of primary fermentation products by HPLC, revealed a group of relevant compounds that mostly account for inter-strain variability

The acquired data will contribute to obtain a holistic view between molecular biology, analytical chemistry, signal processing and bioinformatics , and using computation approaches, to relate genomics, phenomics and metabolomics



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