Comparative genomics of commercial *Saccharomyces cerevisiae* isolates recovered from vineyard environments

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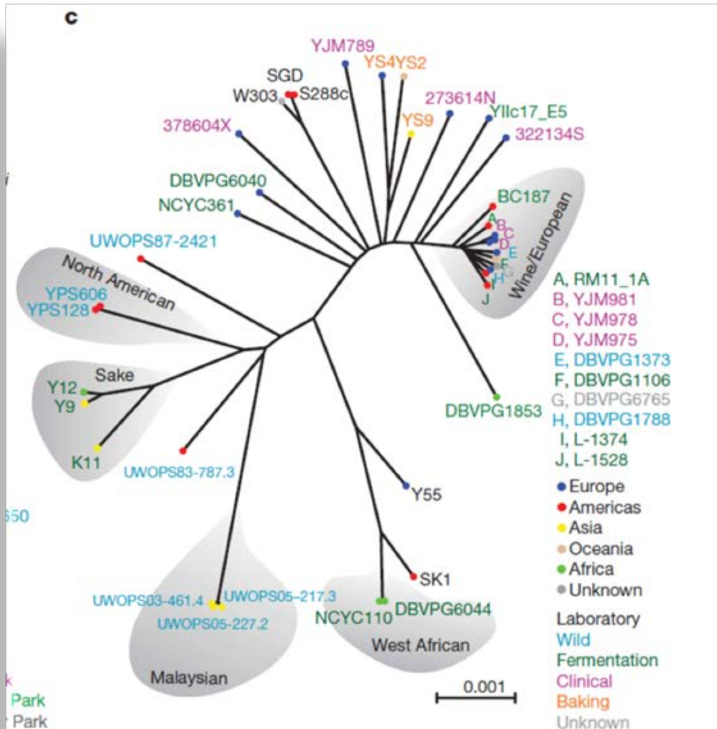
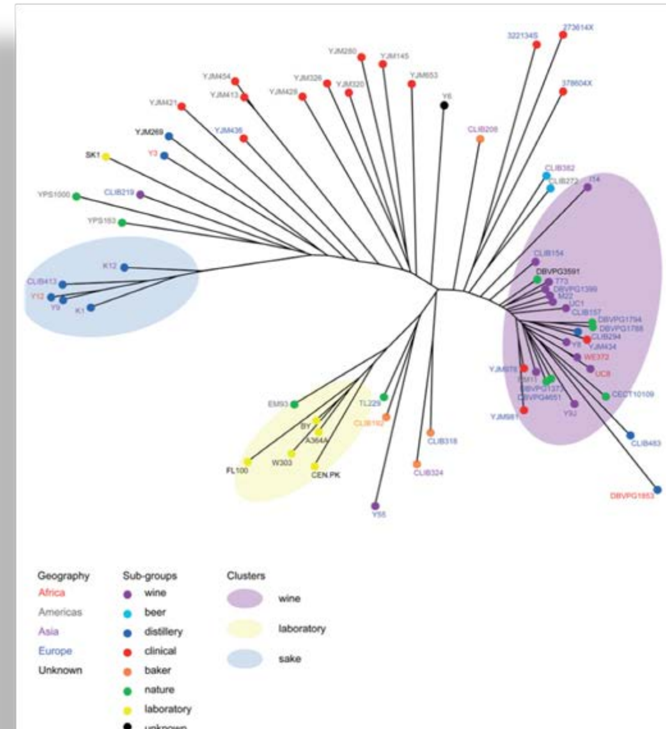
The population structure of *Saccharomyces cerevisiae*



Liti et al., Nature, 2009

235,127 SNPs
14,051 nucleotide insertions or deletions



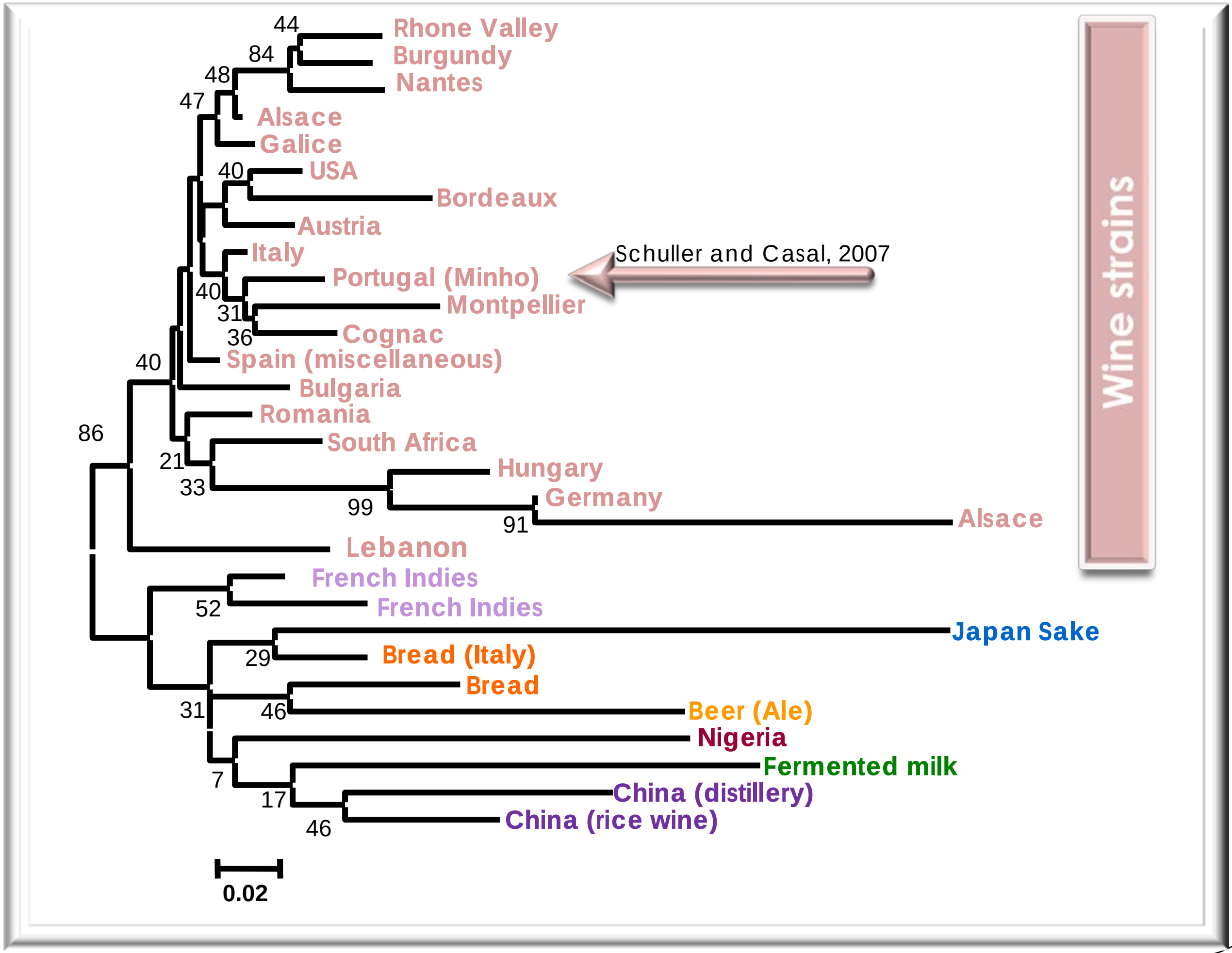
Schacherer et al., Nature, 2009

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

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

The population structure of *Saccharomyces cerevisiae*

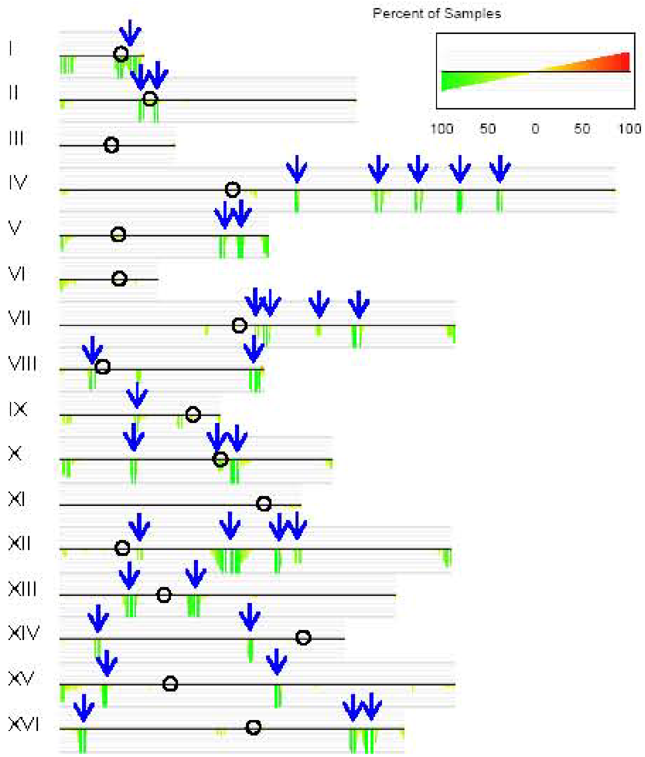
Consensus tree of *S. cerevisiae* populations based on F_{ST} genetic distances



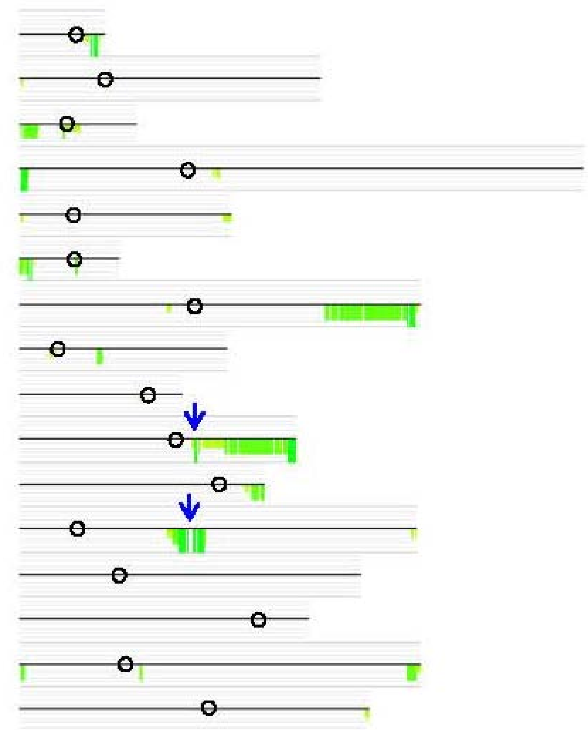
Legras *et. al*., Mol. Ecol. 2006

Intraspecific genome diversity of S. cerevisiae

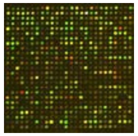
S. cerevisiae winemaking strains



S. cerevisiae clinical strains



Nacional Facility for DNA Microarrays



Carreto *et al.* 2008 BMC Genomics

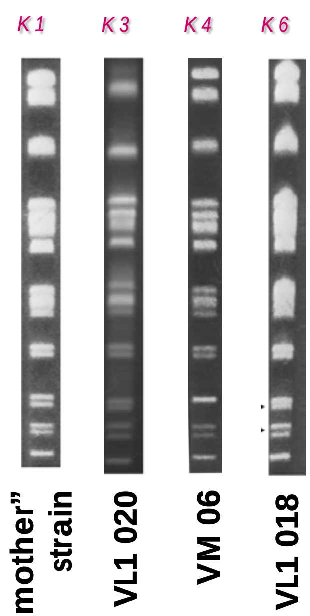
S. cerevisiae *commercial winemaking strains*

- ✓ Extensive use of commercial *Saccharomyces cerevisiae* wine strains
- ✓ Such strains are disseminated from the winery and can be recovered from locations in close proximity (10-200m)

  Valero *et al*., 2005
- ✓ Re-isolation of 100 isolates of the commercial strain VL1 from vineyards close to the winery where this strain has been used during many years

  Schuller and Casal, 2007

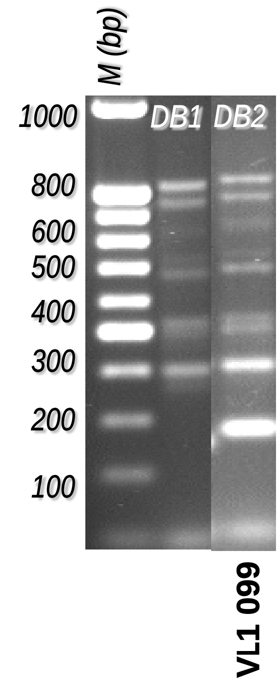
Karyotype patterns

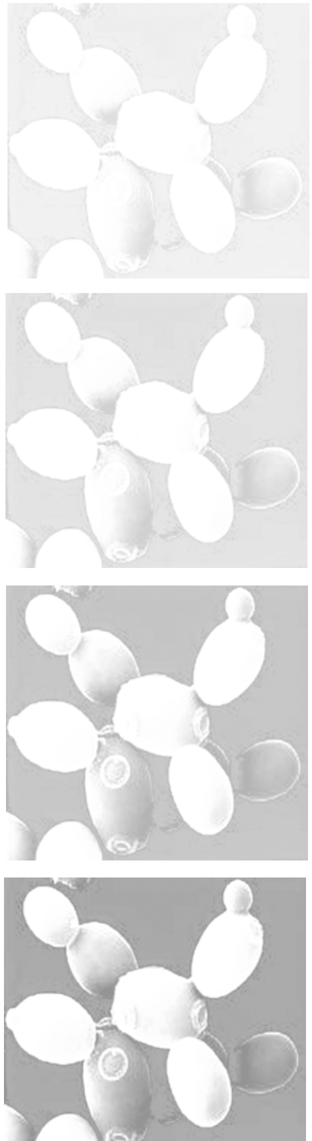


Microsatellite patterns

Loci	Alleles (bp) of distinct microsatellite patterns: M1	M7
ScAAT1	204/219	204/219
ScAAT2	372/381	372/381
ScAAT3	265	265
ScAAT4	329	329
ScAAT5	219/222	222
ScAAT6	256/259	256/259
	"mother" Strain	Vl1 020

Interdelta sequence amplification patterns



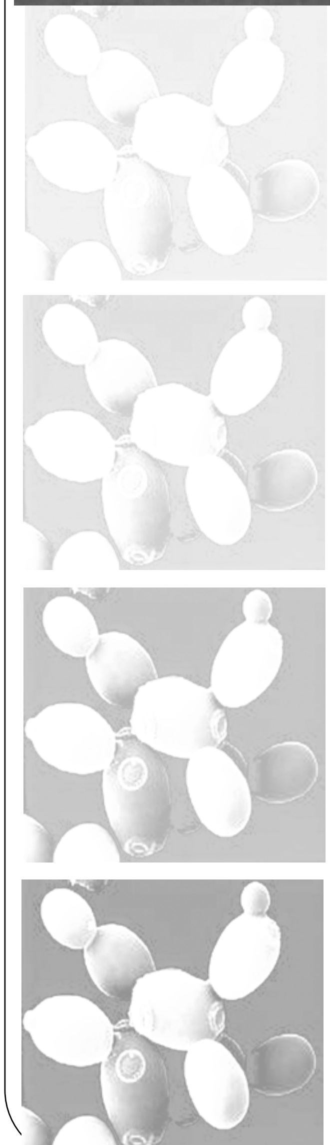


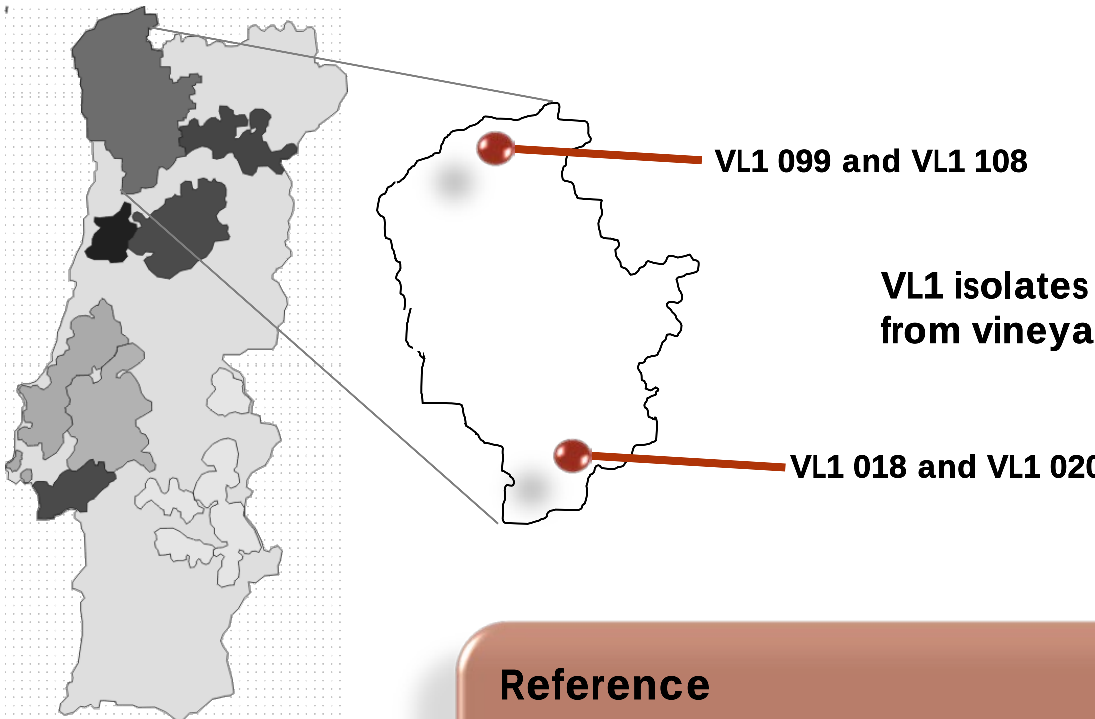
Objectives

Evaluation of genome variations among isogenic isolates of the commercial strain *S. cerevisiae* Zymaflore VL1 that were re-isolated from vineyards surrounding the wineries where this industrial strain was applied, using Comparative Genome Hybridization on array (aCGH);

Conclude about possible adaptive mechanisms that occur during the strain's permanence in vineyard environments

Materials and Methods – S. cerevisiae *isolates*





VL1 isolates recovered from vineyards

Reference

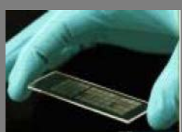
1. **Commercial VL1 "mother" strain**

2. **VM06** (Isolate obtained through clonal expansion of the "mother" strain)

Materials and Methods - *Array Chromosome Genome Hybridization (aCGH)*

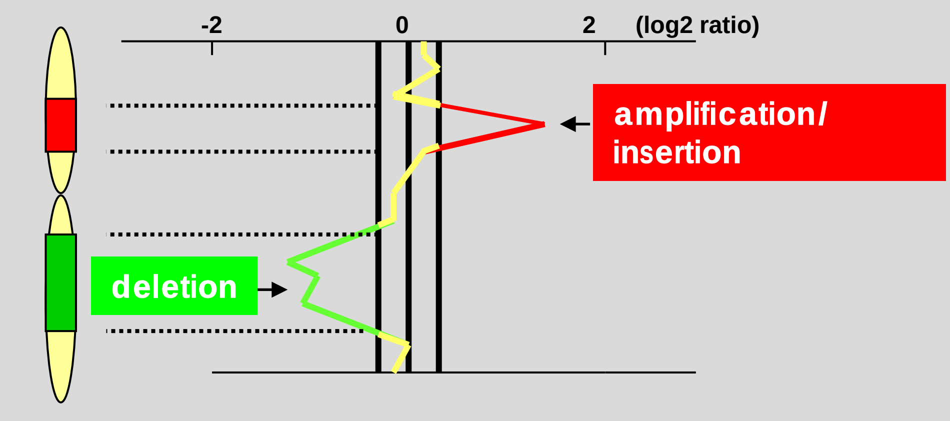
Reference DNA
"VL1 Mother strain"
Cy5

Test DNA
VM06
VL 018
VL 020
VL 099
VL 108
VM06
Cy5



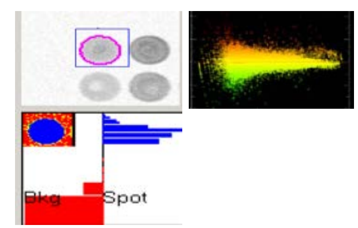
Dye swap hybridizations

Estimation of DNA copy number changes



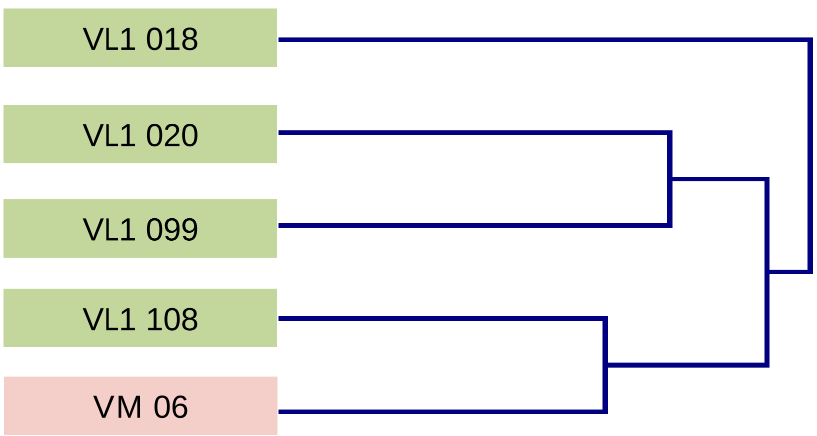
QuantArray software Image analysis - data extraction

BrB software Normalization of data



MeV software Graphical displays of log ratios and visual representation of data
Significance Analysis for Microarrays

Results – *Clustering of aCGH profiles*



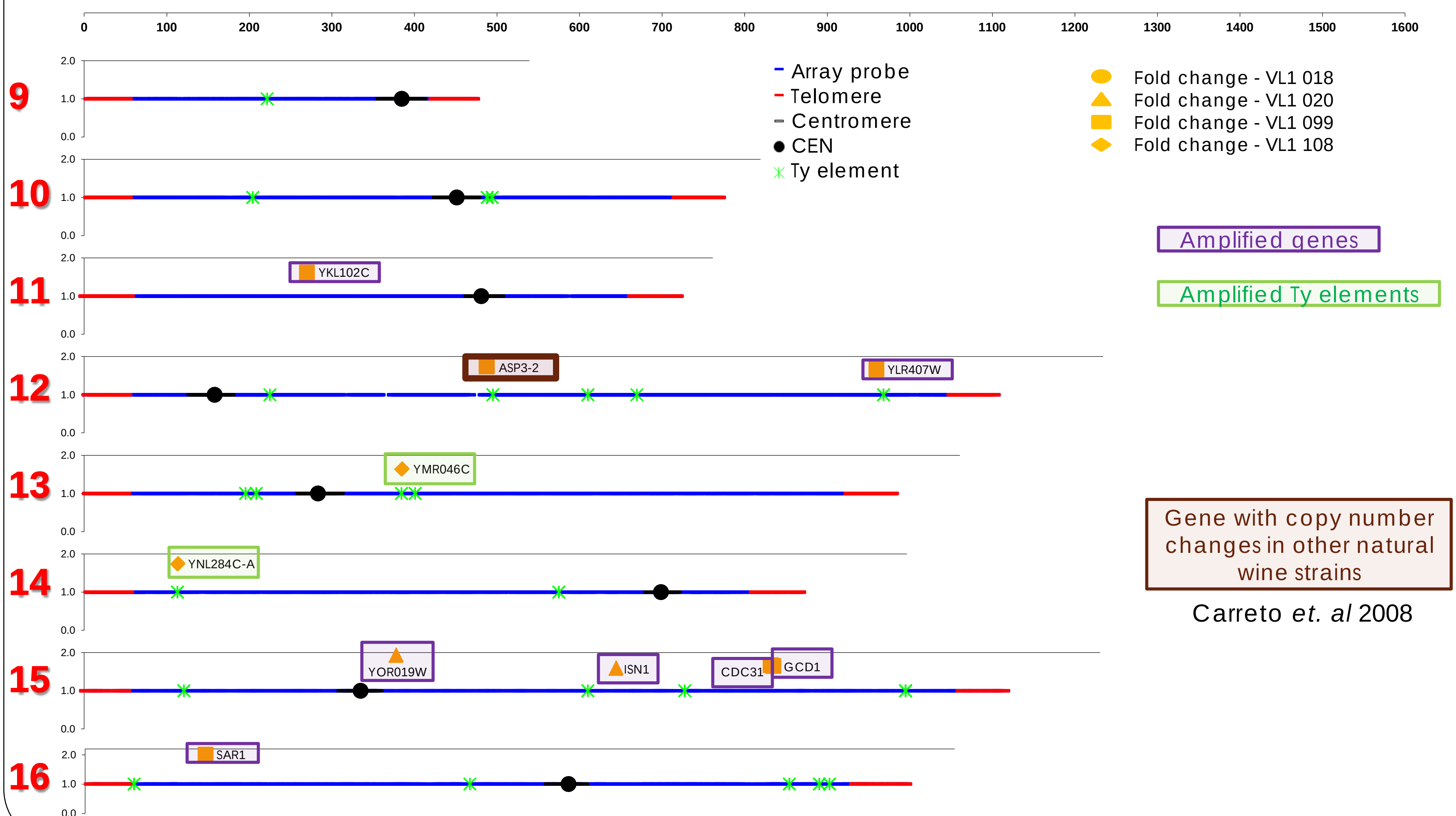
(Hierarchical clustering, Pearson correlation, average linkage)

No clear separation between VL1 isolates obtained from nature ( ) and an isolate derived from the "mother" strain ( )

Results – *Gene Copy number alterations – SAM analysis*

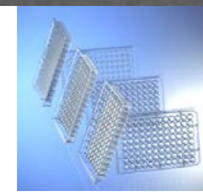
Carreto *et. al* 2008

Results – *Gene Copy number alterations – SAM analysis*



Carreto *et. al* 2008

Results – *Phenotypic characterization*

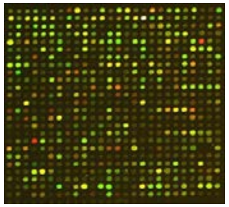


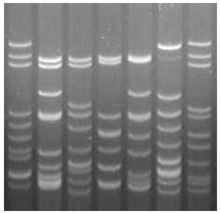
- Wine must + compound
- 30 ºC
- 200 rpm
- quadriplicate

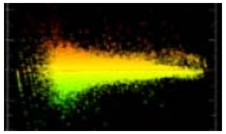
Strain	Phenotypic tests																			
	30ºC	18ºC	40ºC	pH 2	pH 8	KCl 0.75M	NaCl 1.5M	CuSO4 5mM	SDS 0.01%	Etanol 6%	Etanol 10%	Etanol 14%	Iprodion (0.05mg/mL)	Iprodion (0.1mg/mL)	Procymidon (0.05mg/mL)	Procymidon (0.1mg/mL)	KHSO3 (150 mg/l)	$KHSO_3$ (300 mg/l)	Vinho + glucose 0.5%	Vinho + glucose 1%
VL1 018	3	1	3	0	2	2	1	0	0	3	2	1	3	3	3	3	3	1	1	1
VL1 020	3	1	3	0	2	3	1	0	0	3	2	1	3	3	3	3	3	1	1	1
VL1 099	3	1	3	0	2	2	1	0	0	3	2	1	3	3	3	3	3	2	0	0
VL1 108	3	1	3	0	2	2	0	0	0	3	2	1	3	3	3	3	3	2	0	0
VM06	3	1	3	0	2	2	1	0	0	3	2	1	3	3	3	3	3	2	1	1
"Mother" strain	3	0	3	0	2	2	1	1	1	3	2	1	3	3	3	3	3	2	0	1

0 – Abs_{640nm} 0.1
1 – Abs_{640nm} 0.2-0.4
2 – Abs_{640nm} 0.5-1.2
3 – Abs_{640nm} ≥1.3

Summary and conclusions







- Isogenic isolates of the commercial wine yeast strain *Zymaflore VL1* recovered from nature show genetic differences in comparison with the "mother" strain:
  - Ty element amplifications
  - Other gene amplifications
  - Apparent stochastic distribution

- Mechanisms could be involved in the generation of intra-strain phenotypic variability

The transition from nutrient-rich musts to nutritionally scarce natural environments is correlated with microevolutionary changes promoted by Ty elements that may reflect adaptative responses

Acknowledgements

- João Drumonde
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SEVENTH FRAMEWORK PROGRAMME

Innoyeast

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

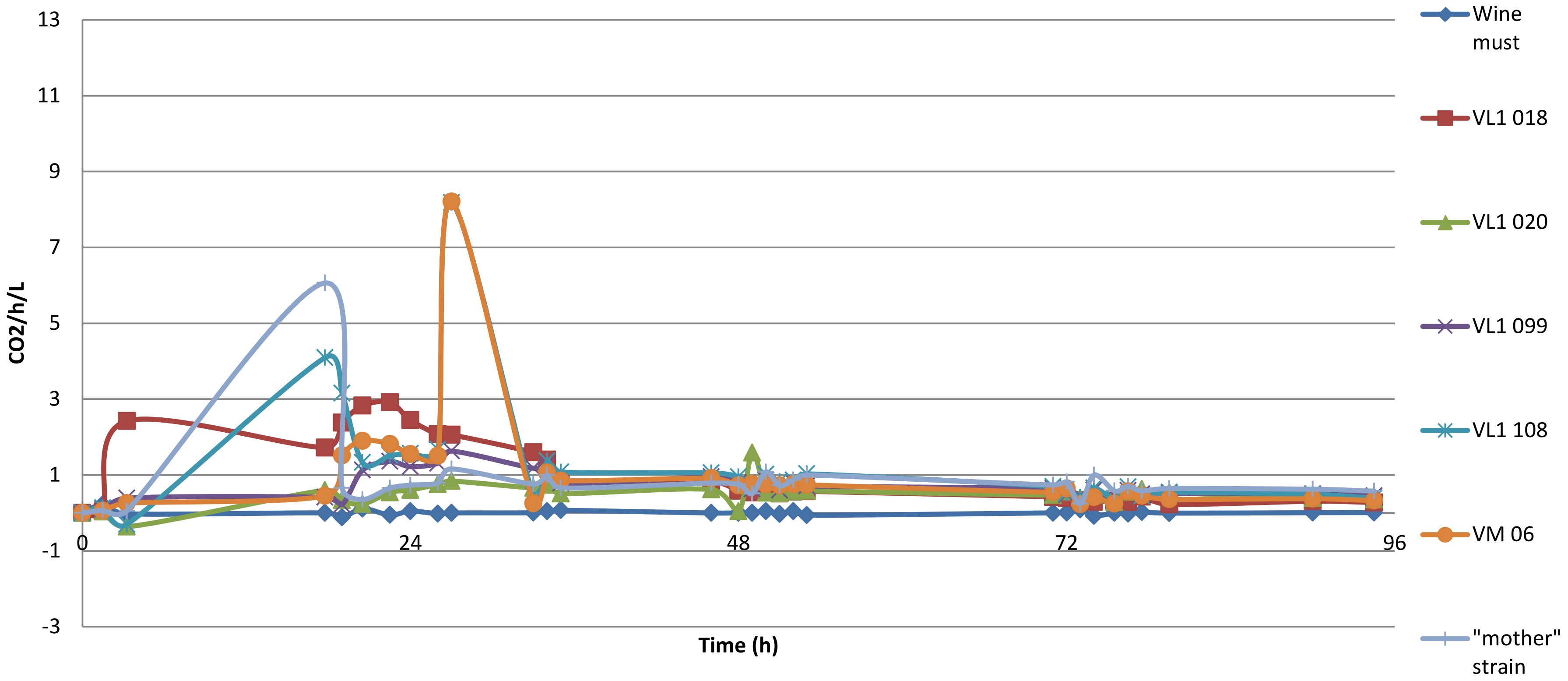
UNIÃO EUROPEIA FUNDOS ESTRUTURAIS

GOVERNO DA REPÚBLICA PORTUGUESA

RESULTS

Fermentation profiles

- Wine must
- 28 ºC
- 150 rpm



RESULTS

Significant altered genes

Strain	Systematic Name	Classical Name	Description	Chromosome
020	YBL031W	SHE1	Mitotic spindle protein that interacts with components of the Dam1 (DASH) complex, its effector Sli15p, and microtubule-associated protein Bim1p; also localizes to nuclear microtubules and to the bud neck in a ring-shaped structure	2
	YOR019W	NA	Protein of unknown function that may interact with ribosomes, based on co-purification experiments	15
	YGL251C	HFM1/MER3	Meiosis specific DNA helicase involved in the conversion of double-stranded breaks to later recombination intermediates and in crossover control; catalyzes the unwinding of Holliday junctions; has ssDNA and dsDNA stimulated ATPase activity	7
	YOR155C	ISN1	Inosine 5'-monophosphate (IMP)-specific 5'-nucleotidase, catalyzes the breakdown of IMP to inosine, does not show similarity to known 5'-nucleotidases from other organisms	15
	YDR034C	LYS14	Transcriptional activator involved in regulation of genes of the lysine biosynthesis pathway; requires 2-aminoadipate semialdehyde as co-inducer	4
	YBR020W	GAL1	Galactokinase, phosphorylates alpha-D-galactose to alpha-D-galactose-1-phosphate in the first step of galactose catabolism; expression regulated by Gal4p	2
099	YDR120C	TRM1	tRNA methyltransferase; two forms of the protein are made by alternative translation starts; localizes to both the nucleus and mitochondrion to produce the modified base N2,N2-dimethylguanosine in tRNAs in both compartments	4
	YLR407W	NA	Putative protein of unknown function; null mutant displays elongated buds and a large fraction of budded cells have only one nucleus	12
	YOR260W	GCD1/TRA3	Gamma subunit of the translation initiation factor eIF2B, the guanine-nucleotide exchange factor for eIF2; activity subsequently regulated by phosphorylated eIF2; first identified as a negative regulator of GCN4 expression	15
	YKL102C	NA	Dubious open reading frame unlikely to encode a functional protein; deletion confers sensitivity to citric acid; predicted protein would include a thiol-disulfide oxidoreductase active site	11
	YOR257W	CDC31/DSK1	Calcium-binding component of the spindle pole body (SPB) half-bridge, required for SPB duplication in mitosis and meiosis II; homolog of mammalian centrin; binds multiubiquitinated proteins and is involved in proteasomal protein degradatio	15
	YHR212C	NA	Dubious open reading frame unlikely to encode a functional protein, based on available experimental and comparative sequence data	8
	YLR157C	ASP3-2	Cell-wall L-asparaginase II involved in asparagine catabolism; expression induced during nitrogen starvation; ORF contains a short non-coding RNA that enhances expression of full-length gene; reference strain S288C has four copies of ASP3	12
	YPL218W	SAR1	GTPase, GTP-binding protein of the ARF family, component of COPII coat of vesicles; required for transport vesicle formation during ER to Golgi protein transport	16

RESULTS

Significant altered genes

Ty elements:

Strain	Systematic Name	Chromosome	Fold Change
018	YMR046C	13	1.6474975
099	YHL009W-A	8	1.5785116
	YHL009W-B	8	1.646452
108	YGR161C-C	7	1.4855264
	YBL005W-A	2	1.5453535
	YDR210C-C	4	1.4554093
	YDR170W-A	4	1.7406232
	YNL284C-A	14	1.7531929
	YMR046C	13	1.7273986