Microevolutionary changes of commercial Saccharomyces cerevisiae strains recovered from vineyard environments identified by comparative genome hybridization on array

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INTRODUCTION

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)

- low coverage genome sequencing
- high density arrays

➤ few well-defined, geographically isolated lineages
➤ many different mosaics of these lineages (wine, laboratory and saké strains)
INTRODUCTION

*S. cerevisiae* commercial winemaking strains

- Extensive use of commercial *S. 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

<table>
<thead>
<tr>
<th>Loci</th>
<th>Alleles (bp) of distinct microsatellite patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td>ScAAT1</td>
<td>M1: 204/219, M7: 204/219</td>
</tr>
<tr>
<td>ScAAT2</td>
<td>M1: 372/381, M7: 372/381</td>
</tr>
<tr>
<td>ScAAT3</td>
<td>M1: 265, M7: 265</td>
</tr>
<tr>
<td>ScAAT4</td>
<td>M1: 329, M7: 329</td>
</tr>
<tr>
<td>ScAAT5</td>
<td>M1: 219/222, M7: 222</td>
</tr>
<tr>
<td>ScAAT6</td>
<td>M1: 256/259, M7: 256/259</td>
</tr>
</tbody>
</table>

Karyotype patterns:
- "mother" strain
- VL1 020
- VM 06
- VL1 018

Microsatellite patterns:
- K1
- K3
- K4
- K6

Interdelta sequence amplification patterns:
- DB1
- DB2

"mother" strain:
- VL1 099

"mother" strain:
- VL1 020
Objectives

- Evaluation of genome variations among isogenic isolates of the commercial strain *Saccharomyces 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 adaptive mechanisms that occur during the strain’s permanence in vineyard environments
Materials and Methods

Saccharomyces cerevisiae isolates

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”

Test DNA
VM 06
VL 018
VL 020
VL 099
VL 108

Estimation of DNA copy number changes

-2 0 2 (log2 ratio)

- deletion

amplification/insertion

Dye swap hybridizations

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
Clustering of aCGH profiles

No clear separation between VL1 isolates obtained from nature (●) and an isolate derived from the “mother” strain (○)

(Hierarchical clustering, Pearson correlation, average linkage)
Results

Gene Copy number alterations – SAM analysis

- Array probe
- Telomere
- Centromere
- CEN
- Ty element

Fold change - VL1 018
Fold change - VL1 020
Fold change - VL1 099
Fold change - VL1 108

Amplified genes
Amplified Ty elements

Ty elements with copy number changes in other wine strains

Carreto et al. 2008

Results

Gene Copy number alterations – SAM analysis

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Carreto et al. 2008
Results

**Gene Copy number alterations – SAM analysis**

- Array probe
- Telomere
- Centromere
- CEN
- Ty element

- Fold change - VL1 018
- Fold change - VL1 020
- Fold change - VL1 099
- Fold change - VL1 108

**Amplified genes**

**Amplified Ty elements**

Gene with copy number changes in other natural wine strains

Carreto et. al 2008
## Results

### Phenotypic characterization

- Wine must + compound
- 30 °C
- 200 rpm
- quadruplicate

<table>
<thead>
<tr>
<th>Strain</th>
<th>30°C</th>
<th>18°C</th>
<th>40°C</th>
<th>pH 2</th>
<th>pH 8</th>
<th>KCl 0.75M</th>
<th>NaCl 1.5M</th>
<th>CuSO4 5mM</th>
<th>SDS 0.01%</th>
<th>Etanol 6%</th>
<th>Etanol 10%</th>
<th>Etanol 14%</th>
<th>Iprodion (0.05mg/ml)</th>
<th>Iprodion (0.1mg/ml)</th>
<th>Procymidon (0.05mg/ml)</th>
<th>Procymidon (0.1mg/ml)</th>
<th>KHSO3 (150 mg/l)</th>
<th>KHSO3 (300 mg/l)</th>
<th>Vinho + glucose 0.5%</th>
<th>Vinho + glucose 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL1 018</td>
<td>3</td>
<td>1</td>
<td>3</td>
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<td>1</td>
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<tr>
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<td>0</td>
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<td>VL1 099</td>
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<td>0</td>
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<td>VL1 108</td>
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<td>VM06</td>
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<tr>
<td>&quot;Mother&quot; strain</td>
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<td>2</td>
</tr>
</tbody>
</table>

0 – Abs<sub>640nm</sub> 0.1
1 – Abs<sub>640nm</sub> 0.2-0.4
2 – Abs<sub>640nm</sub> 0.5-1.2
3 – Abs<sub>640nm</sub> ≥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:
  - Gene amplifications
  - Ty element amplifications
  - Apparent stochastic distribution

- Generation of intra-strain phenotypic variability

The transition from nutrient-rich musts to nutritionally scarce natural environments is correlated with microevolutionary changes that may reflect adaptative responses.
Acknowledgements

• Dorit Schuller
• João Drumonde
• Elza Fonseca
• Inês Mendes
• Nuno Fonseca
• Eugénia Vieira
## RESULTS

### Significant altered genes

<table>
<thead>
<tr>
<th>Strain</th>
<th>Systematic Name</th>
<th>Classical Name</th>
<th>Description</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>020</td>
<td>YBL031W</td>
<td>SHE1</td>
<td>Mitotic spindle protein that interacts with components of the Dam1 (DASH) complex, its effector Sl1.5p, and microtubule-associated protein Bim1p; also localizes to nuclear microtubules and to the bud neck in a ring-shaped structure</td>
<td>2</td>
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<tr>
<td></td>
<td>YOR019W</td>
<td>NA</td>
<td>Protein of unknown function that may interact with ribosomes, based on co-purification experiments</td>
<td>15</td>
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<tr>
<td></td>
<td>YGL251C</td>
<td>HFM1/MER3</td>
<td>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</td>
<td>7</td>
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<tr>
<td></td>
<td>YOR155C</td>
<td>ISN1</td>
<td>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</td>
<td>15</td>
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<tr>
<td></td>
<td>YDR034C</td>
<td>LYS14</td>
<td>Transcriptional activator involved in regulation of genes of the lysine biosynthesis pathway; requires 2-amino adipic semialdehyde as co-inducer</td>
<td>4</td>
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<tr>
<td></td>
<td>YBR020W</td>
<td>GAL1</td>
<td>Galactokinase; phosphorylates alpha-D-galactose to alpha-D-galactose-1-phosphate in the first step of galactose catabolism; expression regulated by Gal4p</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>YDR120C</td>
<td>TRM1</td>
<td>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</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>YLR407W</td>
<td>NA</td>
<td>Putative protein of unknown function; null mutant displays elongated buds and a large fraction of budded cells have only one nucleus</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>YOR260W</td>
<td>GCD1/TRA3</td>
<td>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</td>
<td>15</td>
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<tr>
<td></td>
<td>YKL102C</td>
<td>NA</td>
<td>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</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>YOR257W</td>
<td>CDC31/DSK1</td>
<td>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 mult ubiquitinated proteins and is involved in proteasomal protein degradatio</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>YHR212C</td>
<td>NA</td>
<td>Dubious open reading frame unlikely to encode a functional protein, based on available experimental and comparative sequence data</td>
<td>8</td>
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<tr>
<td></td>
<td>YLR157C</td>
<td>ASP3-2</td>
<td>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</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>YPL218W</td>
<td>SAR1</td>
<td>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</td>
<td>16</td>
</tr>
</tbody>
</table>
### RESULTS

#### Significant altered genes

Ty elements:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Systematic Name</th>
<th>Chromosome</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>018</td>
<td>YMR046C</td>
<td>13</td>
<td>1.6474975</td>
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<tr>
<td>099</td>
<td>YHL009W-A</td>
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<td>1.5785116</td>
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<tr>
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<td>YHL009W-B</td>
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<td>1.646452</td>
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<tr>
<td>108</td>
<td>YGR161C-C</td>
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<td>1.4855264</td>
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<td>YBL005W-A</td>
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<td>YDR210C-C</td>
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<td>YDR170W-A</td>
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<tr>
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<td>YNL284C-A</td>
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</tr>
<tr>
<td></td>
<td>YMR046C</td>
<td>13</td>
<td>1.7273986</td>
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</tbody>
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