Gene dosage influences the functional attributes of *de novo* lager yeast hybrids

The 5th International Young Scientists Symposium on Malting, Brewing and Distilling
April 21-23, 2016 – Chico, California, USA
Kristoffer Krogerus
Background

- Pale lager is the most popular beer style worldwide
- Cold fermentation and clean flavour profile
- Made with lager yeast: *Saccharomyces pastorianus*
Background

- Lager yeast, *S. pastorianus*, is a natural hybrid between *S. cerevisiae* and *S. eubayanus*:

  - *Saccharomyces cerevisiae* (cold-sensitive, good fermentation)
  - *Saccharomyces eubayanus* (cold-tolerant, poor fermentation)
  - *Saccharomyces pastorianus* (Good fermentation performance at low temperature, lager brewing)
Background

- Poor diversity among the traditional lager yeasts
- Belong to one of two distinct lineages:
  - Saaz
  - Frohberg

Group 1 (Saaz)
- Weak fermentation
- Very cold-tolerant
- *S. eubayanus* genome dominates

Group 2 (Frohberg)
- Strong fermentation
- Cold-tolerant
- *S. cerevisiae* genome dominates
Can we increase the diversity of lager yeast by creating new lager yeast hybrids?

- Mating *S. eubayanus* with selected *S. cerevisiae* parents?
Our previous research

- *De novo* lager yeast hybrids can
  - outperform their parent strains during fermentation
  - produce beer with higher concentrations of aroma compounds

New lager yeast strains generated by interspecific hybridization

Kristoffer Krogerus · Frederico Magalhães · Vítor Vidalgras · Brian Gilloon

![Graph showing alcohol content over fermentation time for different strains.](image1)

**Ethyl hexanoate**

- Hybrid H1
- Hybrid H2
- Hybrid H3
- Hybrid H4
- A81082 (54)
- C12902 (54)

![Graph showing ethyl hexanoate concentration for different strains.](image2)

**3-Methylbutyl acetate**

- Hybrid H1
- Hybrid H2
- Hybrid H3
- Hybrid H4
- A81082 (54)
- C12902 (54)

![Graph showing 3-methylbutyl acetate concentration for different strains.](image3)
Controlling the properties of the hybrids

- How does the contribution of the parental subgenomes affect important phenotypic traits?
  - Fermentation performance
  - Aroma production
  - Stress tolerance

Group 1 (Saaz)
- Weak fermentation
- Very cold-tolerant
- *S. eubayanus* genome dominates
- **Allotriploid**

Group 2 (Frohberg)
- Strong fermentation
- Cold-tolerant
- *S. cerevisiae* genome dominates
- **Allotetraploid**
Controlling the properties of the hybrids

S. cerevisiae A62

Hybrid A2 (allodiploid)

S. eubayanus C902

Hybrid B3 (allotriploid)

Hybrid C4 (allotetraploid)
Aims

- Compare the performance of these hybrids in 2-litre fermentations using 15 and 25 °P wort.
- How does the DNA content of the lager yeast hybrids affect
  - fermentation performance
  - aroma production
  - resistance to harsh conditions of high gravity wort
- Elucidate the relationship between gene expression and aroma formation in the strains.
Creation

**Sc A62 ura-**  **Se C902 lys-**

Spores or yeast cell cultures of parent strains

Incubation

Selection

Purification

Confirmation
Ploidy estimation

- The DNA content of the hybrids were estimated with flow cytometry

- Parent strains: diploid

- Hybrid A2: diploid

- Hybrid B3: triploid

- Hybrid C4: tetraploid
Sequencing

- Each hybrid strain was analysed with Illumina pair-end DNA sequencing
- These were compared to the parent genomes
  - *Sc* A62 sequence was obtained through the hybrid assembly of Illumina and PacBio sequencing data
  - *Se* C902 sequence from previous studies (Baker et al. 2015)
Sequencing – allodiploid Hybrid A2

- 1 copy of each chromosome from both subgenomes
Sequencing – allotriploid Hybrid B3

- 2 copies of each chromosome from *S. cerevisiae*
  - Exception in chromosome III (1 copy)
- 1 copy of each chromosome from *S. eubayanus*
Sequencing – allotetraploid Hybrid C4

- 2 copies of each chromosome from *S. cerevisiae*
  - Exception in chromosome III (1 copy)
- 2 copies of each chromosome from *S. eubayanus*
  - Exception in chromosome X (1 copy)
Growth at various temperatures

- Hybrids have a broader temperature range of growth
- The same strain can be used for low and high temperature fermentations?
Fermentation performance (15 °C, 15 °P)

- A81062 (Sc)
- C12902 (Se)
- Hybrid C4
- Hybrid B3
- Hybrid A2
Fermentation performance (15 °C, 25 °P)

- A81062 (Sc)
- C12902 (Se)
- Hybrid C4
- Hybrid B3
- Hybrid A2

Alcohol (%) vs. Fermentation time (hours)
Sugar use (15 °P)

Fermentation time (hours)

- A81062 (Sc)
- C12902 (Se)
- Hybrid C4
- Hybrid B3
- Hybrid A2

Maltose

Maltotriose
Flocculation & ethanol tolerance

- Loss of flocculation ability in diploid Hybrid A2

Flocculation potential (%)

Sc A62  Se C902  Hybrid C4  Hybrid B3  Hybrid A2

Relative growth

5% EtOH  10% EtOH

A81062 (Sc)  C12902 (Se)  Hybrid C4  Hybrid B3  Hybrid A2
**Diacetyl**

- Highest diacetyl peaks were observed for diploid Hybrid A2 and tetraploid Hybrid C4
  - No clear pattern between hybrid strains and the parent strains
  - More rapid diacetyl removal in diploid hybrid A2
# Aroma compounds

<table>
<thead>
<tr>
<th></th>
<th>Sc A81062</th>
<th>Se C12902</th>
<th>Hybrid C4</th>
<th>Hybrid B3</th>
<th>Hybrid A2</th>
<th>Sc A81062</th>
<th>Se C12902</th>
<th>Hybrid C4</th>
<th>Hybrid B3</th>
<th>Hybrid A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td>21.31 (±1.48) a</td>
<td>8.34 (±0.57) b</td>
<td></td>
<td>30.20 (±2.80) c</td>
<td>15.34 (±1.65) d</td>
<td>4.99 (±0.30) e</td>
<td>14.24 (±1.33) a</td>
<td></td>
<td>20.66 (±2.08) b</td>
<td>31.94 (±1.21) c</td>
</tr>
<tr>
<td>(10 mg L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Propanol</td>
<td>16.38 (±0.44) a</td>
<td>13.76 (±0.96) b</td>
<td>16.95 (±0.56) c,d</td>
<td>18.08 (±0.36) c</td>
<td>25.34 (±1.13) c</td>
<td>30.16 (±1.15) a</td>
<td></td>
<td>6.67 (±0.43) b</td>
<td>34.85 (±1.17) c</td>
<td>29.02 (±1.94) d</td>
</tr>
<tr>
<td>(800 mg L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Methylpropanol</td>
<td>11.40 (±0.51) a</td>
<td>31.05 (±1.67) b</td>
<td>24.95 (±1.02) c</td>
<td>21.45 (±0.50) d</td>
<td>36.54 (±1.36) c</td>
<td>16.59 (±0.86) a</td>
<td></td>
<td>14.01 (±0.64) b</td>
<td>51.34 (±1.16) c</td>
<td>35.59 (±1.97) d</td>
</tr>
<tr>
<td>(200 mg L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Methylbutanol</td>
<td>17.59 (±0.64) a</td>
<td>28.09 (±1.40) b</td>
<td>32.86 (±1.01) c</td>
<td>28.41 (±0.60) b</td>
<td>32.44 (±1.56) c</td>
<td>13.31 (±0.62) a</td>
<td></td>
<td>11.39 (±0.49) b</td>
<td>42.67 (±0.79) c</td>
<td>31.34 (±1.63) d</td>
</tr>
<tr>
<td>(65 mg L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Methylbutanol</td>
<td>21.09 (±0.99) a</td>
<td>73.27 (±3.44) b</td>
<td>54.05 (±2.10) c</td>
<td>45.62 (±1.05) d</td>
<td>89.42 (±3.84) e</td>
<td>36.00 (±1.31) a</td>
<td></td>
<td>36.30 (±1.97) b</td>
<td>81.81 (±1.62) c</td>
<td>57.43 (±1.60) c</td>
</tr>
<tr>
<td>(70 mg L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Methylbutyl acetate</td>
<td>0.62 (±0.06) a</td>
<td>2.60 (±0.15) b</td>
<td>2.51 (±0.17) b</td>
<td>1.45 (±0.12) c</td>
<td>1.75 (±0.11) d</td>
<td>0.84 (±0.07) a</td>
<td></td>
<td>0.49 (±0.04) b</td>
<td>2.14 (±0.10) c</td>
<td>1.51 (±0.12) d</td>
</tr>
<tr>
<td>(1.2 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Phenylethyl acetate</td>
<td>0.15 (±0.02) a</td>
<td>1.56 (±0.10) b</td>
<td>0.89 (±0.03) c</td>
<td>0.24 (±0.01) a</td>
<td>0.24 (±0.03) a</td>
<td>ND</td>
<td></td>
<td>0.34 (±0.02) b</td>
<td>0.39 (±0.03) c</td>
<td>0.15 (±0.01) d</td>
</tr>
<tr>
<td>(0.3 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>29.30 (±2.42) a</td>
<td>30.83 (±2.03) a</td>
<td>32.90 (±2.08) a</td>
<td>41.42 (±2.72) a</td>
<td>80.15 (±2.72) c</td>
<td>ND</td>
<td></td>
<td>55.40 (±4.60) a</td>
<td>7.49 (±0.83) b</td>
<td>62.84 (±1.62) c</td>
</tr>
<tr>
<td>(30 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>0.40 (±0.02) a</td>
<td>0.10 (±0.01) b</td>
<td>0.48 (±0.03) c</td>
<td>0.42 (±0.05) a</td>
<td>0.38 (±0.02) a</td>
<td>0.58 (±0.06) a</td>
<td></td>
<td>0.06 (±0.01) b</td>
<td>0.33 (±0.03) c</td>
<td>0.36 (±0.05) c</td>
</tr>
<tr>
<td>(0.21 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl octanoate</td>
<td>0.36 (±0.18) a,b</td>
<td>0.20 (±0.04) a,c</td>
<td>0.44 (±0.12) b</td>
<td>0.22 (±0.08) a,c</td>
<td>0.17 (±0.04) c</td>
<td>0.57 (±0.18) a</td>
<td></td>
<td>0.41 (±0.06) b</td>
<td>0.24 (±0.03) c</td>
<td>0.15 (±0.02) c</td>
</tr>
<tr>
<td>(0.5 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl dodecanoate</td>
<td>0.04 (±0.02) a</td>
<td>0.12 (±0.02) b</td>
<td>0.16 (±0.03) b</td>
<td>0.04 (±0.01) a</td>
<td>0.07 (±0.02) a</td>
<td>0.11 (±0.04) a</td>
<td></td>
<td>0.21 (±0.02) b</td>
<td>0.12 (±0.01) a</td>
<td>0.04 (±0.01) c</td>
</tr>
<tr>
<td>(1.5 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**15P**

**25P**
Aroma compounds (15 °P) – acetate esters

3-methylbutyl acetate

2-phenylethyl acetate
Aroma compounds (15 °P) – ethyl esters

Ethyl hexanoate

Ethyl octanoate

© arsheffield

© Première Moisson
Formation of aroma compounds

- Condensation reaction between an alcohol and acyl-CoA
  - Acetate esters
    - *ATF1*
    - *ATF2*
  - Ethyl esters
    - *EEB1*
    - *EHT1*

Pires et al. 2014
Gene copy numbers

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Genes located on chromosome</th>
<th>Hybrid A2</th>
<th>Hybrid B3</th>
<th>Hybrid C4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scer</td>
<td>Seub</td>
<td>Scer</td>
<td>Seub</td>
</tr>
<tr>
<td>II</td>
<td>Sc-\textit{EHT1}</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>IV</td>
<td>Se-\textit{EHT1}</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>VII</td>
<td>Sc-\textit{ATF2}</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>VIII</td>
<td>Se-\textit{ATF2}</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>XV</td>
<td>Sc-\textit{BAT1}</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>XVI</td>
<td>Se-\textit{BAT1}</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

- Estimated from the sequence coverage
- Supported by qPCR analysis
Transcriptional analysis

- Gene expression: information from a gene is used in the synthesis of a functional gene product.
  - Transcription is the first step of gene expression, in which a particular segment of DNA is copied into mRNA.
Transcriptional analysis (TRAC)

- Transcriptional analysis with the aid of affinity capture

Diagram:
- Biotinylated Poly-dT for capture
- Fluorescent probes for detection
- Magnet
- Streptavidin-coated magnetic beads
- Target mRNA
Transcriptional analysis (TRAC)

**Sc-ATF1**
- A81062 (Sc)
- C12902 (Se)
- Hybrid C4
- Hybrid B3
- Hybrid A2

**Se-ATF1**

**3-methylbutyl acetate**

- Beer

15/04/2016
Transcriptional analysis (TRAC)

Sc-EEB1

Se-EEB1

Ethyl hexanoate

mg/L

Beer

15/04/2016
Correlation – Transcription vs Gene Copies

- Positive correlation between gene copy numbers and transcription levels

Pearson correlation: $r = 0.88$
Correlation – Transcription vs Aroma

- Multiple linear regression between maximum transcription levels and aroma concentrations
  - Positive correlation observed for several genes and esters

<table>
<thead>
<tr>
<th>Gene</th>
<th>3-methylbutyl acetate</th>
<th>2-phenylethyl acetate</th>
<th>Gene</th>
<th>Ethyl hexanoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sc-ATF1</td>
<td>NS</td>
<td>NS</td>
<td>Sc-EHT1</td>
<td>NS</td>
</tr>
<tr>
<td>Se-ATF1</td>
<td>$4.8 \cdot 10^{-4}$</td>
<td>$3.1 \cdot 10^{-4}$</td>
<td>Se-EHT1</td>
<td>$-3.8 \cdot 10^{-5}$</td>
</tr>
<tr>
<td>Sc-ATF2</td>
<td>NS</td>
<td>NS</td>
<td>Sc-EEB1</td>
<td>$1.6 \cdot 10^{-5}$</td>
</tr>
<tr>
<td>Se-ATF2</td>
<td>$5.8 \cdot 10^{-4}$</td>
<td>$3.7 \cdot 10^{-4}$</td>
<td>Se-EEB1</td>
<td>NS</td>
</tr>
</tbody>
</table>

3-Methylbutyl acetate (mg/L)
Conclusions

- Physiological properties of hybrid strains can be controlled to some extent with their ploidy and subgenome inheritance.
- We observed an increased fermentation performance and stress tolerance with increased ploidy level.
- Ester formation linked to genome contribution from parent strains.
  - Higher transcription levels of orthologous genes linked to aroma synthesis when present in higher copy numbers.
Take-home message

- Yeast hybrids often **perform better** than the parents
- Create strains with increased flavour diversity
- Endless possibilities for creating new lager yeasts
- No genetic engineering involved
Acknowledgements

VTT:
- Brian Gibson
- Mikko Arvas
- Sirpa Jylhä
- Frederico Magalhães
- Merja Oja
- Virve Vidgren
- Annika Wilhelmson
- Aila Siltala
- Arvi Wilpola
- Eero Mattila

IRCAN:
- Gianni Liti
- Matteo De Chiara
- Jia-Xing Yue

Funding:
- Alfred Kordelin Foundation
- Svenska Kulturfonden
- PBL Brewing Laboratory
- SABMiller
- Academy of Finland
- FP7 Marie-Curie ITN YEASTCELL