GENETIC CHARACTERIZATION OF COMMERCIAL *SACCHAROMYCES CEREVISIAE* ISOLATES RECOVERED FROM VINEYARD ENVIRONMENTS USING COMPARATIVE GENOME HYBRIDIZATION ON ARRAY (aCGH)

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Introduction

Saccharomyces cerevisiae is the mankind´s oldest domesticated organism and play also a central role in biotechnology. The use of commercial *S. cerevisiae* wine strains as fermentation starters has been extensively generalized over the past two decades. Within our previous work we showed that such strains are disseminated from the winery and their permanence in nature induced genetic changes in comparison to the commercial "mother" strain [1].

During its long history of association with human activity, the genomic makeup of this yeast is thought to have been shaped through the action of multiple independent rounds of wild yeast domestication. Recently published results on sequence comparisons by low coverage whole genome sequencing and high-density arrays suggest the existence of few well-defined geographically isolated lineages, and many mosaic lineages [2,3]. In our previous work [4], comparative genome hybridization on array (aCGH) was used to characterize the genome variability of yeast strains isolated from vineyards and cellars in comparison with laboratory strains, commercial wine strains and isolates from opportunistic human infections. Results showed that Ty element insertions determined genomic differences of wine fermentation strains, whereas sub-telomeric instability was associated with the clinical phenotype.

The objective of the present study was to evaluate, by aCGH, intra-strain genome variations among four isolates of the commercial strain Zymaflore VL1 that were re-isolated from vineyards close the wineries, in comparison to the commercial "mother" strain.

Materials and Methods

***S. cerevisiae* strains**

One hundred isolates of the commercial yeast strain Zymaflore VL1 were recovered from vineyards located close to wineries where this strain has been used for winemaking in consecutive years [1]. These isogenic strains show identical mitochondrial DNA restriction fragment length polymorphisms, and are characterized by small differences in karyotype, interdelta sequence amplification and microsatellite patterns, as shown in the figures on the right. These genetic changes might reflect adaptive mechanisms to environmental conditions that yeast cells encounter during their permanence in nature.

Four natural isolates (**VL1 099, VL1 108, VL1 018 and VL1 020**) were used and compared against two reference strains (commercial VL1 **"mother" strain**, and **VM 06**, an isolate obtained through clonal expansion of the "mother" strain).

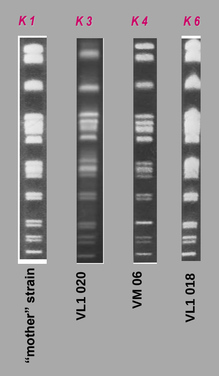
Array Chromosome Genome Hybridization (aCGH)

For comparative genome hybridization array experiments, ULS-Cy3 labelled DNA of each of the 5 strains (VL1 099, VL1 108, VL1 018, VL1 020 and VM 06) was combined with ULS-Cy5 labelled DNA from the commercial VL1 "mother" strain. Dye-swap hybridizations were performed for each strain and the fluorescence were quantified by image analysis using QuantArray software as previously described [4]. Data analysis was performed with BRB-ArrayTools v3.4, using median normalization, and hierarchical cluster analysis was performed using MeV from TM4 software. The relative hybridization signal of each ORF was derived from the average of the two dye-swap hybridizations. Deviations from the 1:1 hybridization ratio were taken as indicative of changes in DNA copy number.

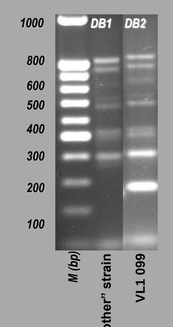
Phenotypic characterization

Phenotypic analysis included the evaluation of traits used in yeast taxonomy or for wine yeast strain selection. Yeast cells were inoculated in 4 replicate wells of a 96-well microplate (cellular density of 0.1, as previously described [5]). Final A_{640} was measured after 22h of growth. Several tests were used such as growth in synthetic must media at different temperatures and pH values, tolerance to several stresses such as osmotic and saline, ethanol resistance/tolerance, and others.

Karyotype patterns



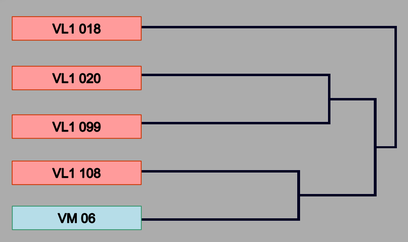
Interdelta sequence amplification patterns



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	VI1 020

RESULTS

1 Clustering of aCGH profiles

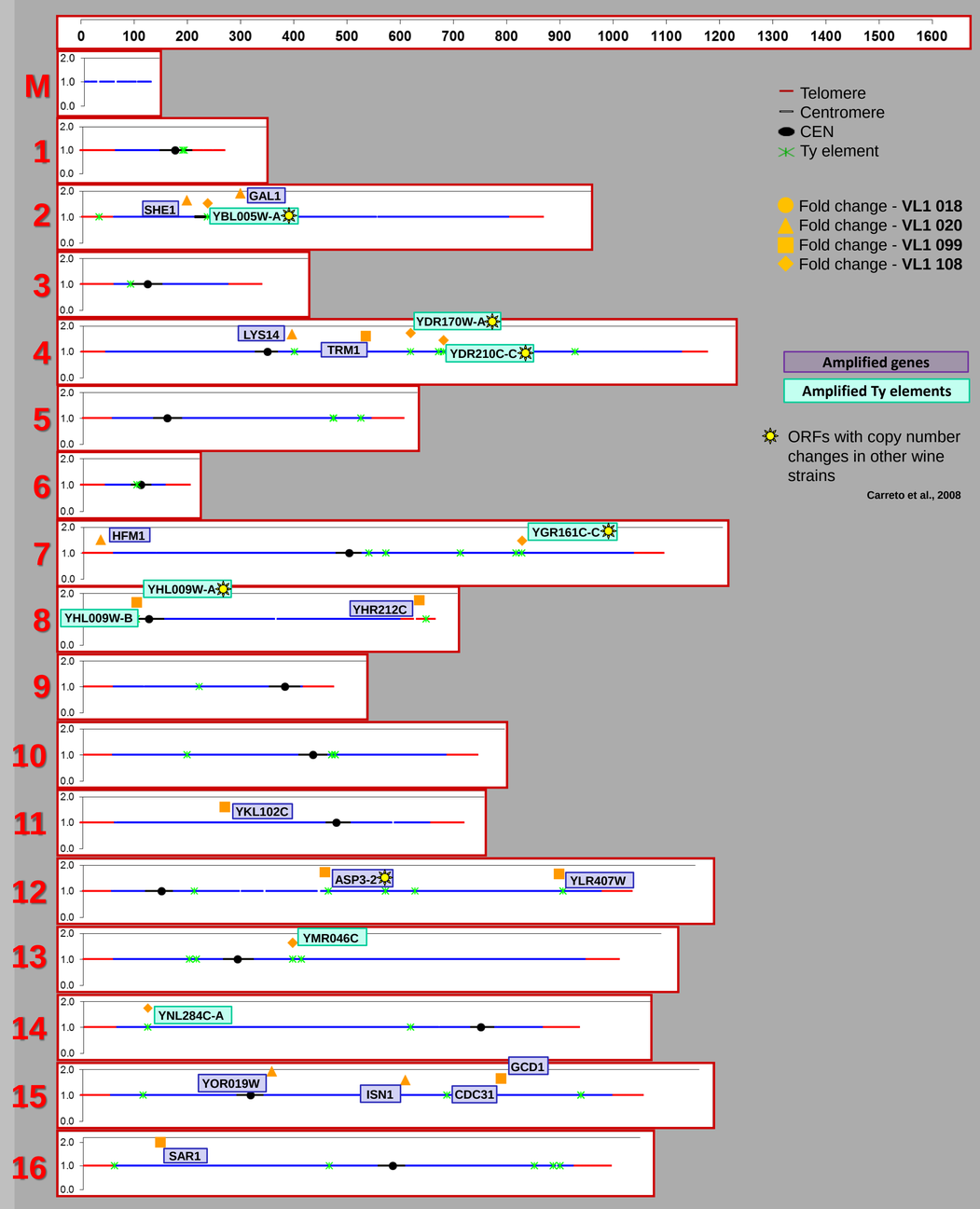


(Hierarchical clustering, Pearson correlation, average linkage)

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

2 Gene copy number alterations – SAM analysis

Graphical representation of gene copy number alterations for 16 chromosomes (including mitochondrial DNA), obtained by SAM analysis of aCGH data:



- ***Differences were apparent between strains recovered from nature and commercial VL1 "mother" strain (reference):***
  - ***ORF copy number changes only occurred in strains that were recovered from nature; strain VM06, a derivative of the "mother" strain showed no copy number changes;***
  - ***Copy number alterations showed a stochastic distribution among strains and chromosomes;***
  - ***Amplification of 22 ORFs were detected (between 1 and 2 fold changes), whereas 8 ORFs corresponded to amplified Ty elements;***
  - ***ASP3-2 and four Ty elements (YBL005W-A, YDR210C-C , YGR161C-C, YHL009W-A) showed copy number alterations in other wine strains (Carreto et al. 2008);***
  - ***Genes that were amplified were related with mitosis (SHE1) or meiosis (HFM1), with lysine biosynthesis (LYS14), galactose catabolism (GAL1), and asparagine catabolism (ASP3-2). This last gene is induced in case of nitrogen starvation, suggesting that these strain could use asparagine as alternative nitrogen source during their presence in nature.***

3 Phenotypic characterization

Strain	Phenotypic tests																		
	30 ºC	18 ºC	40 ºC	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
VM 06	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

(22h of growth, 200 rpm, 4 replicates, in 96-well microplates)

red squares – phenotypic differences between strains

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

- ***Phenotypic differences between the strains were observed for 7 out of 20 tests***
- ***Some phenotypic traits distinct isogenic strains from the commercial "mother" strain:***
  - ***The commercial "mother" strain was unable to grow at 18 ºC, but evidenced some growth in the presence of $CuSO_4$ 5mM and SDS 0.01%***
  - ***Variable growth patterns were found for NaCl 1.5M, $KHSO_3$ (300 mg/l), wine + glucose 0.5% and wine + glucose 1%***

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, with various functions, that could reflect adaptive mechanisms to environmental conditions, such as ASP3-2***
  - ***Apparent stochastic distribution***
- ***These differences could be related to mechanisms involved in the generation of intra-strain phenotypic variability. We hypothesize that the transition from nutrient-rich musts to nutritionally scarce natural environments induces adaptive responses and microevolutionary changes promoted by Ty elements.***

References

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Acknowledgements

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