Comparative genomics of commercial Saccharomyces cerevisiae isolates recovered from vineyard environments

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XXXVI Jornadas Portuguesas de Genética
Coimbra, 1st June, 2011
The population structure of *Saccharomyces cerevisiae*

Liti et al., Nature, 2009

235,127 SNPs
14,051 nucleotide insertions or deletions

Schachter et al., Nature, 2009

1.89 x 10^6 SNP (30,097 SNPs per strain)
3,985 deletions (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*

*Carreto et al. 2008 BMC Genomics*
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

<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>
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

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

Estimation of DNA copy number changes

-2                          0                          2 (log2 ratio)

- Dye swap hybridizations
- **amplification/insertion**
- **deletion**

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**Image analysis - data extraction**

**QuantArray software**

**BrB software**

**MeV software**

**Normalization of data**

**Graphical displays of log ratios and visual representation of data**

**Significance Analysis for Microarrays**
Results - 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

- 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

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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

**Phenotypic tests**

<table>
<thead>
<tr>
<th>Strain</th>
<th>30°C</th>
<th>18°C</th>
<th>40°C</th>
<th>pH2</th>
<th>pH8</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>
<td>0</td>
<td>2</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>VL1 020</td>
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<td>1</td>
</tr>
<tr>
<td>VL1 099</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>2</td>
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<td>3</td>
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<td>0</td>
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</tr>
<tr>
<td>VL1 108</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>2</td>
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<td>0</td>
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<td>VM06</td>
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<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
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</tr>
<tr>
<td>“Mother” strain</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
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<td>3</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

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

0 - Abs$_{640nm}$ 0.1
1 - Abs$_{640nm}$ 0.2-0.4
2 - Abs$_{640nm}$ 0.5-1.2
3 - Abs$_{640nm}$ ≥1.3
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
- Elza Fonseca
- Ricardo Duarte
- Inês Mendes
- Nuno Fonseca
- Eugénia Vieira
RESULTS

Fermentation profiles

- Wine must
- 28 ºC
- 150 rpm

![Graph showing fermentation profiles of different wine must samples with time in hours on the x-axis and CO2/h/L on the y-axis. The graph includes lines for different strains: VL1 018, VL1 020, VL1 099, VL1 108, VM 06, and a "mother" strain.](image-url)
## 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 Sli15p, and microtubule-associated protein Bim1p; also localizes to nuclear microtubules and to the bud neck in a ring-shaped structure</td>
<td>2</td>
</tr>
<tr>
<td>020</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>
</tr>
<tr>
<td>020</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>
</tr>
<tr>
<td>020</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>
</tr>
<tr>
<td>020</td>
<td>YDR034C</td>
<td>LYS14</td>
<td>Transcriptional activator involved in regulation of genes of the lysine biosynthesis pathway; requires 2-aminoadipate semialdehyde as co-inducer</td>
<td>4</td>
</tr>
<tr>
<td>020</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>099</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>099</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>099</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>
</tr>
<tr>
<td>099</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>099</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 proteosomal protein degradation</td>
<td>15</td>
</tr>
<tr>
<td>099</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>
</tr>
<tr>
<td>099</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>099</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>
## 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>
</tr>
<tr>
<td>099</td>
<td>YHL009W-A</td>
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<td>1.5785116</td>
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<tr>
<td></td>
<td>YHL009W-B</td>
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<tr>
<td>108</td>
<td>YGR161C-C</td>
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<td></td>
<td>YBL005W-A</td>
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<td>YDR210C-C</td>
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<td>1.4554093</td>
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<td>YDR170W-A</td>
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<td>1.7406232</td>
</tr>
<tr>
<td></td>
<td>YNL284C-A</td>
<td>14</td>
<td>1.7531929</td>
</tr>
<tr>
<td></td>
<td>YMR046C</td>
<td>13</td>
<td>1.7273986</td>
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