Getting started: Adaptation of wine yeast to early fermentation conditions

George van der Merwe
Associated Professor

CCOVI: February 13th, 2013
WINE PRODUCTION

Wine yeast development
  - Molecular response to wine fermentation
  - Molecular response to Icewine fermentation

Microbial wine spoilage
  - Molecular response to sparkling wine (secondary) fermentation
  - Brettanomyces
Wine yeast: *Saccharomyces*
What do yeasts do?

- **DNA**
- **RNA**
- **Proteins**
- **Substrate**
- **EtOH; flavour compounds**
- **Enzymes**
- **Biomass**
- **Nutrients & precursors**

Gene expression
### Growth of *Saccharomyces cerevisiae*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimal growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>25-30°C</td>
</tr>
<tr>
<td>pH</td>
<td>5.0 – 5.5</td>
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<tr>
<td>Ethanol concentration</td>
<td>&lt; 1.4 % v/v</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>Ammonia/Glutamine</td>
</tr>
<tr>
<td>Oxygen</td>
<td>Aerobic</td>
</tr>
<tr>
<td>Water activity</td>
<td>0.998</td>
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</table>
# Impact of vinification on yeast growth

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimal growth</th>
<th>Vinification</th>
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<tbody>
<tr>
<td>Temperature</td>
<td>25-30°C</td>
<td>Variable</td>
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<tr>
<td>pH</td>
<td>5.0</td>
<td>&lt; 3.4</td>
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<tr>
<td>Ethanol concentration</td>
<td>&lt; 1.4 % v/v</td>
<td>Increasing to 11-16 % v/v</td>
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<tr>
<td>Nitrogen</td>
<td>Ammonia/Glutamine</td>
<td>Nitrogen depletion</td>
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<tr>
<td>Oxygen</td>
<td>Aerobic</td>
<td>Anaerobic</td>
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<tr>
<td>Water activity</td>
<td>0.998</td>
<td>Low (0.982-0.939)</td>
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</table>
ADY & inoculation

**Dosage:**
20-30 g/hL of must.

**Rehydration:**
Add 1 kg of yeast to 10/1 of diluted must (±7° B) at 35-38°C. Allow to stand for 10 minutes.

Stir to disperse the yeast and cool to within 10°C of the fermentation temperature by the addition of cold must, before adding to the fermentation.
Typical wine fermentation

High solute concentration
Low pH
Low temperature
Oxygen

In Boulton et al. (1998)

Increasing EtOH concentration

Log of Absorbance = yeast cell density

Fig. 4-11. Fermentation profile of grape juice. Absorbance (○); ethanol (▲); Brix (□).

Log of Absorbance = yeast cell density

In Boulton et al. (1998)
Fermentation stresses inhibit yeast performance

- Temperature
- High acidity
- Osmotic pressure
- Anaerobiosis
- Nutrient changes
- Ethanol tolerance

Growth, protection & survival

Physiological & metabolic adaptation
Adaptation of *S. cerevisiae* to its environment

- Temperature
- High acidity
- Osmotic pressure
- Anaerobiosis
- Nutrient changes
- Ethanol tolerance

Stimulus

Sensing & Signal Transduction

- Transcriptional regulation
- Protein regulation
- Metabolic adaptation

Cellular adaptation
Impact of fermentation stresses

- Slow start
  - Increased lag phase
  - Wine, Icewine & Sparkling wine production
- Inefficient fermentations (stuck/sluggish)
  - Delay in sugar utilization and nutrient uptake; affects product quality
    - Off-flavour production
    - Spoilage organisms
- Winery efficiency
  - Cellar operations suffer; decreased/delayed production
  - Impacts bottom line......$$$

Molecular Cellular Biology

University of Guelph
Measuring the yeast’s response?

- **Product**: DNA → RNA → Proteins
- **Substrate**: Nutrients & precursors
- **Enzymes**: proteins
- **DNA**: Gene expression
- **RNA**: Substrate
- **Proteins**: Substrate → Enzymes
- **Biomass**: Product → EtOH; flavour compounds
- **EtOH; flavour compounds**: Biomass
How can we “see” a protein in a yeast cell?

HXT3

Yeast Chromosome

Tagging cassette

Green Fluorescent Protein
GFP-tagged hexose transporter

Glucose/Fructose

Hxt3

GFP

Glucose/Fructose

Environment

Cytoplasm
**Vid30c & changing nutrient conditions**

**VID/GID genes**
- Vid30 complex (Vid30c)
- Participate in adaptation to changing nutrient conditions
  - Involved in turnover of Hxt3 and Hxt7

Chris Snowdon
Vid30c impacts Hxt7 turnover

Parent *HXT7*-GFP

*vid30c HXT7*-GFP

Nitrogen starvation (h)

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<th>0</th>
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<th>12</th>
<th>24</th>
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<tr>
<td>Parent HXT7-GFP</td>
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<tr>
<td>vid30c HXT7-GFP</td>
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Snowdon et al. (2008) *FEMS Yeast Res* 8:204-216
Vid30c participates in Hxt3 turnover

<table>
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<th>Shift from glucose to EtOH (h)</th>
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<tbody>
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Parent HXT3-GFP

vid30c HXT3-GFP

Snowdon and van der Merwe (2012) *PLoS ONE* 7(12): e50458
**VID/GID genes**
- Vid30 complex (Vid30c)
- Participate in adaptation to changing nutrient conditions
  - Involved in turnover of Hxt3 and Hxt7

**Etp1 complex**
- Etp1 needed for ethanol tolerance
Ethanol tolerance

- 7.5\% (w/v) ethanol considered ethanol stress
  - Impacts membrane fluidity
  - Denatures proteins
  - Greatly decreases cell viability

- Yeast’s response
  - Adjusts membrane fluidity
  - Increase expression of chaperone proteins
    - Induces transcription of HSP genes
ETP1/YHL010c is a novel gene needed for the adaptation of *Saccharomyces cerevisiae* to ethanol

Christopher Snowdon, Ryan Schierholtz, Peter Poliszczuk, Stephanie Hughes & George van der Merwe

Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada

![Image of yeast growth plates](image)

**FEMS Yeast Res. 9 (2009) 372–380**
Etp1 is needed for HSP gene activation

Snowdon et al. (2009)
Etp1 is needed for Hxt3 turnover

<table>
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<tr>
<th>Switch from glucose to ethanol (hours)</th>
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<th>1.5</th>
<th>3</th>
<th>4.5</th>
<th>6</th>
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</table>
| Parent *HXT3*-GFP                    | ![Image](image1)
| *etp1* *HXT3*-GFP                   | ![Image](image2) |

Snowdon *et al.* (2009)
Etp1 function in fermentation....

- Construct homo- and heterozygous mutants of *ETP1* in M2
- Chardonnay fermentations
- Hypothesis:
  - Needed for ethanol tolerance
  - Expects homozygous mutant to ferment well until high levels of ethanol is produced before mutant stops fermenting
    - Function when ethanol levels are high (around 7.5%)
    - Impact *HSP* gene expression later in fermentation
Etp1 is essential for efficient Chardonnay fermentation

Daily Weight Loss (g)

Days

M2

ETP1/etp1

etp1/etp1

Hillier and GvdM (unpublished)
ETP1 needed for early adaptation to Chardonnay fermentation

Hillier and GvdM (in preparation)
Loss of *ETP1* perturbs sugar metabolism during fermentation

* p-value ≤ 0.05

Hillier and GvdM (in preparation)
Loss of *ETP1* perturbs amino acid metabolism during fermentation

* p-value ≤ 0.05

Hillier and GvdM (in preparation)
**ETP1** & transcriptional adaptation to Chardonnay fermentation

Biological triplicates; n = 3

Hillier and GvdM (in preparation)
# genes compared to parent: | 10 hours | 24 hours | 48 hours | 144 hours |
--- | --- | --- | --- | --- |
Higher in *etp1/etp1* | 101 | 376 | 493 | 870 |
Lower in *etp1/etp1* | 142 | 227 | 374 | 635 |
Total genes | 243 | 603 | 867 | 1505 |

All genes: p-value ≤ 0.05; fold-change > 2
Groups of related genes affected by *ETP1* deletion

<table>
<thead>
<tr>
<th># genes in <em>etp1/etp1</em> compared to parent:</th>
<th>10 hours</th>
<th>24 hours</th>
<th>48 hours</th>
<th>144 hours</th>
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<tbody>
<tr>
<td></td>
<td>Up</td>
<td>Down</td>
<td>Up</td>
<td>Down</td>
</tr>
<tr>
<td>Amino acid &amp; Nitrogen metabolism</td>
<td>2</td>
<td>13</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>Cold &amp; anaerobiosis</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>Cell wall</td>
<td>6</td>
<td>1</td>
<td>20</td>
<td>1</td>
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</table>
Yeast cell wall
Yeast cell wall

Baba et al. (1989)

Mannoprotein

β-Glucan & Chitin

Integral membrane protein

Cytoplasm

Periplasmic space

Cell wall

Plasma membrane
Adaptation of S. cerevisiae to its environment

Stimulus

Sensing & Signal Transduction

Temperature
High acidity
Osmotic pressure
Anaerobiosis
Nutrient changes
Ethanol tolerance

Cellular adaptation

Transcriptional regulation
Protein regulation
Metabolic adaptation
Physicochemical factors and wine production: Cold & Anaerobiosis

- Temperature of grape must during fall harvest
  - Cold soak
  - Colder fermentations for white wines

- Oxygen as major threat to wine production
  - Oxidation of flavour compounds
  - Low levels of dissolved oxygen at start of fermentation quickly scavenged by yeast following inoculation
  - “Oxygenation” during wine production
    - Pump-overs; micro-oxygenation; yeast RAPIDLY consumes oxygen during fermentation
Adaptation of yeast to cold and anaerobic conditions

- Impact on plasma membrane
  - Decrease in fluidity; decreased membrane function
  - Inability to produce new membrane lipids in absence of oxygen
  - Alteration to existing lipid composition to increase fluidity and membrane function

- Remodeling of cell wall
  - Alteration of cell wall components and proteins
  - Induced transcription of cell wall mannoprotein genes
    - \textit{DAN/TIR/PAU}
Sensing cell wall stress
# Etp1 impacts gene expression during fermentation

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<tr>
<td></td>
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<tr>
<td>WSC2</td>
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<tr>
<td>WSC3</td>
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<td>.</td>
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<tr>
<td>MID2</td>
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<tr>
<td>M KK1</td>
<td>.</td>
<td>.</td>
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<tr>
<td>SLT2/M PK1</td>
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</tr>
<tr>
<td>CHS2</td>
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<td>CHS7</td>
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<tr>
<td>CRH1</td>
<td>+3.29</td>
<td>+4.16</td>
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<td>YEA4</td>
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<td>RCR1</td>
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<td>KRE6</td>
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<tr>
<td>GAS5</td>
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<td>+3.86</td>
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Hillier and GvdM (in preparation)
Etp1 impacts levels of Mpk1

Hillier and GvdM (in preparation)
Sensing high sugars and anaerobic environments......
Stress-induced production, processing and stability of a seripauperin protein, Pau5p, in *Saccharomyces cerevisiae*

Zongli Luo & Hennie J.J. van Vuuren

Wine Research Centre, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada

Fig. 3. Immunoblotting of cell lysates from wine yeast strain LY15 (chromosomally encoding Pau5-TAP). Cells were collected at various time points as indicated during Chardonnay must fermentations. (a) and (b) are results from two independent fermentations. Loading controls were visualized by staining as described in ‘Materials and methods’.
Functional analyses of PAU genes in *Saccharomyces cerevisiae*

Zongli Luo and Hennie J. J. van Vuuren

Wine Research Centre, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada
The Hog1 Mitogen-Activated Protein Kinase Mediates a Hypoxic Response in Saccharomyces cerevisiae

Mark J. Hickman,* † Dan Spatt* and Fred Winston* †

*Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115 and † Lewis−Sigler Institute and Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544
Etp1 impacts gene expression during fermentation

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</tr>
<tr>
<td>PAU1</td>
<td>.</td>
<td>-9.96</td>
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<td>PAU2</td>
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Hillier and GvdM (in preparation)
Etp1 impacts levels of Hog1

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Hillier and GvdM (in preparation)
Conclusions

- *ETP1* is needed for a normal fermentation to occur
  - Significant impact on transcriptional adaptation process

- *ETP1* deletion affects protein levels of Hog1 and Mpk1 early in fermentation
  - Leads to significant down-regulation in *PAU* gene transcription early in fermentation
  - Cell remodelling genes are mis-regulated

- Etp1 is most likely involved in the ubiquitin-dependent turnover of proteins
  - Specific target(s)?
Acknowledgements

**GvdM lab members**

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Stephanie Hughes
Erik Nielson
Stephanie Hallows
**Peter Poliszczuk**
Nate Ferguson

**Collaborators**

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Barry Shelp (U. of Guelph)
Hennie van Vuuren (UBC)
**Terence van Rooyen (NCTW)**
Hung Lee (U. of Guelph)

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University of Guelph
CFI
NSERC
OMAFRA
Genome Canada
ORF-RE
Etp1 impacts gene expression sulphur metabolic genes

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