I Jornadas de Bioquímica
as faces da bioquímica na Universidade do Minho
24 e 25 de Maio de 2010

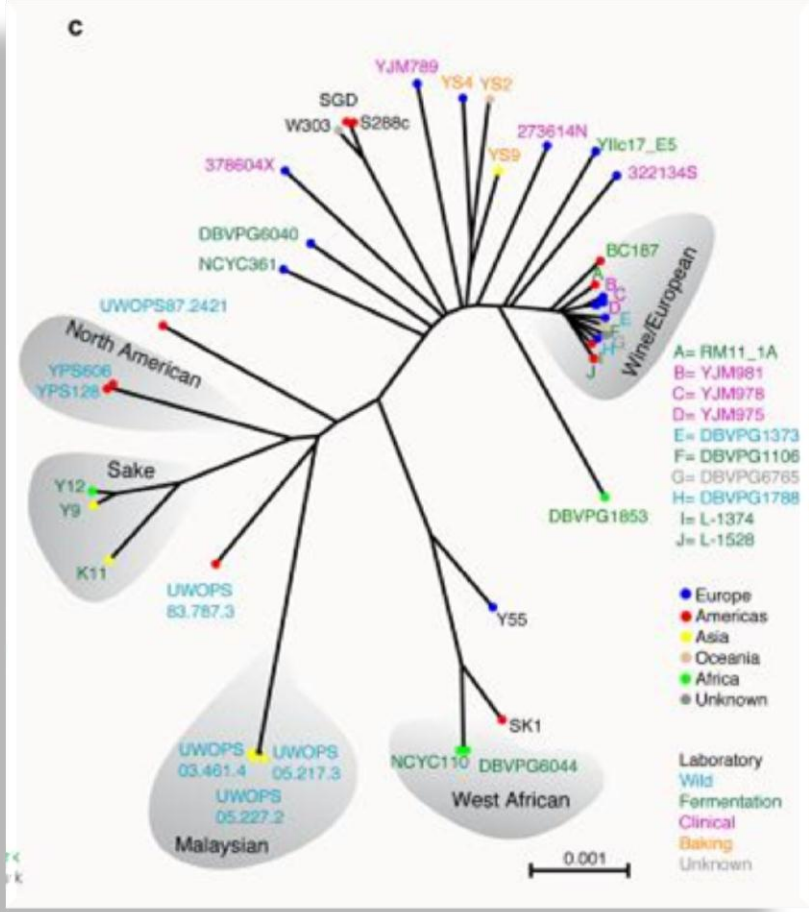
# Better yeast for better wine – *Saccharomyces cerevisiae* strains for winemaking

**Dorit-Elisabeth Schuller**
**Centro de Biologia Molecular e Ambiental, Universidade do Minho, Braga**

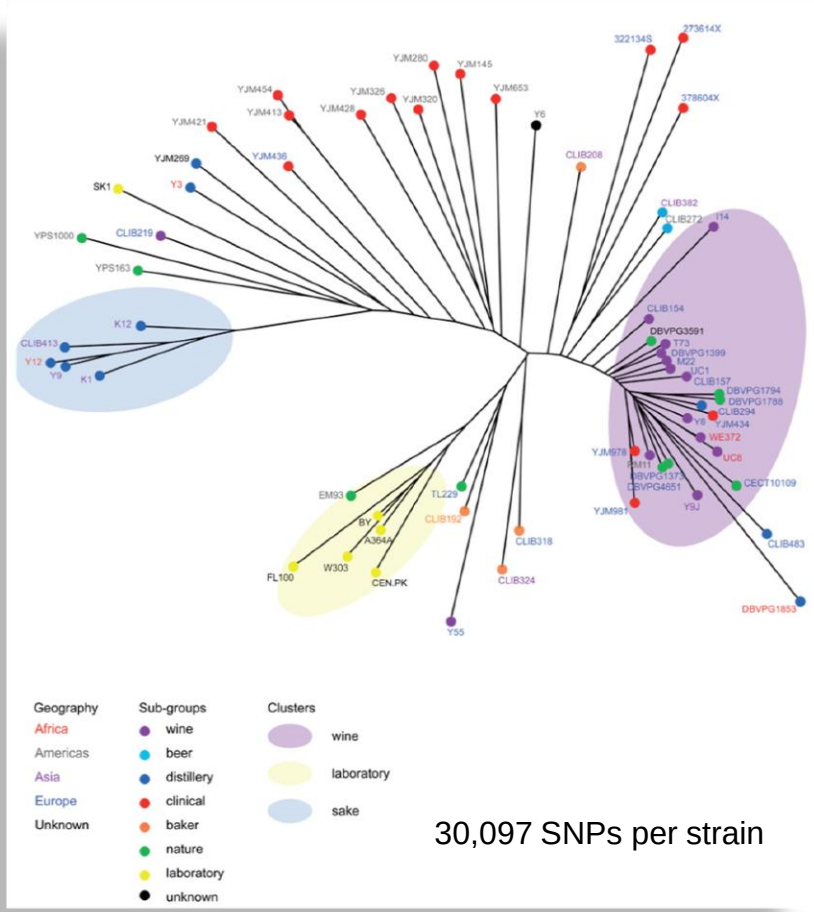
1996


**Identification of polymorphisms among individuals within a species**

**To study the genetic basis of phenotypic differences elucidate**
**To evaluate the evolutionary history of the species**




Liti et al., Nature, 2009




30,097 SNPs per strain

Schacherer et al., Nature, 2009

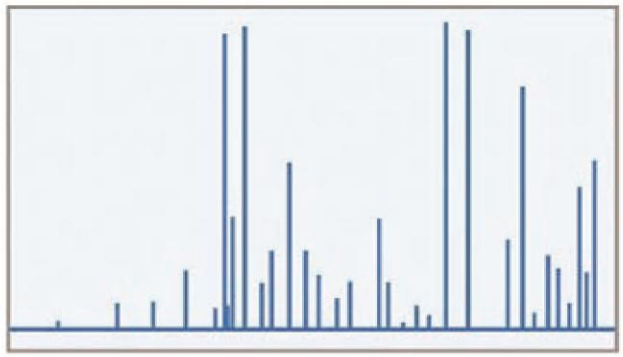
# Fermentation technologies are deeply rooted in the history of most societies

*Saccharomyces cerevisiae* strains from winemaking environments

- high ethanol production and tolerance
- growth at high sugar concentrations
- high fermentative activity (complete sugar consumption)
- growth at low temperatures
- glycerol production
- low sulphur dioxide production
- hydrogen sulphide production
- **production of flavouring compounds**

Today: Winemakers use commercial winemaking strains

# Fermentation technologies are deeply rooted in the history of most societies

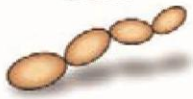


Volatile compounds in grape juice

Fermentation

*Saccharomyces cerevisiae*

*Oenococcus oeni*

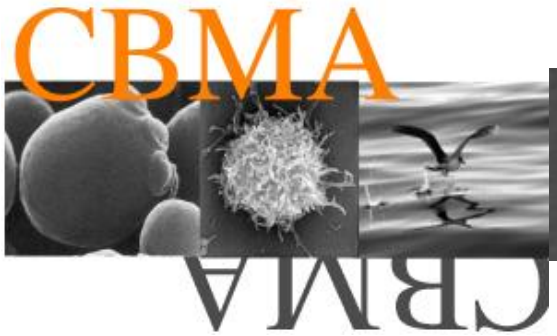


*Which of these are the impact aroma and flavour compounds ?*

Volatile compounds in wine

**Adapted from**

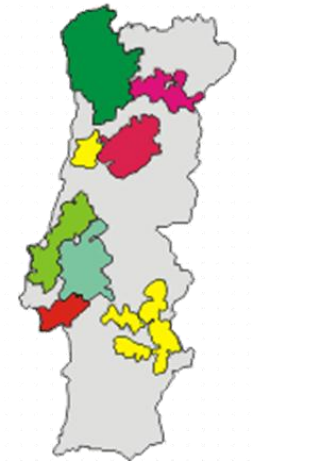
**Swiegers et al, Australian Journal of Grape and Wine Research, 2005**

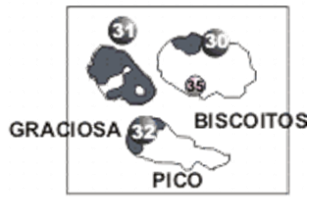



## Main topics

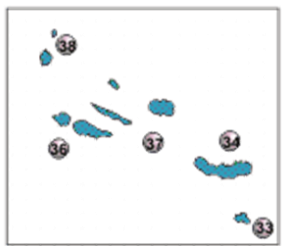
- Biogeography and populational analysis of indigenous *S. cerevisiae* strains from winemaking environments
- Constitution of a *S. cerevisiae* bio-databank comprising ca. 700 strains from distinct winemaking regions
- Selection of novel *S. cerevisiae* winemaking strains
- Selection of novel *S. cerevisiae* winemaking strains - Linking genetic profiles and phenotypic information
- Genomic approaches to unravel the genetic characteristics of *S. cerevisiae* winemaking strains

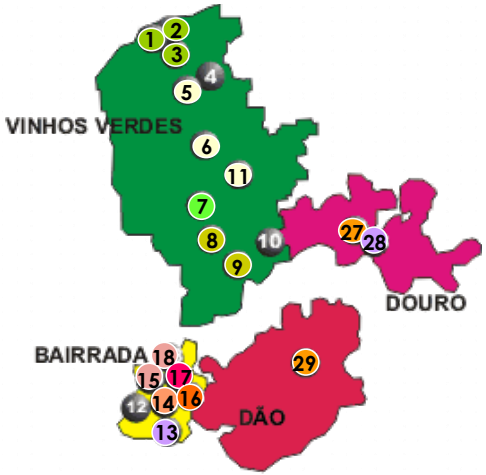
# Biogeography and populational analysis of indigenous *S. cerevisiae* strains from winemaking environments




AÇORES
ZONAS NÃO DEMARCADAS





## - *sampling sites* -

- Arinto
- Aragonês
- Avesso
- Alvarinho
- Baga
- Castelão
- Terrantez
- M. Gomes
- Verdelho
- Loureiro
- Touriga Nacional
- Bical

# - Summary of S. cerevisiae strains from Portuguese winemaking regions-

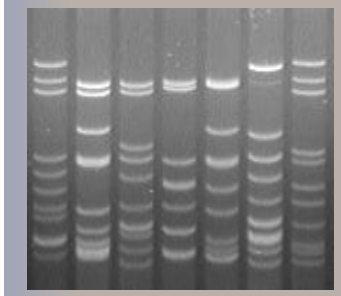
| | N°of grape samples | Spontaneous fermentations | N° of isolates | *N° of different Saccharomyces cerevisiae strains* |
|---|---|---|---|---|
| Vinhos Verdes | 282 | 115 | 3450 | 516 |
| Bairrada | 126 | 22 | 630 | 137 (*) |
| Estremadura | 38 | 34 | 1020 | 9 |
| Alentejo | 53 | 34 | 1020 | (*) |
| Palmela | 6 | 5 | 150 | 0 |
| Ribatejo | 12 | 7 | 210 | (*) |
| Douro | 12 | 6 | 180 | (*) |
| Dão | 6 | 6 | 180 | (*) |
| Açores | 88 | 55 | 1650 | (*) |
| **Total** | **623** | **285** | **8520** | **662** |

(*) underway

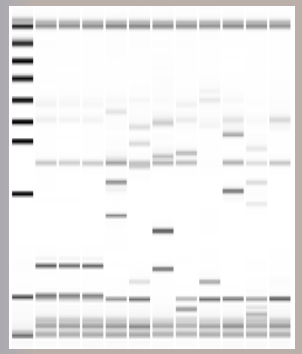
# - molecular typing methods -

Preliminary screening

## Mitochondrial DNA restriction analysis (mtDNA RFLP)



## Interdelta sequence amplification



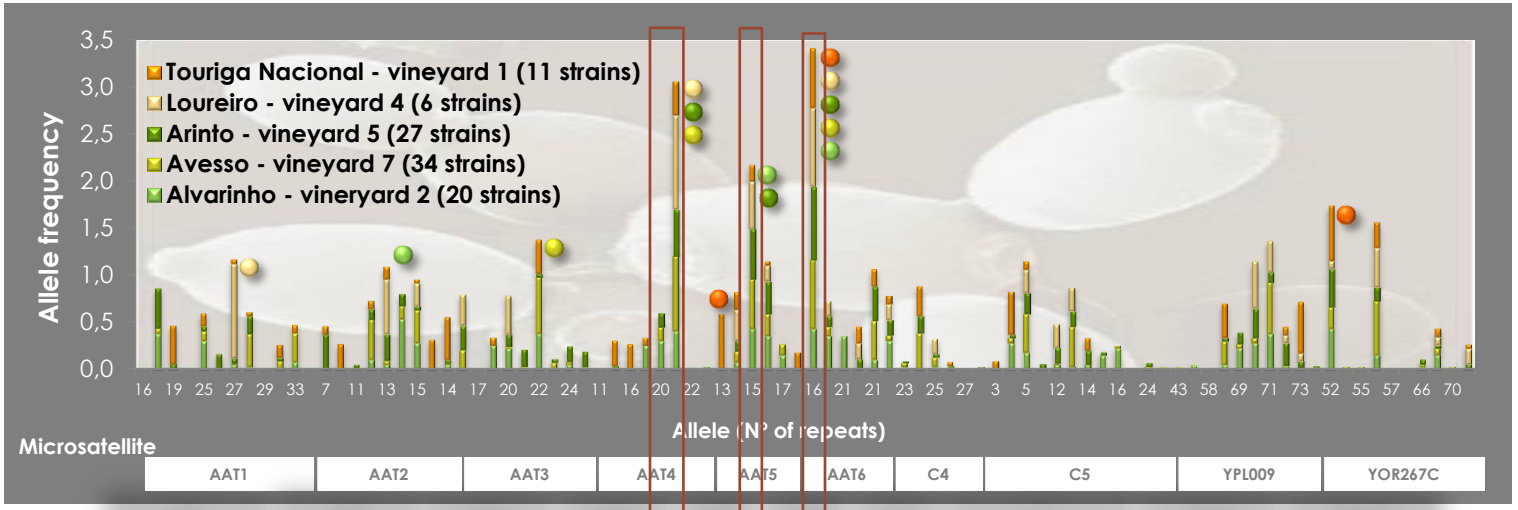
In depth characterization

## Microsatelite analysis

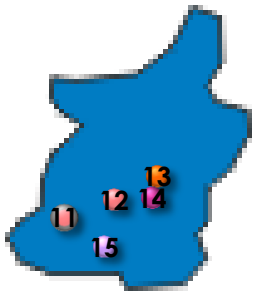

| Microsatellite | Chrom. | Position/ORF | Repeat | Ref. |
|---|---|---|---|---|
| ScAAT 1 | XIII | 86 901 – 87 129 | ATT | Pérez and Gallego, 2001 |
| ScAAT 2 | II | YBL084c | ATT | |
| ScAAT 3 | IV | YDR160w | ATT | |
| ScAAT 4 | VII | 431 334 – 431 637 | ATT | |
| ScAAT 5 | XVI | 897 028 - 897 259 | TAA | |
| ScAAT 6 | IX | 105 661 – 105 926 | TAA | |
| YPL009 | XV | YOR156c | TAA | Legras et al., 2005 |
| ScYOR267C | XV | YOR267c | TGT | |
| C4 | XV | 110 701-110 935 | TAA+TAG | |
| C5 | VI | 210 250-210 414 | GT | |

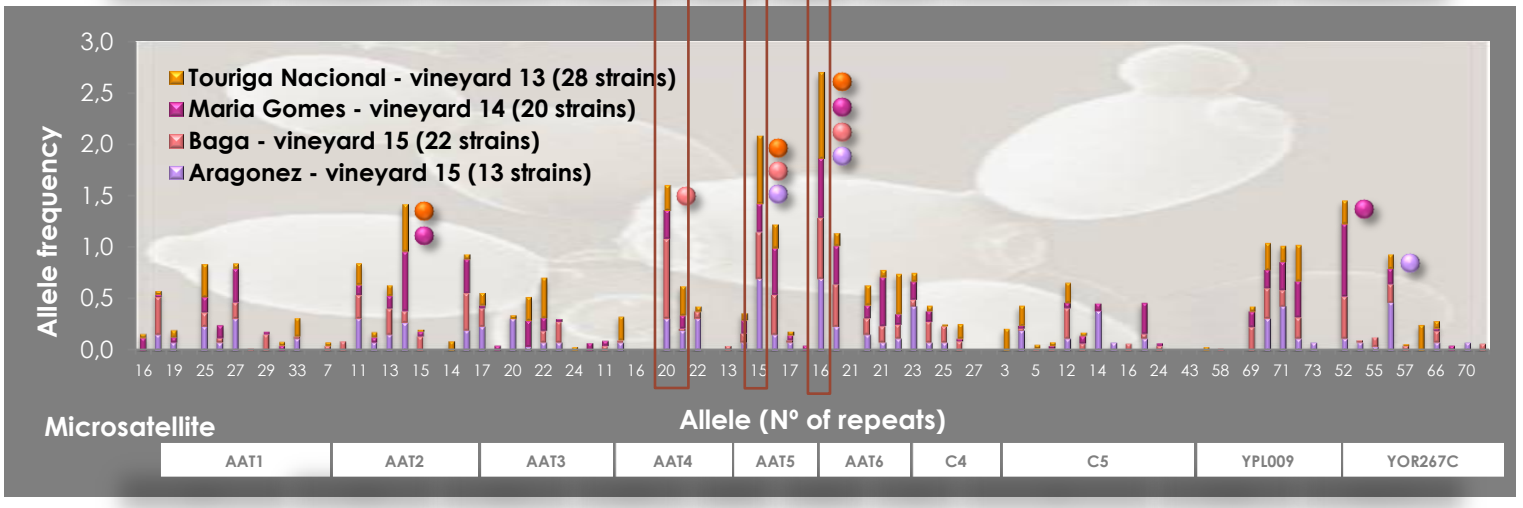
# - Populational analysis of S. cerevisiae populations from distinct winemaking regions and grape varieties -

**Vinho Verde**


**Bairrada**



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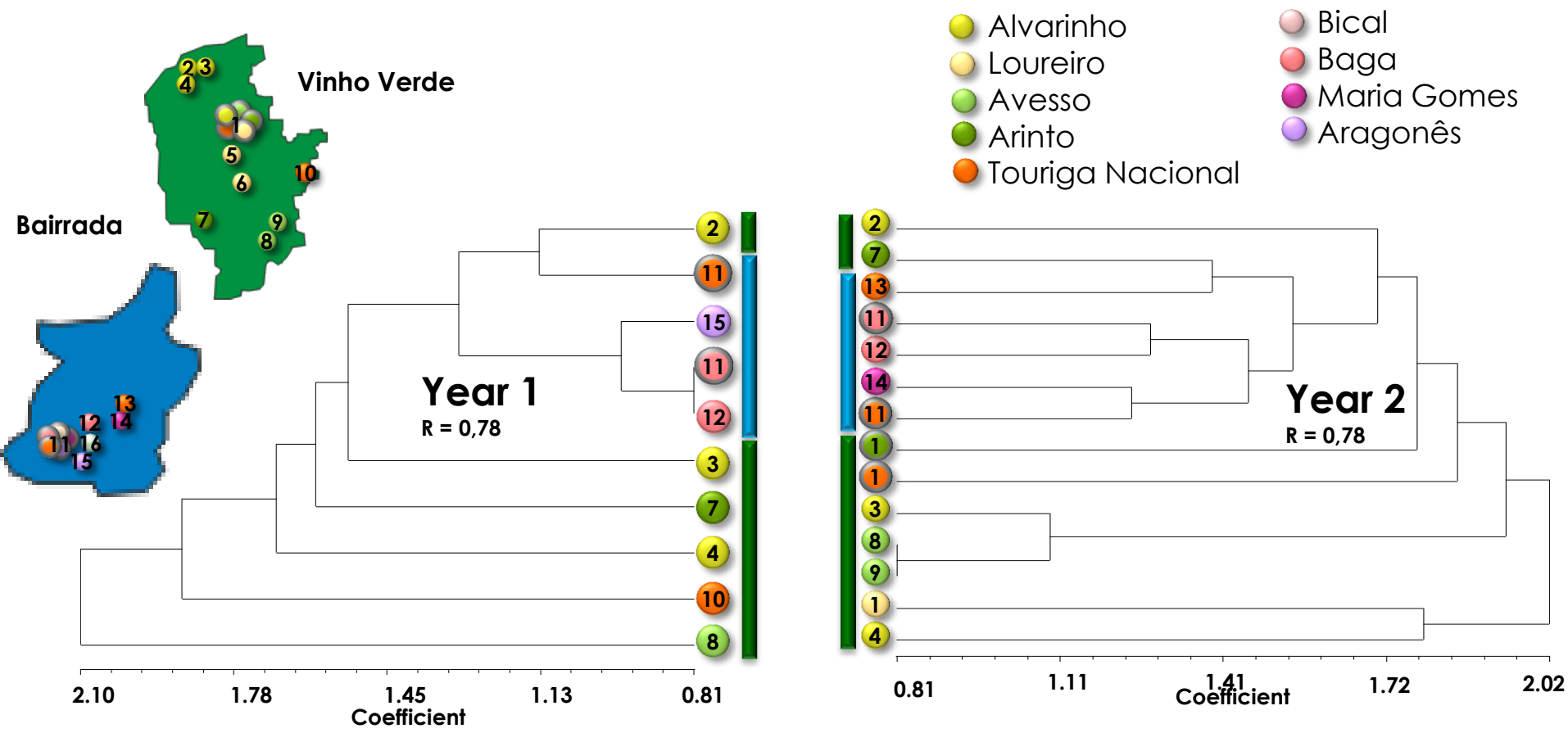
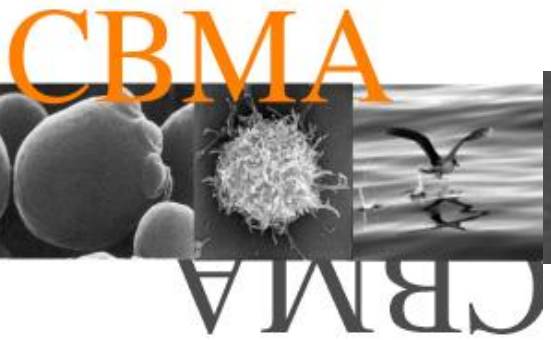



- *Cluster analysis (UPGMA) based on a Euclidean distance dissimilarity matrix of allelic frequencies.*
- *Genetic proximity is correlated with geographic distance between wine regions*
- *Within wine regions, S. cerevisiae populations are associated with the grape variety*

*Schuller et al., 2005*

*Schuller et al., 2007*



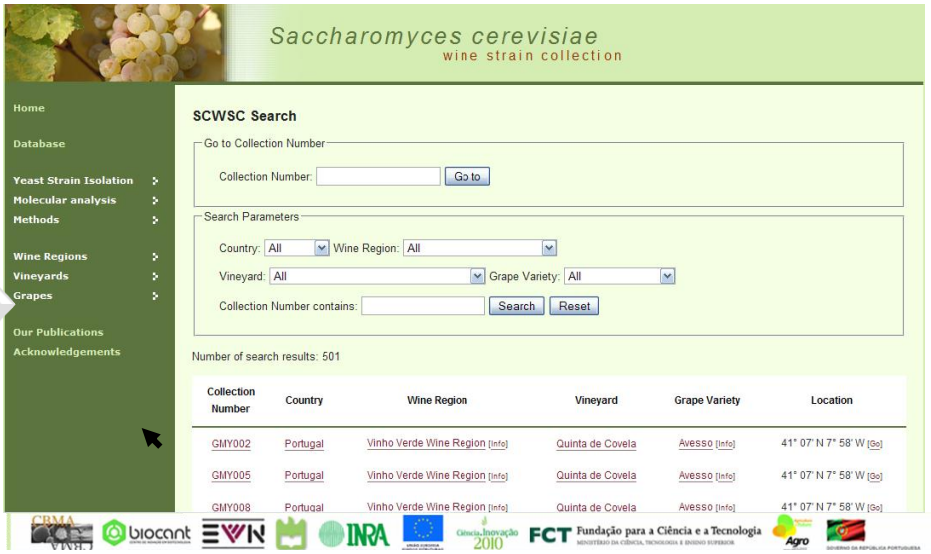

# Constitution of a S. cerevisiae bio-databank comprising ca. 600 strains from distinct winemaking regions

## Queries

- Isolate
- Grape varieties
- Vineyard
- Wine Region
- Sampling year

http://scwsc.bio.uminho.pt

## Allelic combinations for each strain





*Schuller et al., 2005*
*Schuller et al., 2007*
*Valero et al., 2007*
*Schuller and Casal, 2007*

VINALIA® Soluções de Biotecnologia para a Vitivinicultura, lda

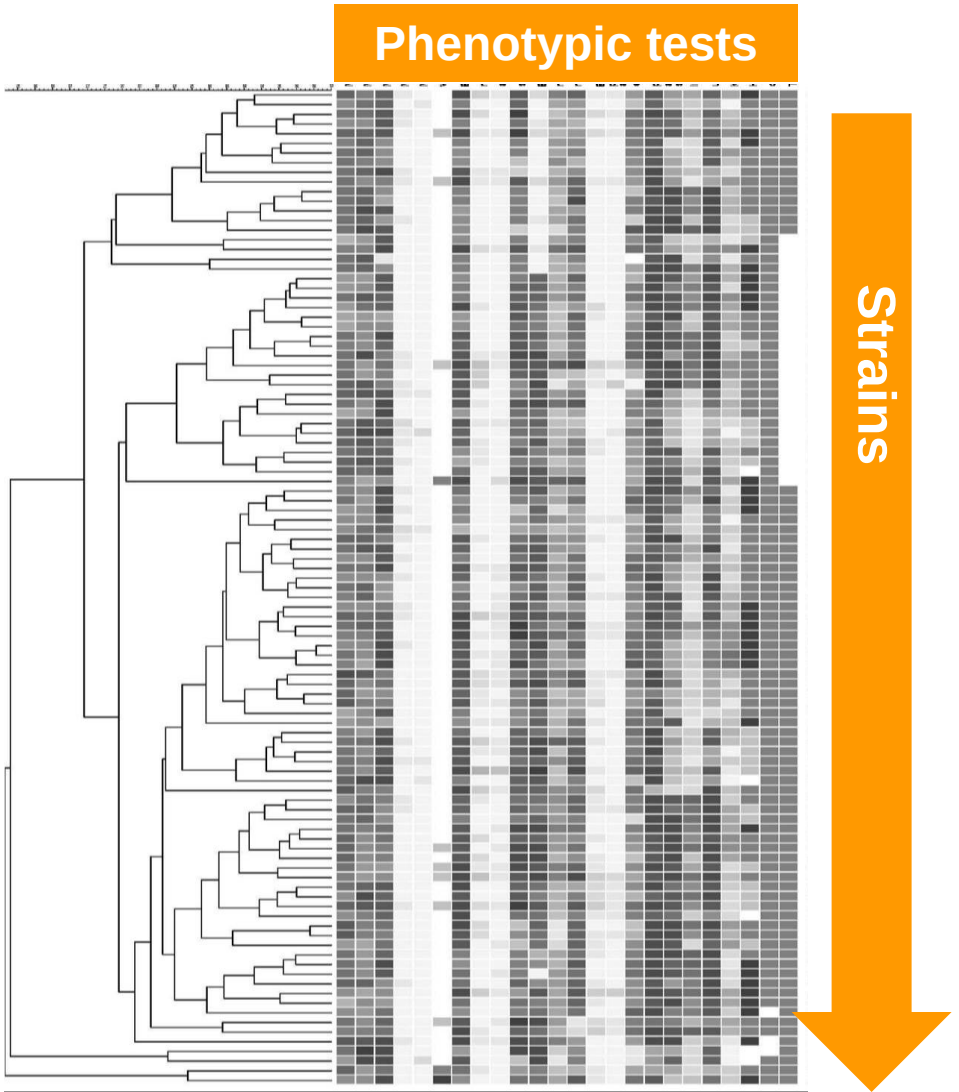
# Linking genetic profiles and phenotypic information by computational approaches

Glucose
Ribose
Arabinose
Sucrose
Galactose
Raffinose
Maltose
Glycerol
Potassium acetate

Peptone
Ammonium sulphate
Imidazole
Urea

Ethanol tolerance
Temperature
Osmotic stress
$H_2S$ production
Cerulenin resistance
TFL resistance

**Cells in the heatmap represent values of O.D.**



# Modelling the relation between individual phenotypes and genotype information

| Phenotypic tests (sub-groups) | 4 ºC | 4 ºC | 18ºC | 30ºC | 30ºC | 45ºC | 45ºC | YNB + galactose | YNB + galactose | YNB + maltose | YNB + maltose | 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 | $0.5 \leq \frac{A_{640}}{0.6} \geq$ | $A_{640}$ = 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 | linea | linear | linear | linear | tree | kNN | linear | linear | linear | linear | linea | 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 |

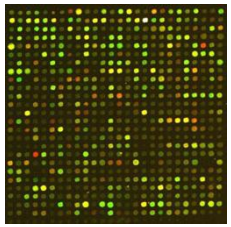
AUC = area under receiver operation score

**Franco-Duarte *et al.*, 2009**

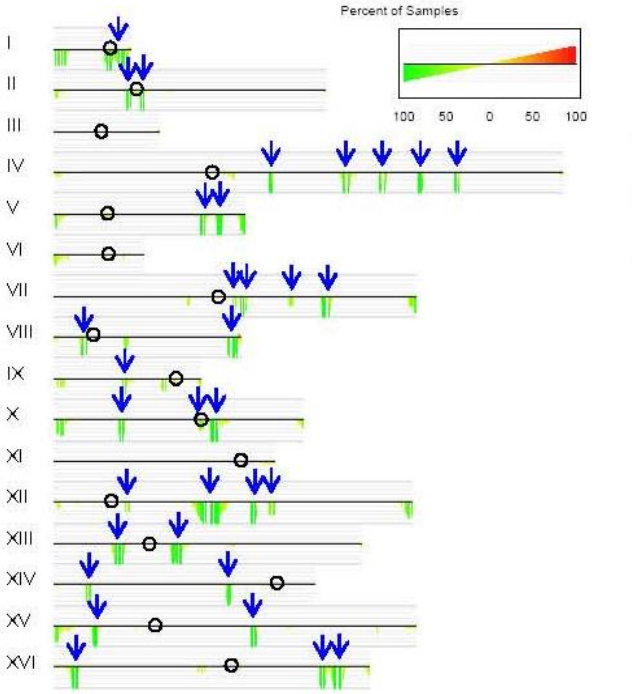
# Comparative genomics of yeast strains isolated from diverse ecological niches unveils important genome diversity

*- unravel intraspecific natural genome diversity -*

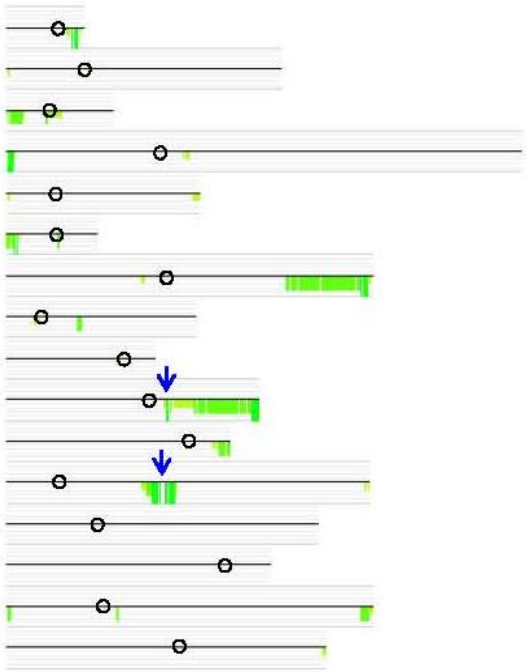
*Nacional Facility for DNA Microarrays*



*S. cerevisiae* winemaking strains




*S. cerevisiae* clinical strains



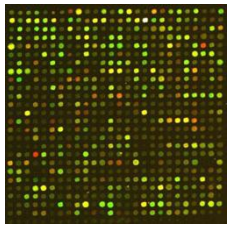
Variability is associated with the sub-telomeric regions of some chromosomes

Wine strains : reduced number of Ty elements and the flanking genes

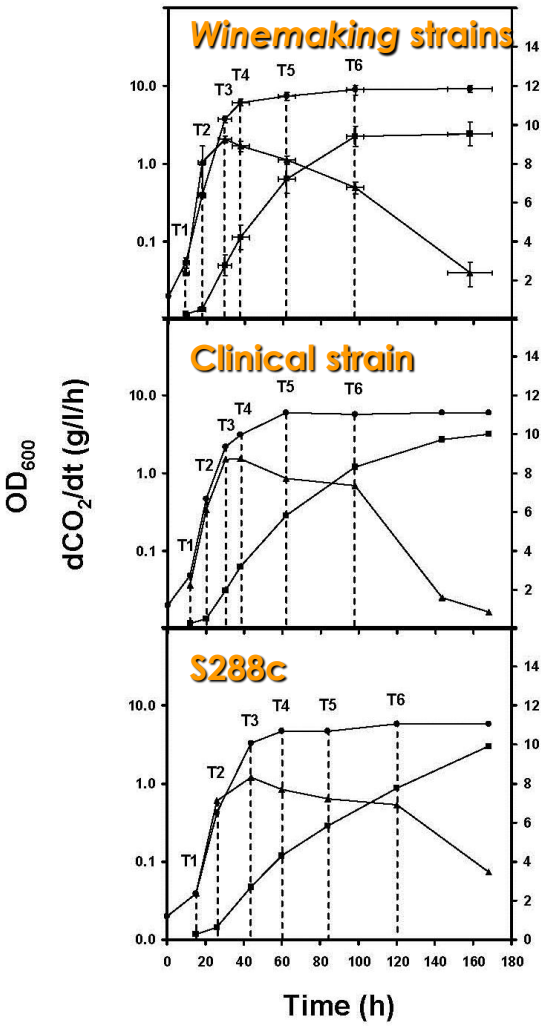
**Carreto *et al.*, 2008**

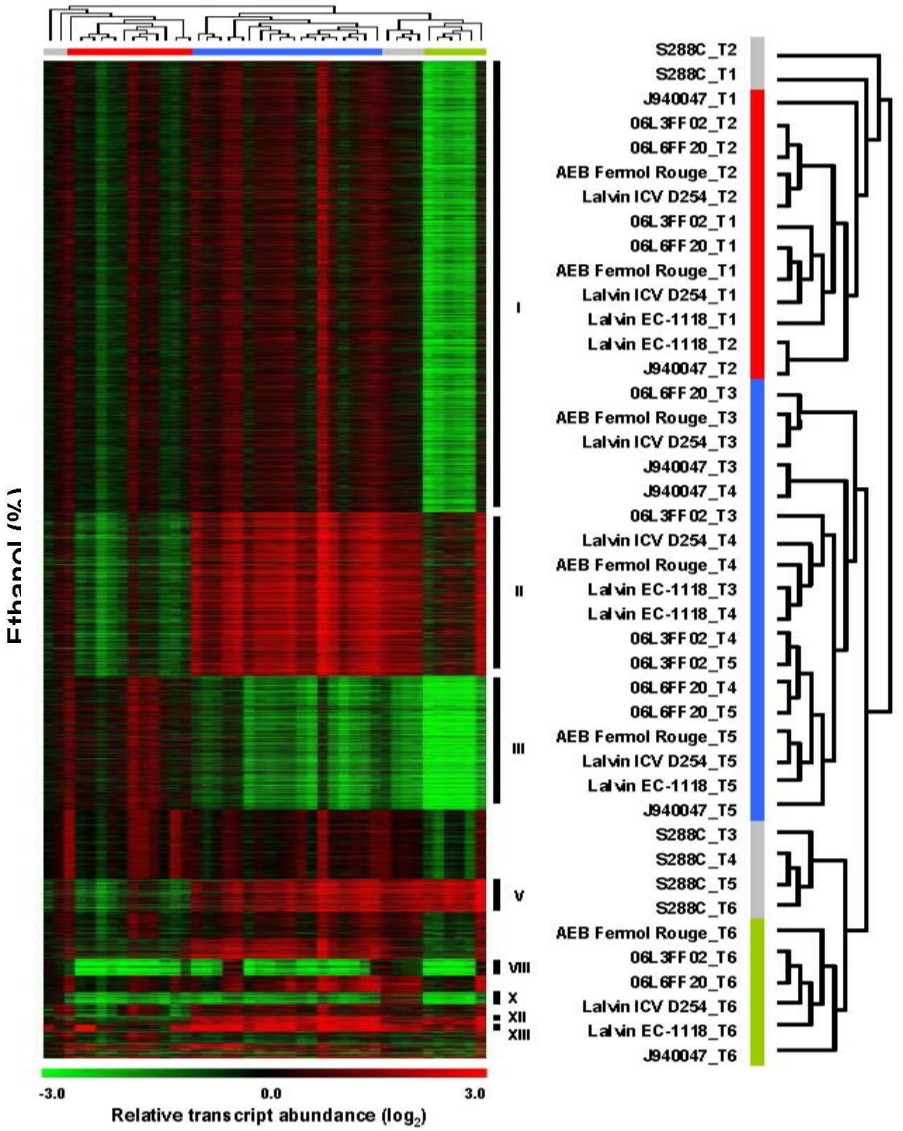
# Differential expression of co-regulated genes distinguishes *Saccharomyces cerevisiae* strains during fermentation

*Nacional Facility for DNA Microarrays*



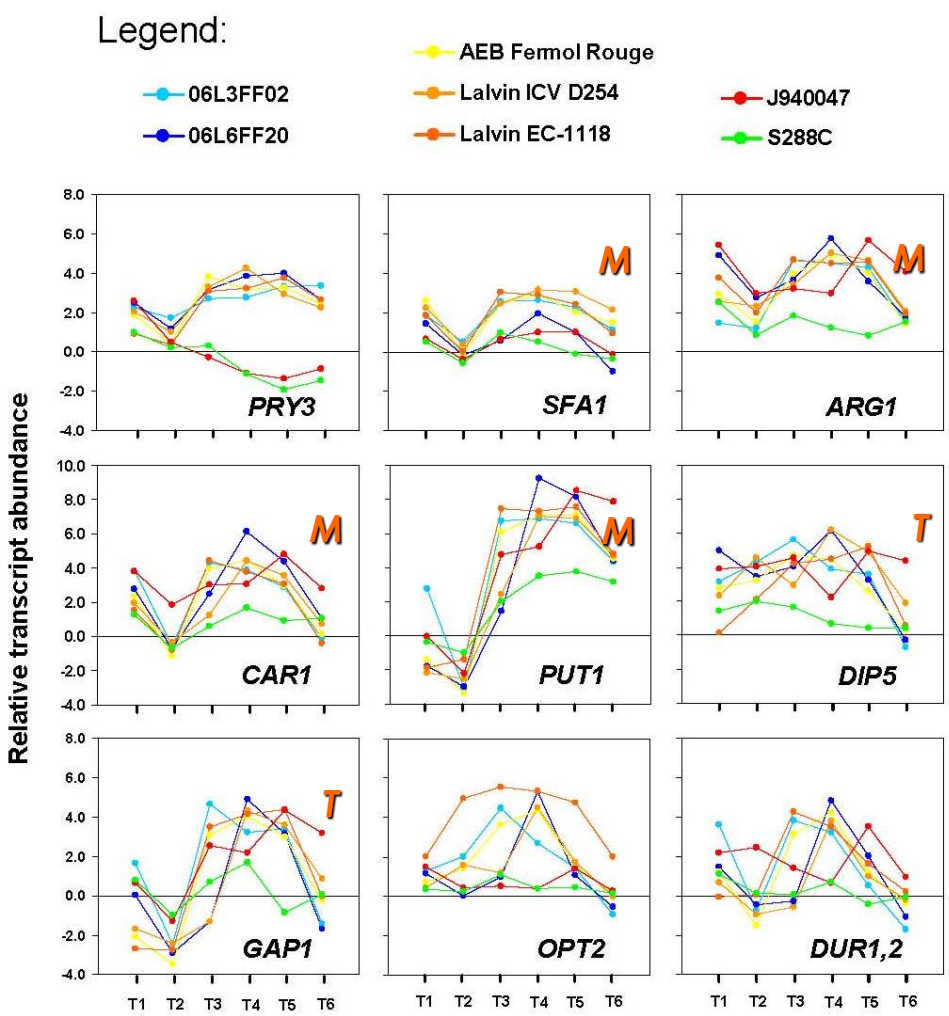
**Carreto *et al.*, in prep.**



# Differential expression of co-regulated genes distinguishes *Saccharomyces cerevisiae* strains during fermentation

*Nacional Facility for DNA Microarrays*




Carreto *et al.*, in prep.

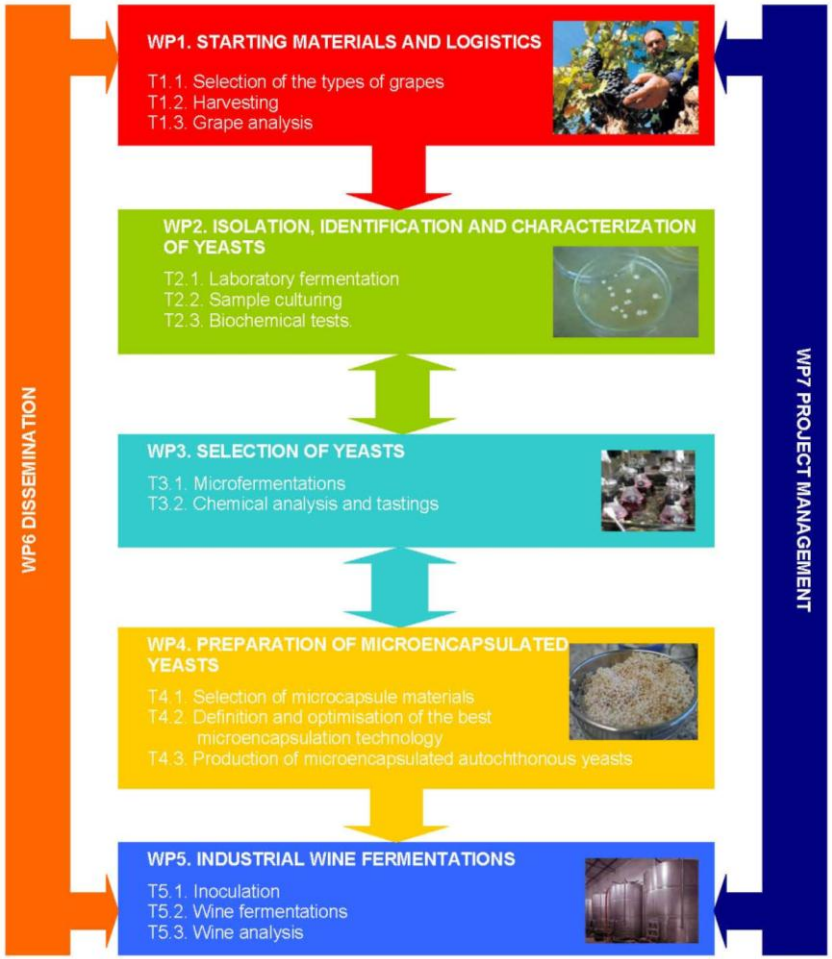
*M Amino acid metabolism*
*T Amino acid transport*

# Ongoing projects

## INNOYEAST - Innovation and improvement of European wine industry competitiveness by the research and development of native microencapsulated wine yeasts to produce quality wines

www.innoyeast.eu

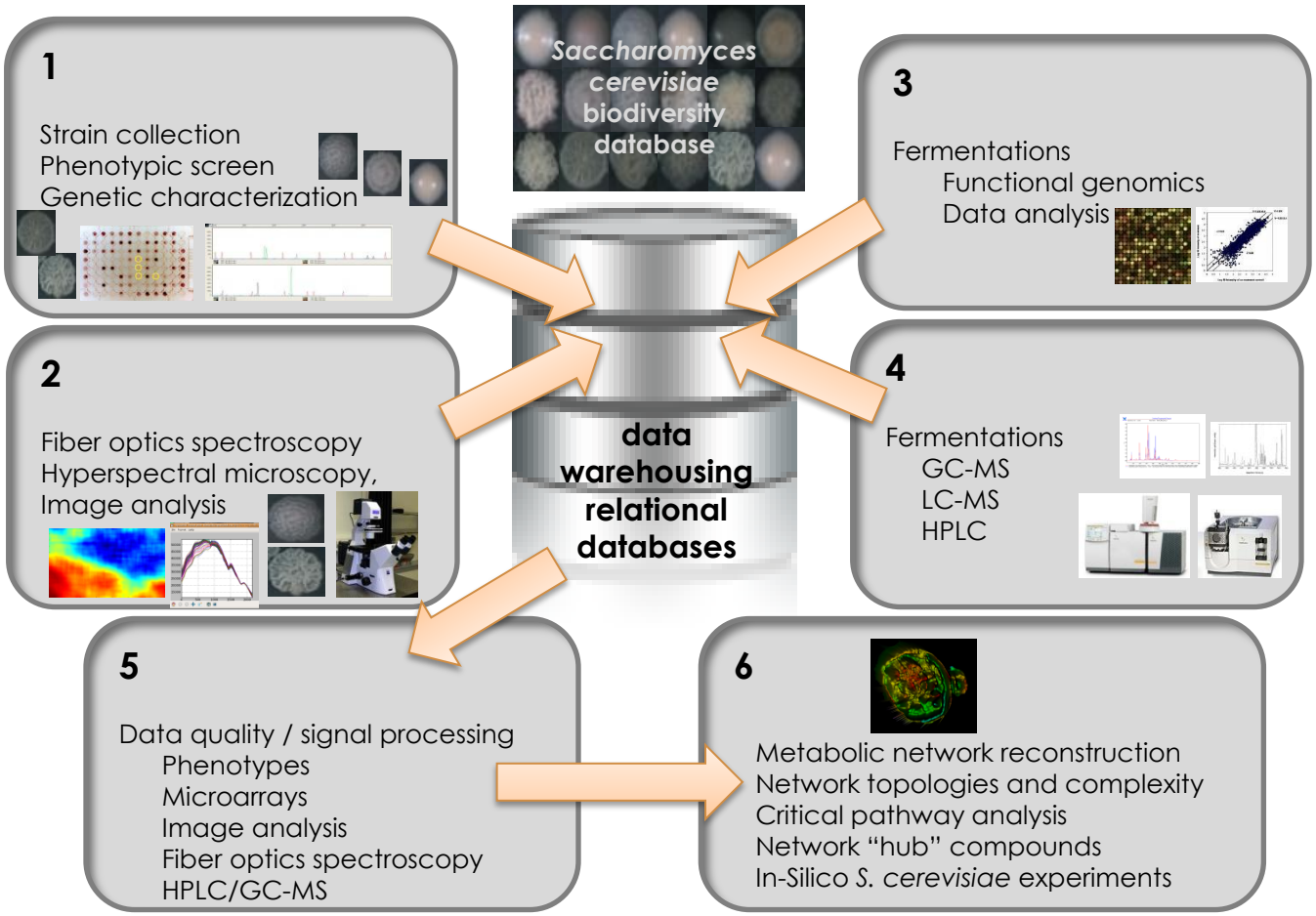
SEVENTH FRAMEWORK PROGRAMME



# PhenoMet - Integrative pheno-metabolomic and genomic approaches for Saccharomyces cerevisiae winemaking yeasts

PTDC/AGR-ALI/103392/2008

## Project diagram





CBMA
Centro de Biologia Molecular e Ambiental

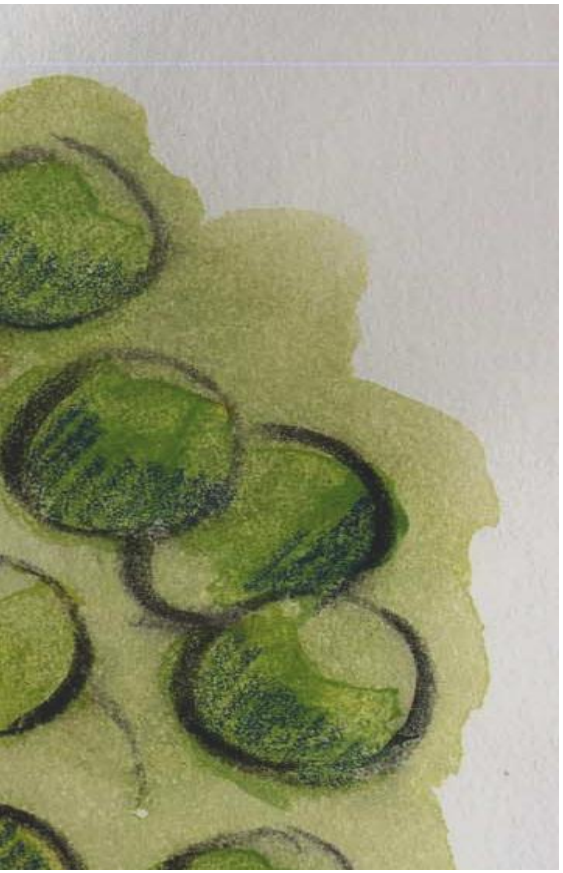
CBQF
Centro de Biotecnologia e Química Fina

IBB
Institute for Biotechnology and Bioengineering

INETI
Instituto Nacional de Engenharia Tecnologia e Inovação

CESAM
Centre for Environmental and Marine Studies

# Acknowledgements

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- Eugénia Vieira
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- Nuno Fonseca
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José Domingues
João Melícias
Frederico Gomes
Leonor Novais

Magda Silva Graça
Sofia Machado
Barbara Dellinger

Adega Cooperativa de Cantanhede
Comissão de Viticultura
da Região dos Vinhos Verdes
Companhia das Quintas
Estação Vitivinícola Amândio Galhano
PROVAM – Produtores de
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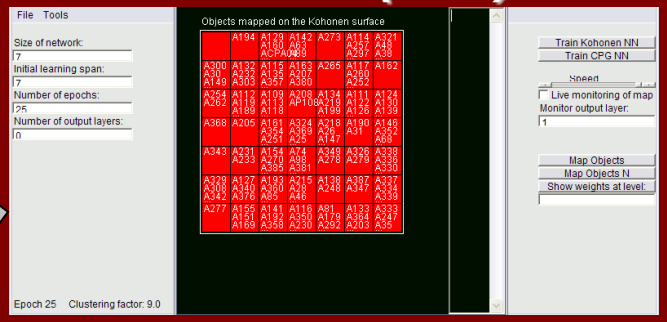
# Phenotypic characterization of a S. cerevisiae strain collection

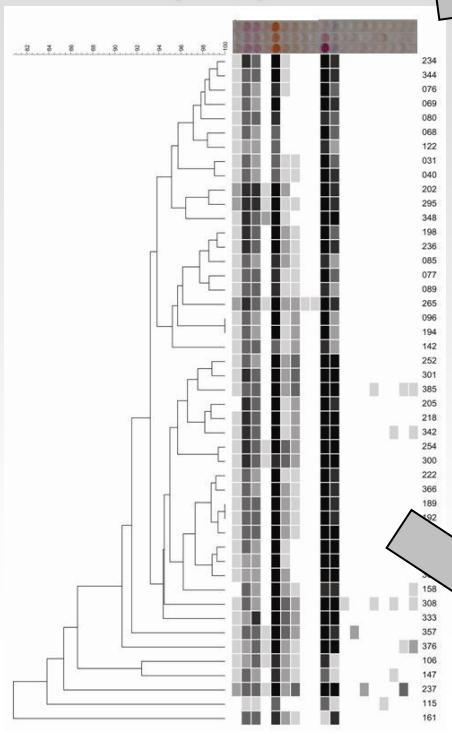
***Current research and future perspectives***

## Neural networks (SOM)




**Selection of a genetically most diverse set of strains**

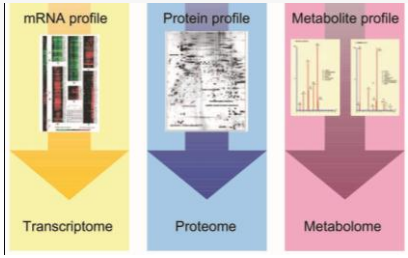
## Enzymatic activities (API)



## Fermentative and aromatic profiling

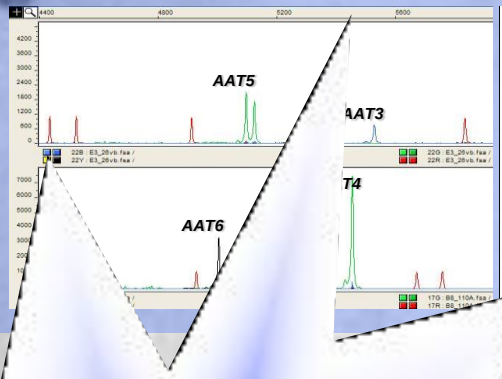
## Genetic profiles

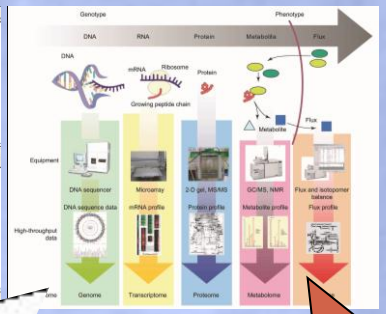
## Molecular markers that correlate with specific phenotypes / traits



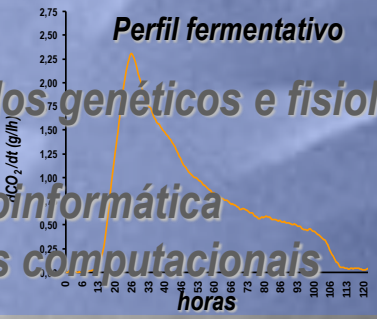
# Abordagens a desenvolver ....

## Genética




## Comportamento fermentativo



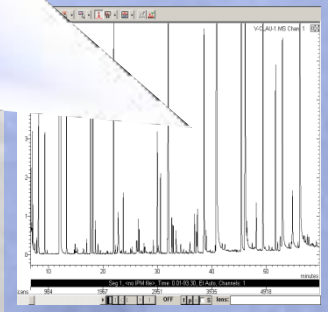

Integração de dados genéticos e fisiológicos

Bioinformática

Métodos computacionais

Previsão de características desejáveis a partir de dados genéticos

## Analítica



# Conclusions

The finding of 501 *S. cerevisiae* strains reveals a fascinating genetic diversity in vineyard environments.

Microsatellite analysis permitted a high resolution populational screen, showing that

genetic differences among *S. cerevisiae* populations derived from both "diagnostic" vineyard-, specific alleles and the accumulation of small allele-frequency differences across ten microsatellite loci;

each vineyard contains differentiated *S. cerevisiae* populations, hypothesizing the occurrence of specific native strains that can be associated with a *terroir*.

# Acknowledgements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Sofia Machado
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## São Miguel


## Terceira

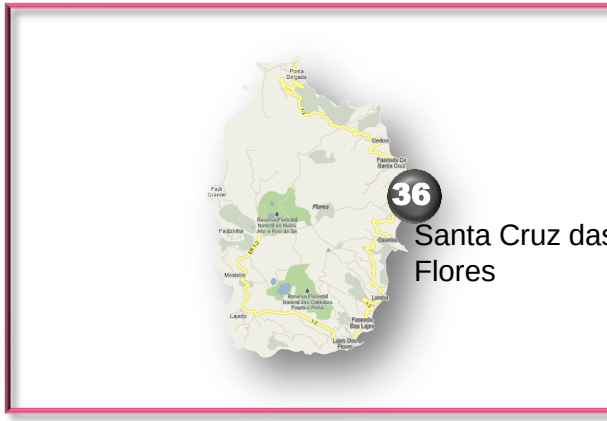

## Graciosa


## Pico


## Faial


## S. Jorge


## Flores



# bioYtec

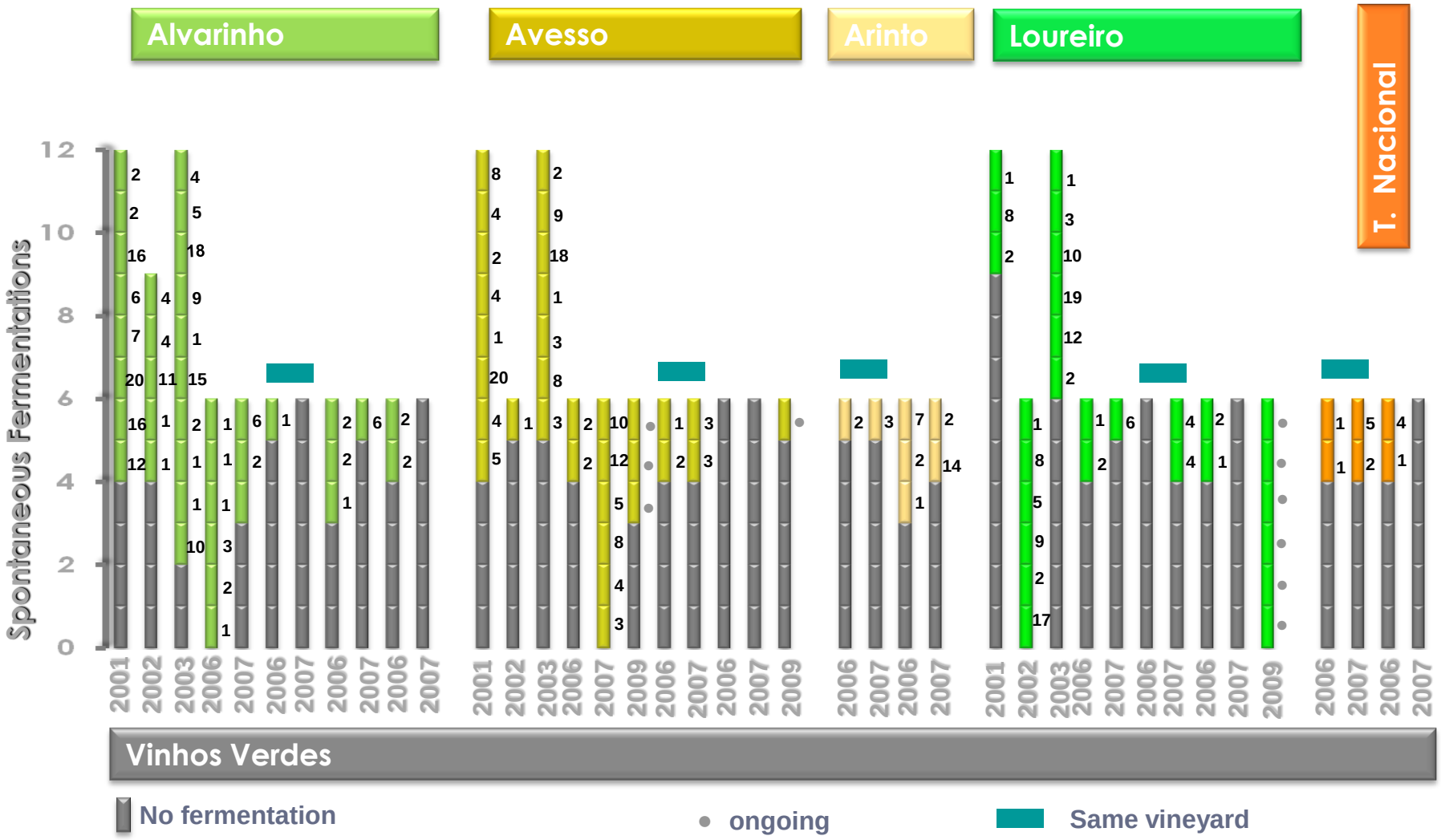
## Molecular Biotechnology

- **Fermentation technology:**

- **Ongoing projects:**
  - Integrative Pheno-Metabolomic and genomic approaches for *Saccharomyces cerevisiae* winemaking yeasts
  - The indigenous microbiome of Portuguese wine fermentations
  - Implementation of a National Facility for DNA Microarrays

Ciência.Inovação 2010 Programa Operacional Ciência e Inovação 2010 MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR
FCT Fundação para a Ciência e a Tecnologia MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR
UNIÃO EUROPEIA FUNDOS ESTRUTURAIS
Agricultura & Futuro Agro

# *S. cerevisiae* strains involved in spontaneous fermentations



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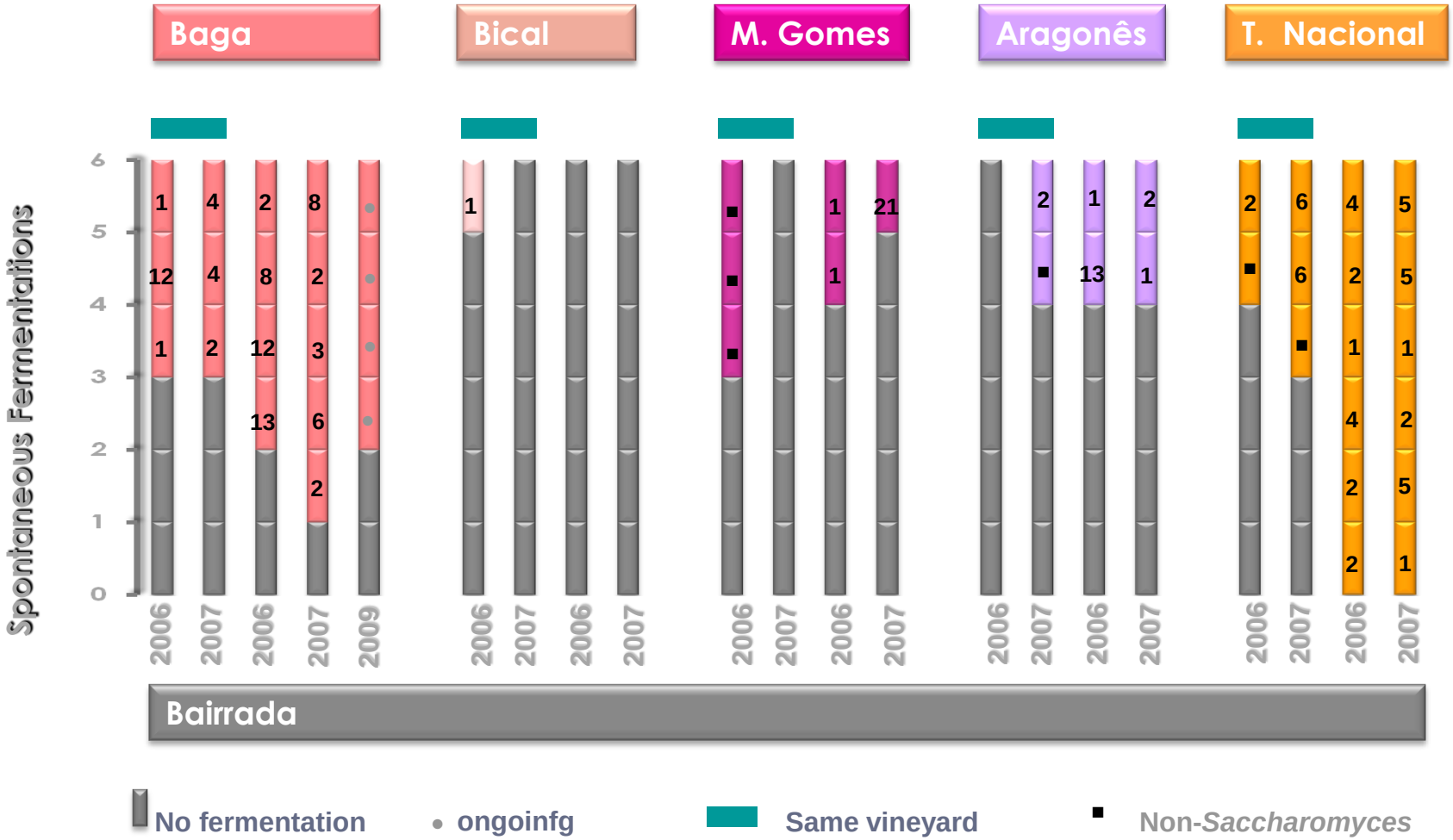

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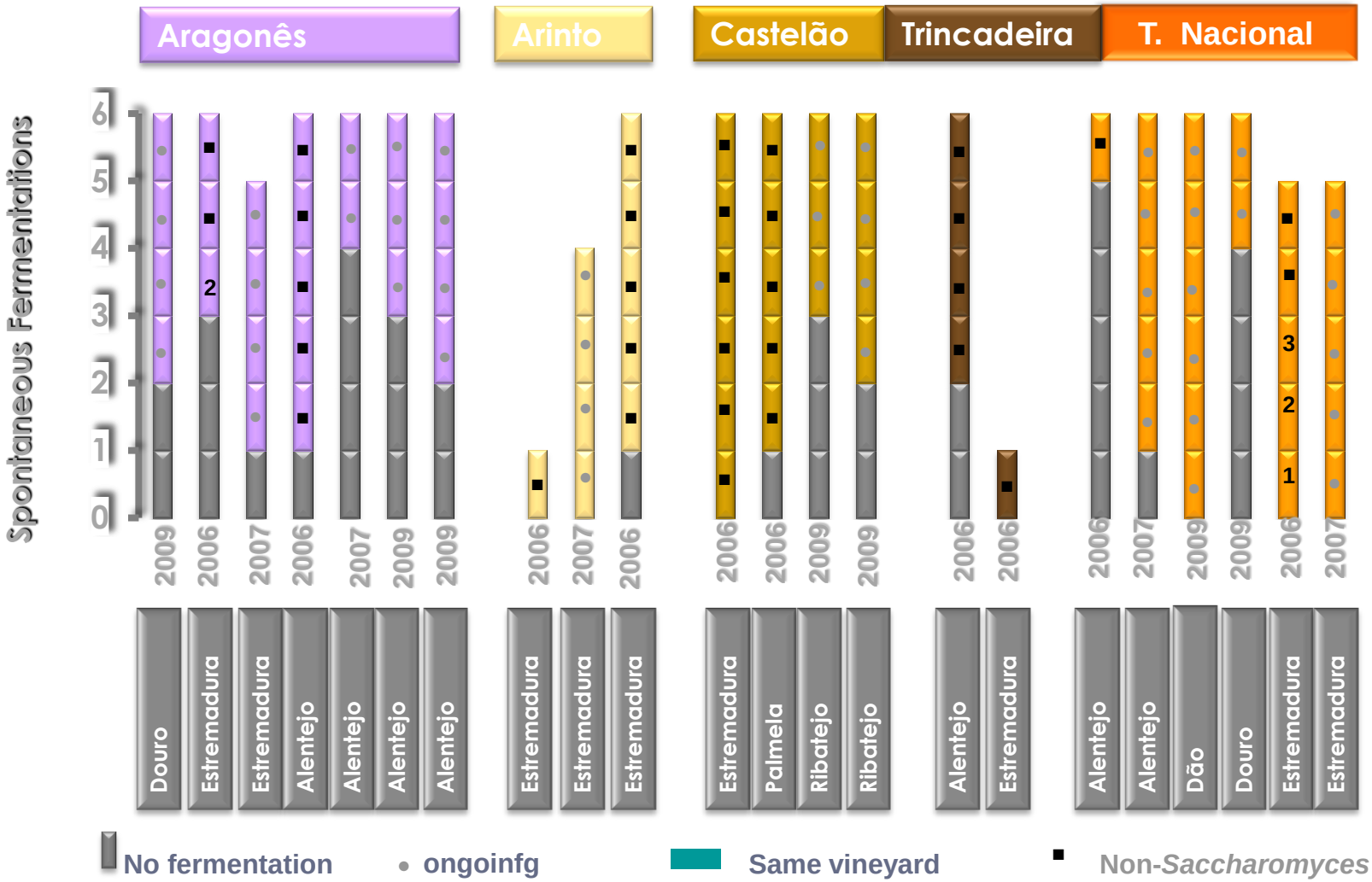
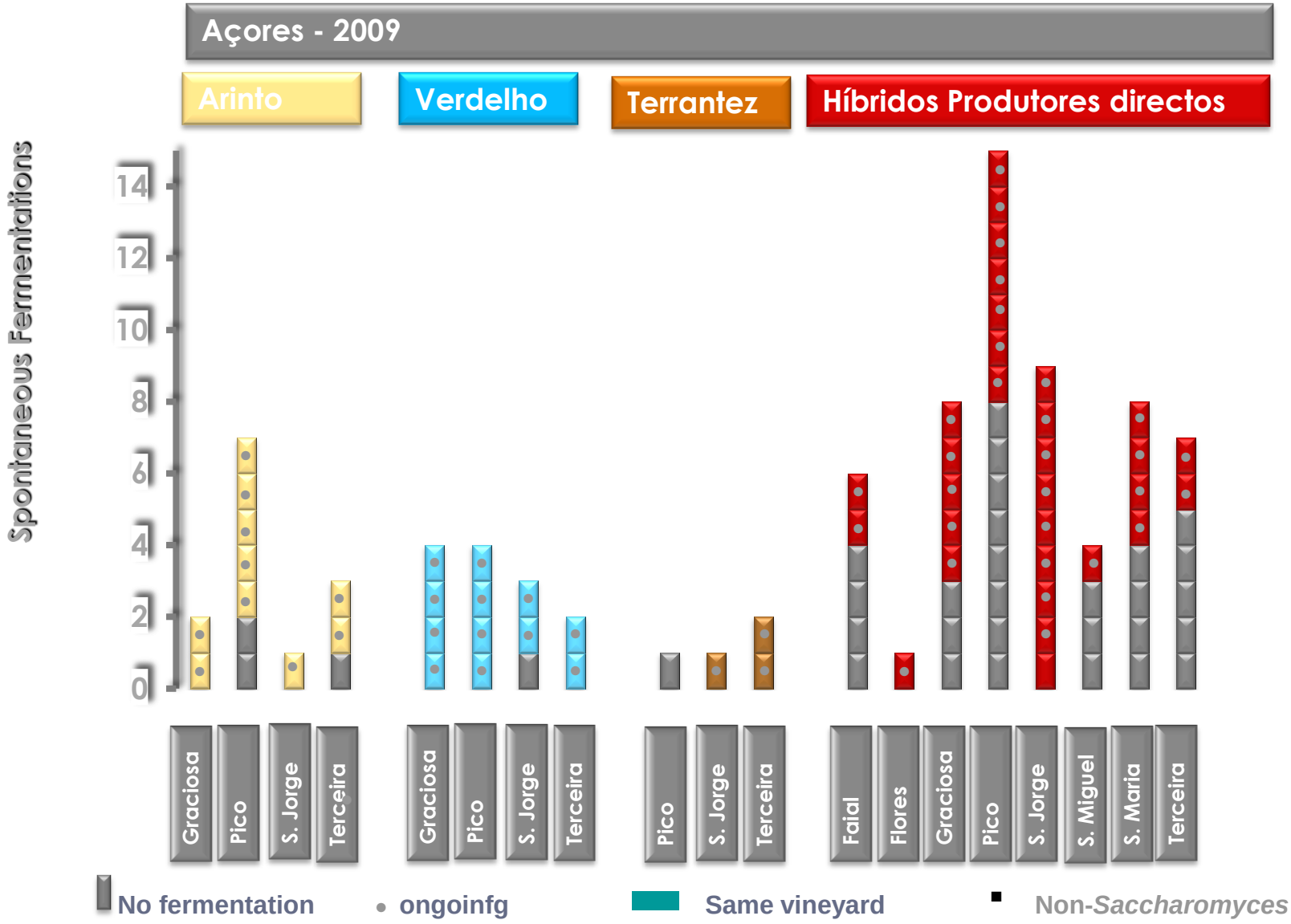
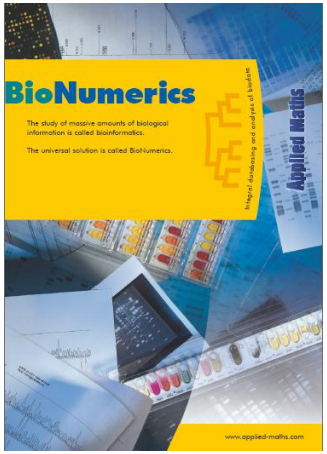


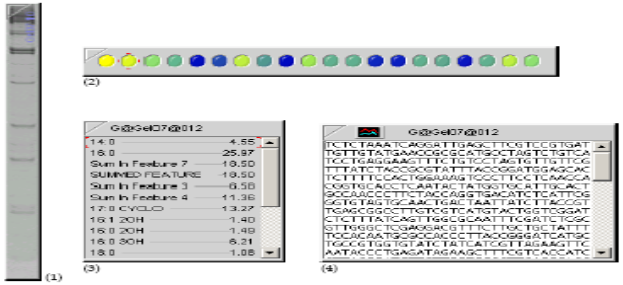
# *S. cerevisiae* strains involved in spontaneous fermentations



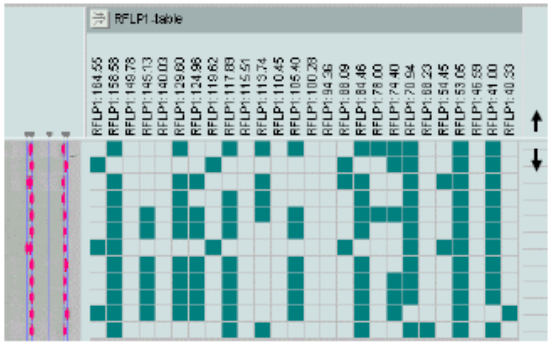

| | Traditional grape varieties | Hybrid varieties from non-abandoned vineyards | Hybrid varieties from abandoned vineyards |
|---|---|---|---|
| Number os samples | 24 | 34 | 30 |
| Number of fermentations | 8 | 16 | 25 |
| ay of the beggining of the fermentation (average) | 2,0 | 2,4 | 2,3 |
| Duration of the fermentations (average) | 25,9 | 29,3 | 18,6 |
| Finished fermentations (%) | 33,3 | 47,1 | 83,3 |
| Fermentations completed by *S. cerevisiae* (%) | 75,0 | 56,3 | 56,0 |
| Fermentations completed by *non-Saccharomyces* species (%) | 25,0 | 43,7 | 44,0 |
| Total number f *S. cerevisiae* strains | 36 | 53 | 80 |
| Minimum number of *S. cerevisiae* strains / sample | 1 | 1 | 1 |
| Maximum number of *S. cerevisiae* strains / sample | 11 | 21 | 23 |

# The BIONUMERICS software for databasing and cluster analysis



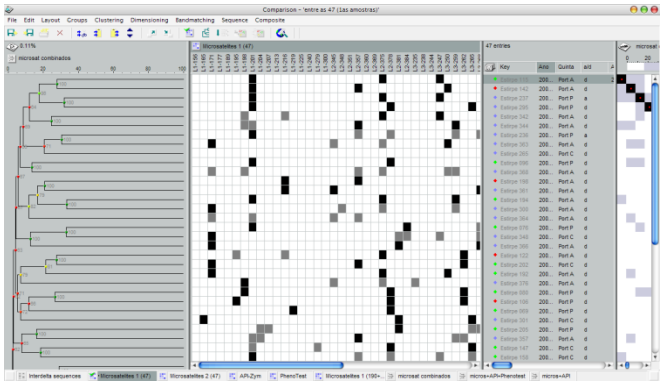



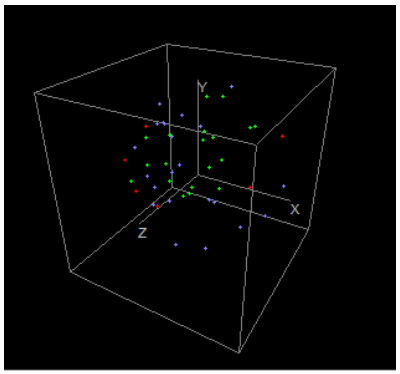
Entries from multiple fingerprint and character types



Binary/numerical value experiment file(s)

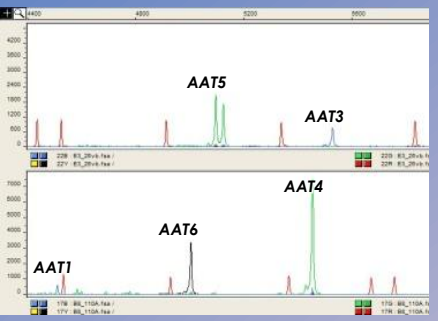
Approaches with single/multiple (polyphasic) fingerprint and character types





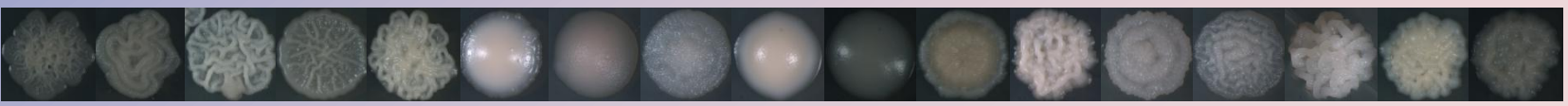
# - distribution of the most frequent microsatellite alleles -

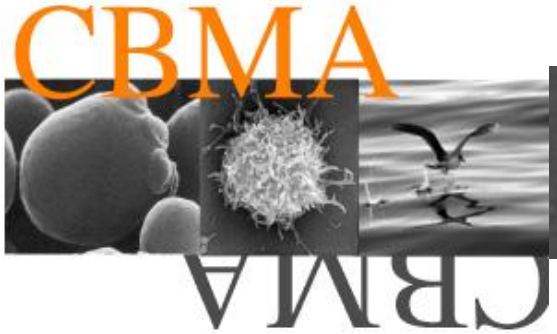
Most frequent alleles




| Locus | N° of alleles | Allele (n° of repeats) |
|---|---|---|
| ScAAT 1 | 43 | 12 13 15 16 17 18 19 20 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 39 40 41 43 47 49 51 53 54 59 |
| ScAAT 2 | 13 | 3 4 5 6 7 8 10 11 12 13 14 15 16 |
| ScAAT 3 | 21 | 9 10 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 34 49 55 |
| ScAAT 4 | 19 | 6 8 9 10 11 12 13 14 15 16 18 19 20 21 22 23 24 26 27 |
| ScAAT 5 | 5 | 13 14 15 16 17 |
| ScAAT 6 | 11 | 13 14 15 16 17 18 19 20 21 23 28 |
| C4 | 11 | 20 21 22 23 24 25 26 27 36 37 41 |
| C5 | 26 | 3 4 5 6 8 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 28 30 31 34 |
| YPL009 | 24 | 43 51 53 55 57 58 60 61 62 63 65 66 69 70 71 72 73 76 77 79 80 81 82 89 |
| ScYOR267 | 26 | 19 22 25 30 31 32 33 35 36 37 39 41 42 43 44 46 47 48 49 50 51 52 53 54 55 58 |

Total: 192 alleles






# bioYtec

## Molecular Biotechnology

- **Fermentation technology: wine**

### Main topics

- Biogeography and populational analysis of indigenous *S. cerevisiae* strains from winemaking environments
- Constitution of a *S. cerevisiae* bio-databank comprising ca. 700 strains from distinct winemaking regions
- Selection of novel *S. cerevisiae* winemaking strains
- Linking genetic profiles and phenotypic information by computational approaches
- Genomic approaches to unravel the genetic characteristics that are responsible for distinct fermentative behavior (*S. cerevisiae* winemaking strains *versus* other S. cerevisiae strains)