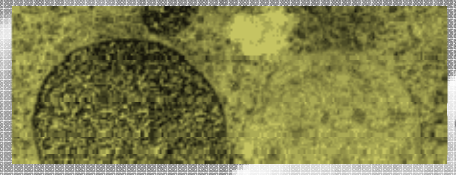

XV JORNADAS DE BIOLOGIA DE LEVEDURAS "PROFESSOR NICOLAU VAN UDEN"

Porto – 15/16.06.2007

BIOINFORMATIC APPROACHES FOR THE GENETIC AND PHENOTYPIC CHARACTERIZATION OF A *SACCHAROMYCES CEREVISIAE* WINE YEAST COLLECTION

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Introduction

- *Saccharomyces cerevisiae* is the most well known eukaryotic model, mankind's oldest domesticated organism and the world's premier commercial microorganism for biotechnological applications

- Genetic/genomic studies are traditionally carried out with a few laboratory strains and natural isolates are still poorly investigated

- With the advent of molecular typing methods, genetic variability and relatedness among natural isolates of *S. cerevisiae* can be established, and linked to phenotypic characteristics

- Bridging the genotype-to-phenotype gap using bioinformatics approaches will become very important for the industrial scale production for biotechnologically valuable products

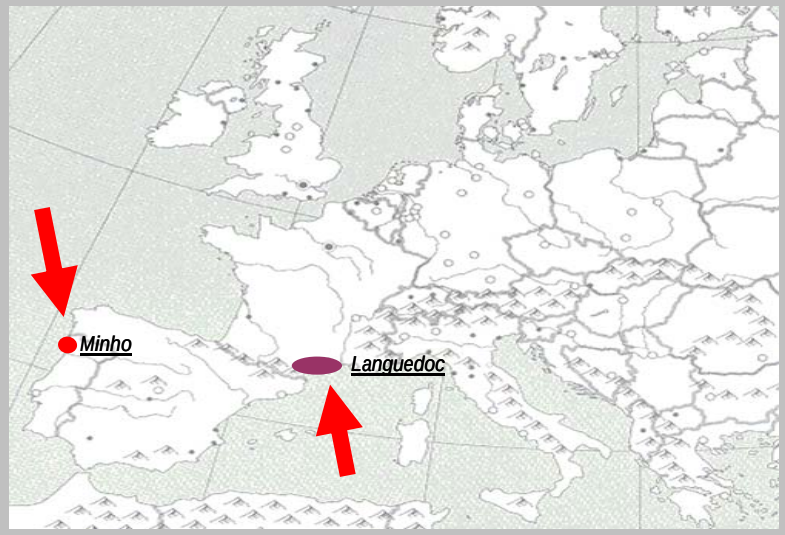
Objectives

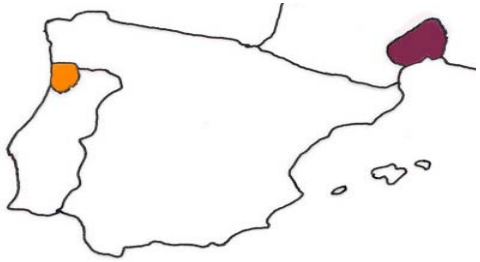
- To compose and systematize a *Saccharomyces cerevisiae* strain collection including genetic profiles from multiple molecular typing methods and phenotypic information

- To investigate genetic relatedness among natural populations

- To compare genetic and phenotypic variation using bioinformatics approaches as a basis for future analysis to estimate a strain's biotechnological potential

Biogeographical large-scale survey – *Saccharomyces cerevisiae* Wine Yeast Collection

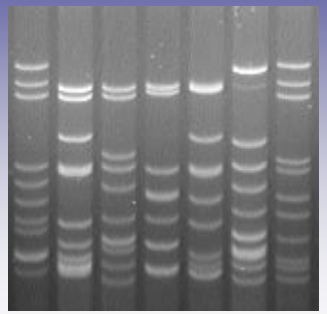




	Minho 2001 – 2006	Languedoc 2001-2003	Total
Number of isolates	2520	2160	6380
S. cerevisiae strains (unique genetic profiles)	340	100	440

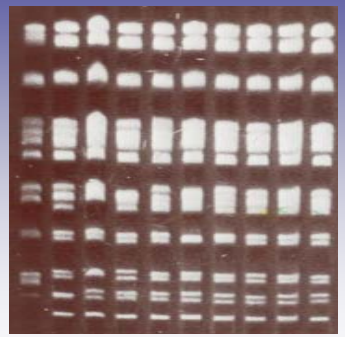
Molecular methods for S. cerevisiae strain characterization

Mitochondrial DNA restriction analysis (mtDNA RFLP)



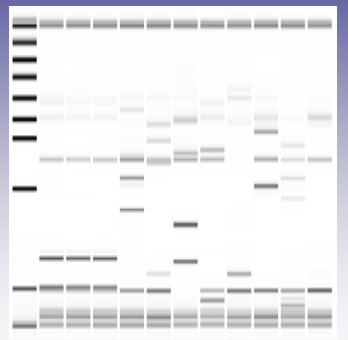
(Querol et al.*, 1992; Lopez* et al.*, 2001)*

Electrophoretic karyotyping (PFGE)



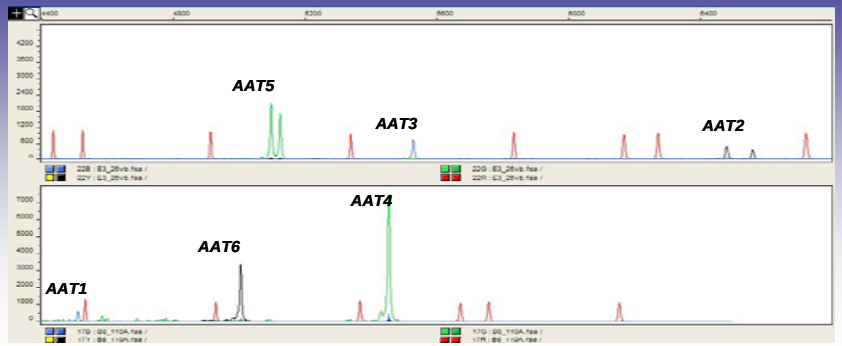
(Carle and Olson., 1995; Blondin and Vezinhet, 1988)

PCR based -Interdelta sequence amplification



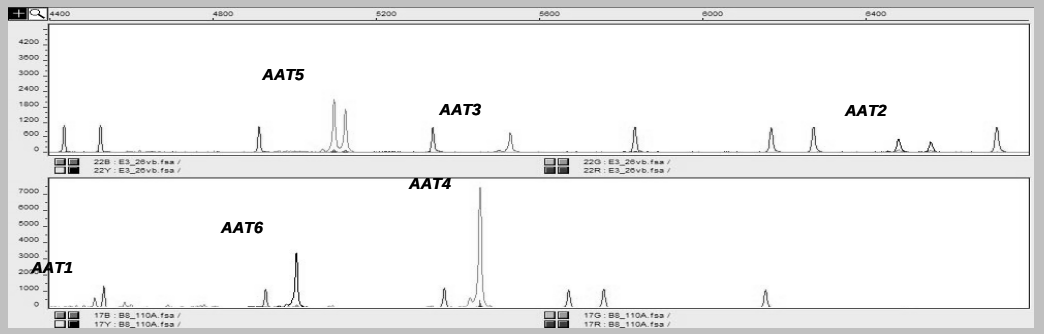
(Ness et al.*, 1993) (Legras* et al.*, 2003)*

Microsatellite analysis



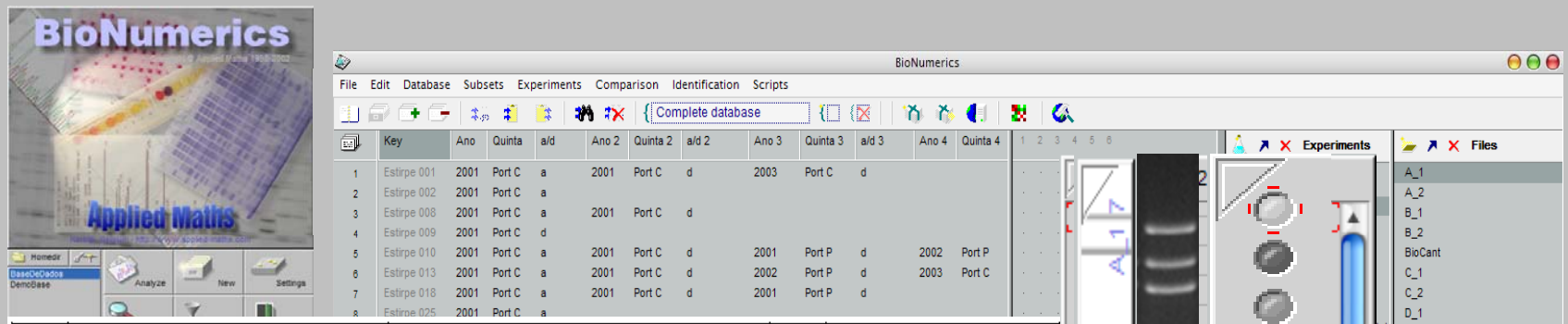
Molecular methods for S. cerevisiae strain characterization

Microsatellite analysis



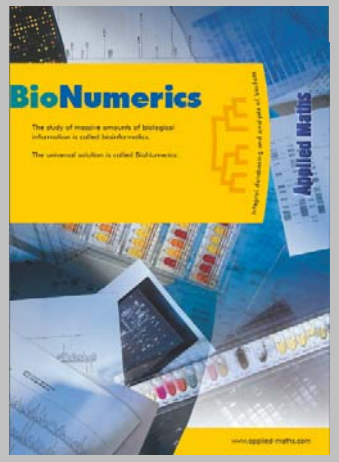
Microsatellite	*Chromosome*	*Position/ORF*	*Repeat*	*N⁰ of alleles*	*Ref.*
ScAAT1	XIII	86 901 – 87 129	ATT	29	Pérez and Gallego, 2001
ScAAT2	II	YBL084c	ATT	18	
ScAAT3	IV	YDR160w	ATT	19	
ScAAT4	VII	431 334 – 431 637	ATT	17	
ScAAT5	XVI	897 028 - 897 259	TAA	6	
ScAAT6	IX	105 661 – 105 926	TAA	10	
YPL009	XV	YOR156c	TAA	13	Legras et al., 2005
SCYOR267C	XV	YOR267c	TGT	12	
C4	XV	110 701-110 935	TAA+TAG	9	
C5	VI	210250-210414	GT	19	
C11	X	518870-519072	GT	18	

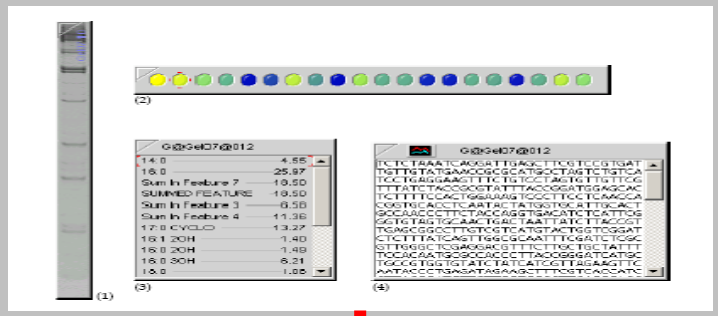
The BIONUMERICS software for databasing and cluster analysis



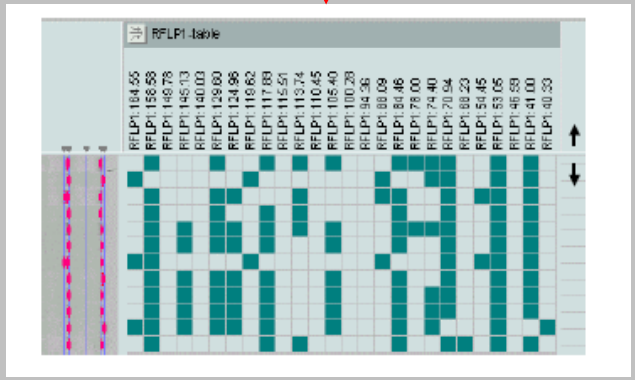
No.	ENZYME ASSAYED FOR	SUBSTRATE	pH	RESULT	
				POSITIVE	NEGATIVE
1	Control			Colorless or color of the sample if it has an intense coloration	
2	Alkaline phosphatase	2-naphthyl phosphate	8.5	Violet	Colorless or Very pale yellow *
3	Esterase (C 4)	2-naphthyl butyrate	6.5	Violet	
4	Esterase Lipase (C 8)	2-naphthyl caprylate	7.5	Violet	
5	Lipase (C 14)	2-naphthyl myristate	"	Violet	
6	Leucine arylamidase	L-leucyl-2-naphthylamide	"	Orange	
7	Valine arylamidase	L-valyl-2-naphthylamide	"	Orange	
8	Cystine arylamidase	L-cystyl-2-naphthylamide	"	Orange	
9	Trypsin	N-benzoyl-DL-arginine-2-naphthylamide	8.5	Orange	
10	α-chymotrypsin	N-glutaryl-phenylalanine-2-naphthylamide	7.5	Orange	
11	Acid phosphatase	2-naphthyl phosphate	5.4	Violet	
12	Naphthol-AS-BI-phosphohydrolase	Naphthol-AS-BI-phosphate	"	Blue	
13	α-galactosidase	6-Br-2-naphthyl-αD-galactopyranoside	"	Violet	
14	ß-galactosidase	2-naphthyl-ßD-galactopyranoside	"	Violet	
15	ß-glucuronidase	Naphthol-AS-BI-ßD-glucuronide	"	Blue	
16	α-glucosidase	2-naphthyl-αD-glucopyranoside	"	Violet	
17	ß-glucosidase	6-Br-2-naphthyl-ßD-glucopyranoside	"	Violet	
18	N-acetyl-ß-glucosaminidase	1-naphthyl-N-acetyl-ßD-glucosaminide	"	Brown	
19	α-mannosidase	6-Br-2-naphthyl-αD-mannopyranoside	"	Violet	
20	α-fucosidase	2-naphthyl-αL-fucopyranoside	"	Violet	

The BIONUMERICS software for databasing and cluster analysis



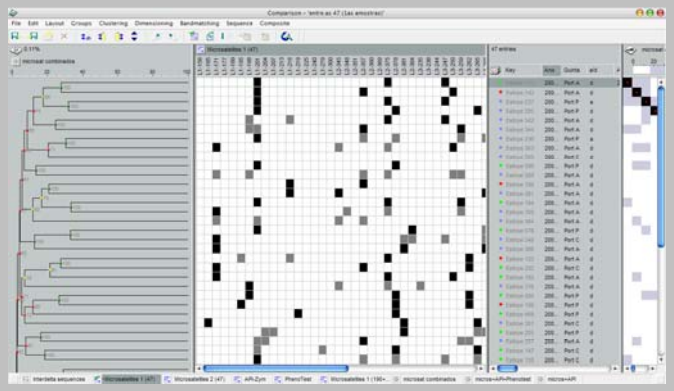


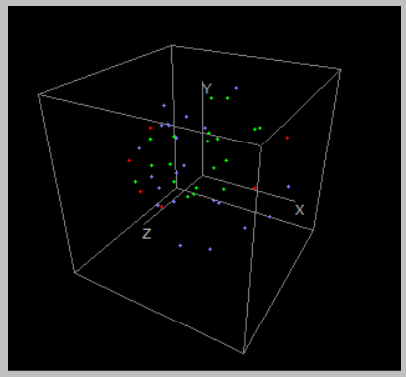
Entries from multiple fingerprint and character types



Binary/numerical value experiment file(s)

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



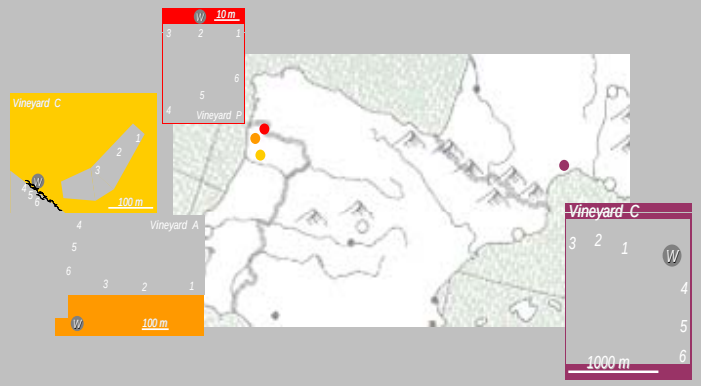


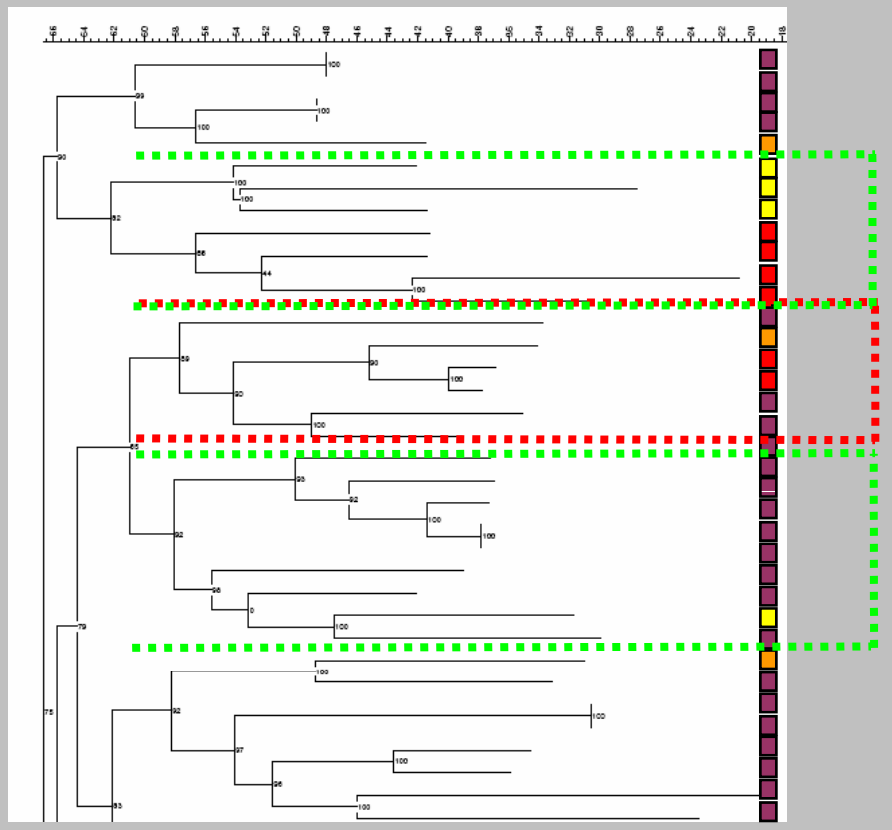
Genetic similarities based on microsatellite data

Analysis of individual strains

What are the genetic differences among strains from distinct geographical regions?

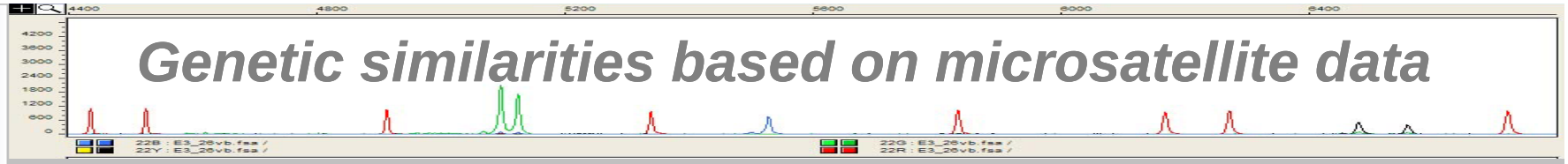
Correlation between geographic distance and genetic similarity ?

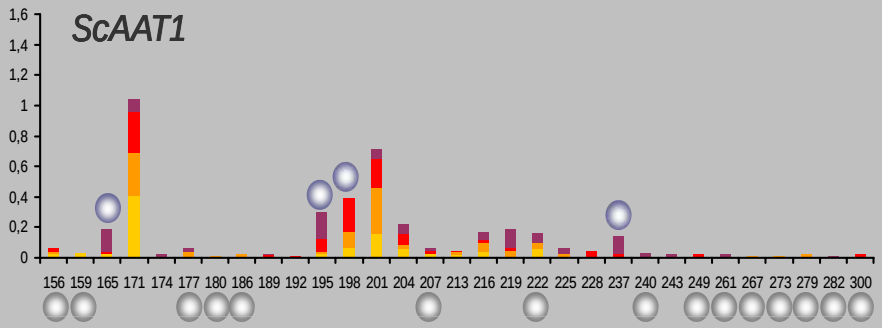


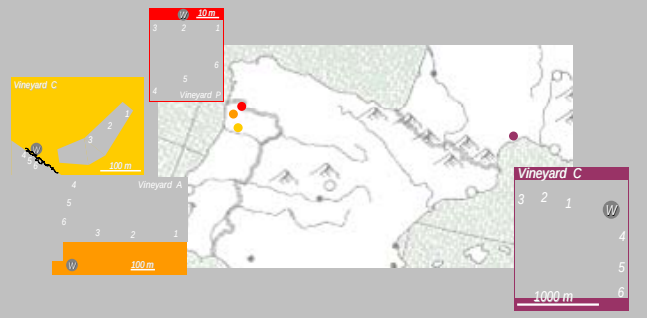


Neighbor-joining clustering
r=0,75

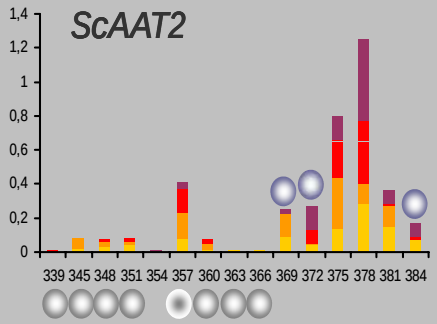
Genetic similarities based on microsatellite data

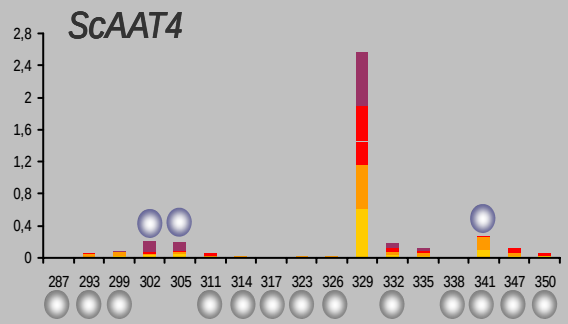


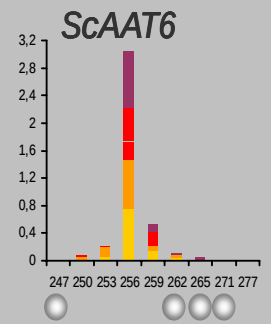


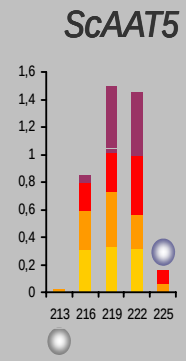


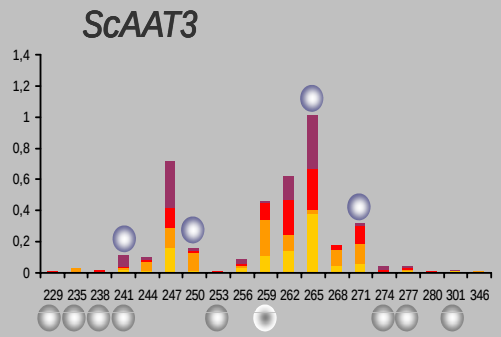
Allelic frequency











Allele (bp)

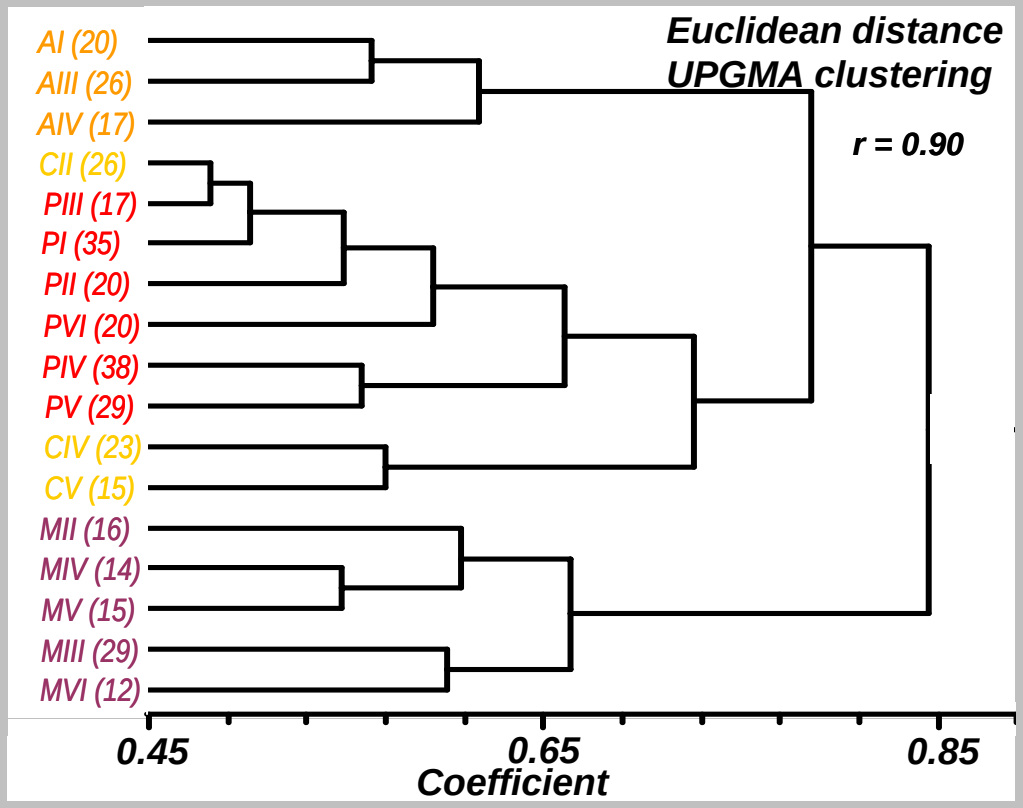
New allele

Vineyard – specific allele

Wine Region – specific allele

Genetic similarities based on microsatellite data

Comparison of populations



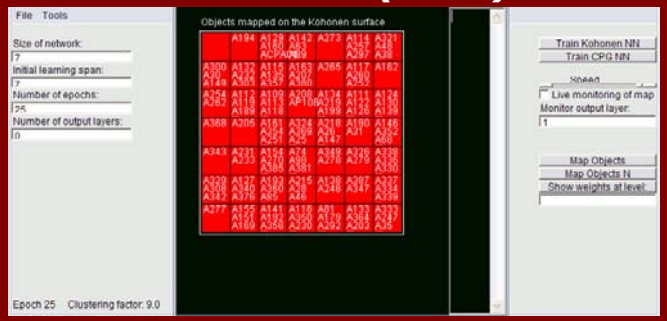
- ***Populational substructure***
- ***Genetic differences delimit specific populations - characteristic for each vineyard***
- ***Correlation between geographic distance and genetic similarity ?***

( ) number of strains analyzed

Schuller and Casal, 2007

Comparison between genetic and phenotypic variability

Neural networks (SOM)

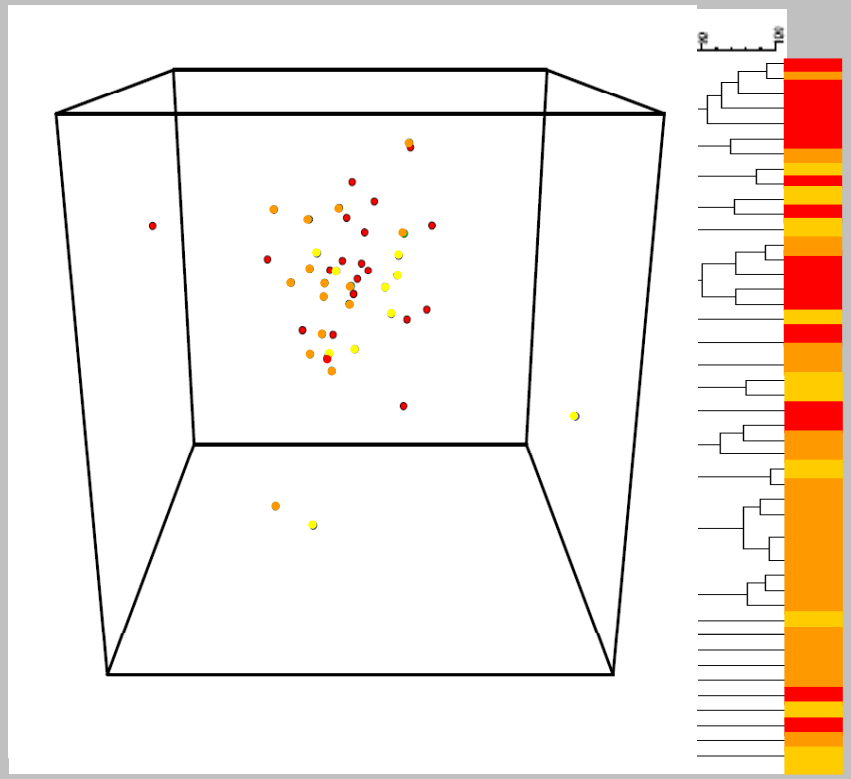


Selection of a genetically most diverse set of strains

340 → **47**

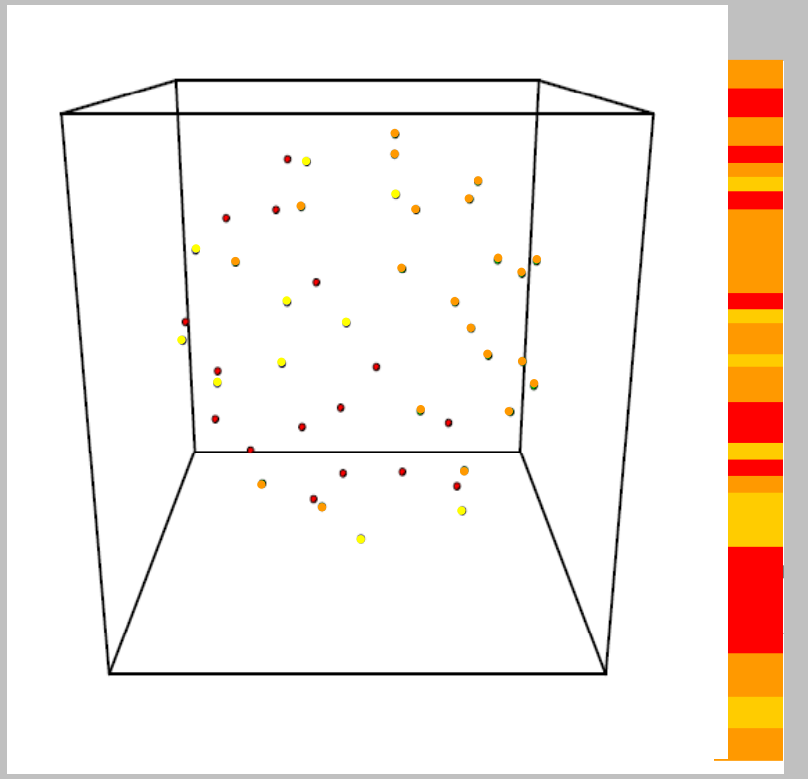
Comparison between genetic and phenotypic variability

API-ZYM analysis

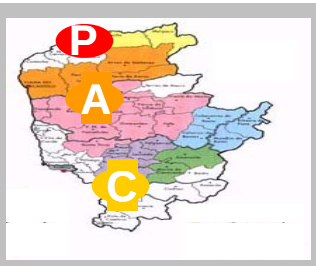


Pearson correlation
UPGMA clustering
R=0,80

Microsatellite analysis



Pearson correlation
UPGMA clustering
R=0,64



Ongoing work and future perspectives

- **Include additional phenotypic data with biotechnological relevance (for example wine making), using most objective phenotyping approaches**

- **Expand computational methods (artificial intelligence) in order to make predictions regarding biotechnological relevant characteristics of natural strains**

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