How and What to Brew with
*S. eubayanus*

Homebrew Con 2016

Jared Spidel
Saccharomyces spp.

Many species described, but turned out to be divergent strains or hybrids of previously described species

https://archive.org/details/cu31924000078810

Seven known species of Saccharomyces
S. cerevisiae and S. uvarum widely associated with fermentations
S. paradoxus, S. mikatae, S. kudriavzevii, S. arboricola, S. eubayanus never found in association fermented beverages

Kurtzmann, Fell, Boekhout (eds.) The Yeasts, 5th edn, Vol. 2. 2011
Discovery of *S. eubayanus*

Discovered by yeast hunters in search of new *Saccharomyces* species

Found associated with southern beech trees in Patagonia\(^1\), oak and deciduous trees in Tibet and western China\(^2\), North American beech trees in Wisconsin\(^3\), and the forests of North Island New Zealand\(^4\).

Genetic analysis demonstrated *S. eubayanus* is long lost parent of lager yeast *S. pastorianus*.

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Interspecies Hybridization

Domestication sometimes resulted in hybridization between two species.
Mule, wheat, peppermint, grapefruit
Usually sterile and require human cultivation or husbandry
Prior to late 19\textsuperscript{th} century all fermentations were a mixture of yeast strains and species. Under stress, \textit{Saccharomyces} reproduces sexually. Results in diploid (2 sets of chromosomes) hybrids. Can also result in polyploidy hybrids. Over time, resulting from selective pressure, duplicate chromosomes can chimerize via homologous recombination or can be simply deleted.
Yeast Domestication

Since the early 20\textsuperscript{th} century suspected that \textit{S. pastorianus} was not true species – low sporulation, low viability

Mid-1980s genetic analysis demonstrated as a hybrid of \textit{S. cerevisiae} and likely the brewing contaminant \textit{S. bayanus}

In 2011 non-\textit{S. cerevisiae} identified as \textit{S. eubayanus}

\textit{S. bayanus} is a hybrid of \textit{S. cerevisiae}, \textit{S. uvarum}, and \textit{S. eubayanus}

A model of the formation of \textit{S. pastorianus} and the hybrid strains of \textit{S. bayanus}. First, wild \textit{S. eubayanus} and ale-type \textit{S. cerevisiae} hybridized to form an allotetraploid that gave rise to \textit{S. pastorianus}. Second, domestication imposed strong selective pressure for strains with the most desirable brewing properties. Third, in the brewing vats with high densities of \textit{S. pastorianus}, cell lysis releases large DNA fragments that occasionally transform, fourth, contaminating wild strains of \textit{S. eubayanus} because of the lack of pure culture techniques. Fifth, multiple hybridization events with wild strains of \textit{S. uvarum} gave rise to CBS 380T and NBRC 1948. This model does not exclude prior or parallel involvement of \textit{S. uvarum} in brewing or contamination.\textsuperscript{1}

A Brief History of Lager Brewing

In 15th-century Bavaria, brewers began fermenting and storing beer at colder temperatures, and in 1553 summer brewing was banned.

The process of selecting cryotolerant yeast resulted in new strains.

The mixture of microbes in primitive "yeast" contained other Saccharomyces species that mated with S. cerevisiae to create hybrid strains, and under certain conditions these hybrid strains out-competed wild-type S. cerevisiae.

Duke Albrecht V of Bavaria confined brewing between St. Michael’s Day (Sept 29) and St. George’s Day (April 23).
The Silk Road

After discovery in Patagonia, theories about how S. eubayanus traveled to Bavaria Never found in Europe... yet Identification of Chinese/Tibetan strains closer related to S. pastorianus (99.82% vs 99.35% identity to Weihenstephan 34/70)

Travelled to Europe via Silk Road?

Many of the Chinese/Tibetan strains poorly utilize maltose²

Its only chance at survival was hybridizing with S. cerevisiae

Emil Christian Hansen isolated the first lager strain (S. carlsbergensis; CBS1513), and freely distributed it to other breweries.

“One November 12, 1883 the Old Carlsberg Brewery started to use in its production Unterhefe Nr. 1. In 1884 the entire production of 200,000 hl beer was based on pure strains of yeast, as was the almost equal quantity manufactured at the New Carlsberg Brewery of Carl Jacobsen. Within a few years the use of clones of bottom fermenting yeast in beer production became the standard procedure throughout the world. By 1892 Pabst, Schlitz and Anheuser-Busch in North America alone manufactured 2.3 million hl with pure yeast strains as did an additional 50 breweries on that continent.”

Isolating Pure Yeast Cultures

At the turn of the 20th century, Paul Lindner isolated two individual *S. pastorianus* strains. Named Saaz (type 1) and Frohberg (type 2) after their respective regions. Bohemian and Carlsberg breweries adopted Saaz strains, while most other breweries in Denmark, the Netherlands and Germany adopted the Frohberg strains.

Saaz and Frohberg Strains

Saaz strains are more cryo-tolerant and flocculent than Frohberg strains, although less attenuative due to inability to ferment maltotriose.

“[Saaz] lager yeasts showed greater amounts of acetaldehyde (perceived as fruity at these concentrations) whereas the [Frohberg] strain produced far more ethylacetate (pear drops flavor) and also more isoamyl alcohol/acetate (banana flavor).”

Some Quick Genetics

Saaz stain CBS1513 contains 47 total chromosomes (triploid), mostly from S. eubayanus¹

Frohberg strain WS34/70 is tetraploid – basically two diploid S. cerevisiae and S. eubayanus genomes²

Some variability in chromosome number within Frohberg strains³

Mitochondria of both strains derived from S. eubayanus²,⁴

Fermentation by *S. eubayanus*

Comparison of Saaz and Frohberg strains with *S. eubayanus*

Little fermentation by *S. eubayanus* at warm temperatures

Saaz and *S. eubayanus* behave very similarly

Frohberg had high attenuation, but took longer to begin fermentation at colder temperatures

Fermentation (alcohol % by volume) of 15 °P all-malt wort at 22 °C or 10 °C. Strains are the *S. cerevisiae* ale strains (Sc), the *S. eubayanus* type strain (Se), the *S. pastorianus* Frohberg-type lager yeast (Fr) and the *S. pastorianus* Saaz-type lager yeast (Sa)

Fermentation by *S. eubayanus*

No fermentation of maltotriose by Saaz strains or *S. eubayanus*

Saaz strains produced esters ethyl acetate (fruit, solvent), 3-methylbutyl acetate (banana, pear) and ethyl caprylate (apple, aniseed) below taste threshold levels in finished beers (30 mg/L, 1.2 mg/L, and 1 mg/L, respectively)

Frohberg and *S. eubayanus* fermentation flavor profiles very similar, but Saaz and *S. eubayanus* fermentation kinetics very similar

<table>
<thead>
<tr>
<th>Strain</th>
<th>Maltose (g/L)</th>
<th>Maltotriose (g/L)</th>
</tr>
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<tbody>
<tr>
<td>none</td>
<td>15 °P wort</td>
<td>68.5</td>
</tr>
<tr>
<td>Ale</td>
<td>A56 22 °C</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>A56 10 °C</td>
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<tr>
<td></td>
<td>A60 22 °C</td>
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<tr>
<td>Frohberg</td>
<td>A03 10 °C</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>A03 22 °C</td>
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</tr>
<tr>
<td></td>
<td>A15 10 °C</td>
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</tr>
<tr>
<td></td>
<td>A15 22 °C</td>
<td>0.3</td>
</tr>
<tr>
<td>Saaz</td>
<td>A11 10 °C</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>A11 22 °C</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>A12 10 °C</td>
<td>18.1</td>
</tr>
<tr>
<td></td>
<td>A12 22 °C</td>
<td>13.4</td>
</tr>
<tr>
<td><em>S. eubayanus</em></td>
<td>C902 10 °C</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>C902 22 °C</td>
<td>46.9</td>
</tr>
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<table>
<thead>
<tr>
<th>Strain</th>
<th>Ethanol (original)</th>
<th>Acetaldehyde</th>
<th>1-Propanol</th>
<th>2-Methylpropanol (isobutanol)</th>
<th>3-Methylbutanol (isoamyl alcohol)</th>
<th>2-Methylbutanol</th>
<th>2-Phenylethyl alcohol</th>
<th>Ethyl acetate</th>
<th>3-Methylbutyl acetate (isoamyl acetate)</th>
<th>Ethyl caproate (ethyl hexanoate)</th>
<th>Ethyl caprylate (ethyl octanoate)</th>
<th>2-Phenylethyl acetate</th>
<th>Ethyldecanoate</th>
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<tr>
<td>Ale</td>
<td>A56 22 °C</td>
<td>6.1</td>
<td>14</td>
<td>13.5</td>
<td>14.4</td>
<td>26.4</td>
<td>7.8</td>
<td>25.2</td>
<td>1.8</td>
<td>0.1</td>
<td>0.4</td>
<td>0.5</td>
<td>3.4</td>
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<td>A60</td>
<td>22 °C</td>
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<td>99.9</td>
<td>13.5</td>
<td>14.4</td>
<td>26.4</td>
<td>7.8</td>
<td>25.2</td>
<td>1.8</td>
<td>0.1</td>
<td>0.4</td>
<td>0.5</td>
<td>3.4</td>
</tr>
<tr>
<td>Frohberg</td>
<td>A03 10 °C</td>
<td>3.4</td>
<td>0.5</td>
<td>13.5</td>
<td>14.4</td>
<td>26.4</td>
<td>7.8</td>
<td>25.2</td>
<td>1.8</td>
<td>0.1</td>
<td>0.4</td>
<td>0.5</td>
<td>3.4</td>
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<td>A15</td>
<td>22 °C</td>
<td>6.5</td>
<td>32.2</td>
<td>13.5</td>
<td>14.4</td>
<td>26.4</td>
<td>7.8</td>
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<td>0.1</td>
<td>0.4</td>
<td>0.5</td>
<td>3.4</td>
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<tr>
<td>Saaz</td>
<td>A11 10 °C</td>
<td>6.5</td>
<td>65.5</td>
<td>13.5</td>
<td>14.4</td>
<td>26.4</td>
<td>7.8</td>
<td>25.2</td>
<td>1.8</td>
<td>0.1</td>
<td>0.4</td>
<td>0.5</td>
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<td>7.8</td>
<td>25.2</td>
<td>1.8</td>
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<td>0.5</td>
<td>3.4</td>
</tr>
</tbody>
</table>

How to Brew with *S. eubayanus*
S. eubayanus (CBS 12357/PYCC 6148)
Grows well on YPD-agar plates, in YPD medium, and 1.040 wort
Grows similarly at 4 °C (39 °F) & 22 °C (72 °F)
In my hands, density tends to be higher than S. cerevisiae
In 1.040 wort and grown on stir plate at 18–22 °C, S. cerevisiae averages 100-150x10^6 cells/ml, S. eubayanus averages ~300x10^6 cells/ml
**S. eubayanus Flavor Profile**

German Pilsner
High IBU to balance low attenuation
Clean malt and hop profile to really taste the yeast’s flavor profile
Apple/pear esters
Some sharp phenols
Unrefined, muddy flavor

**Eubayanus Pils**

100 % Pilsner Malt
- 66 °C (151 °F), 1 hour

Hops: 40 IBU, 0.78 BU:GU
Sterling: 13.6 IBU @ 60 min, 16.4 IBU @ 20 min, 9.8 IBU @ 10 min

Fermented 10 °C (50 °F), 3 weeks, racked and lagered

SG: 1.051
FG: 1.020
61% attenuation
4.1% ABV

Match these flavors to the best recipe
Collecting Brewing Data

Little information on practical brewing with *S. eubayanus*

Info from lab data using 15 °P (1.061) wort (unhopped?) fermented at 10 °C (50 °F) 1-2 weeks

Analysis on unconditioned beer

Optimal mash temp/time, fermentation temp/time, IBUs, types of malt and hops, etc?

Rather than test one variable at a time (hundreds or thousands of beers), setup brewing competition

The Patagonian Brewing Experience

BUZZ, Stoney Creek, Keystone Hops clubs

Brew anything – except maltose is only fermentable and only yeast is *S. eubayanus*

Transferred yeast to White Labs
Design of Experiments

Test of Means - one factor experiment

Multi-Factor Experiments

- Full Factorial experiment – $2^k$, $3^k$, $4^k$, etc; $k = \# \text{ factors}$
- 10 factors at 2 levels requires 1024 runs
- DOE - fractional factorial designs may be used

Randomization of factor levels between runs

Factors:

- Original Gravity
- Starter – Yes or No
- Starter Type
- All Grain/Extract
- Malt Bill
- Mash Time
- Mash Temp
- IBUs
- Fermentation Time
- Fermentation Temp

Figure 1-1 General model of a process or system.
Data Analysis

Analyzed attenuation output as a function of OG, mash temp, mash time, fermentation temp, IBUs, percentage of specialty malts

Attenuation – mean 63.57%, median 64.86%, standard deviation 11.66%, CI 3.30%

Correlation between increased attenuation and mash temp

No significant correlation between fermentation temp and attenuation

Contrary to published reports good attenuation up to 22 °C (72 °F)
CO₂ May Affect Attenuation

Closed versus open fermentation
Closed – lid with airlock
Open – lid set on top of bucket
After 2 weeks, 10-point difference
Changed “closed” to “open” and next day fermentation restarted
More complete experiments needed

2 weeks at 10 °C (50 °F)
### White Labs Analysis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Detection Threshold</th>
<th>Flavor/Aroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diacetyl</td>
<td>50-100 ppb</td>
<td>butter or butterscotch</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>10-25 ppm</td>
<td>green apples, raw apple skin, bruised apples</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>700 ppm</td>
<td>fusel alcohol, solvent-like</td>
</tr>
<tr>
<td>Isobutanol</td>
<td>200 ppm</td>
<td>fusel alcohol, alcoholic, solvent-like</td>
</tr>
<tr>
<td>Amyl Alcohols</td>
<td>60-80 ppm</td>
<td>vinous, solvent-like</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>33 ppm</td>
<td>fruity with solvent undertones</td>
</tr>
<tr>
<td>Isoamyl Acetate</td>
<td>1-3 ppm</td>
<td>banana</td>
</tr>
<tr>
<td>Ethyl Hexanoate</td>
<td>0.2 ppm</td>
<td>apple like (ripe fresh), aniseed, pineapple, green banana</td>
</tr>
<tr>
<td>Ethyl Octanoate</td>
<td>0.9 ppm</td>
<td>apple, sweet, fruity, waxy, wine, floral, fruity, pineapple, apricot, banana, pear</td>
</tr>
<tr>
<td>Ethyl Butyrate</td>
<td>0.2-0.4 ppm</td>
<td>fruity, juicy fruit, pineapple, cognac, papaya</td>
</tr>
</tbody>
</table>

Dashed lines indicate flavor threshold(s); 1-propanol and isobutanol levels all below threshold.
Flavor Compounds DOE

Analyzed flavor compound outputs as function of OG, fermentation temperature, and fermentation time

Correlation between levels of 1-propanol and OG, ethyl acetate and OG, ethyl acetate and fermentation temp, ethyl butyrate and fermentation temp
What to Brew with *S. eubayanus*
The Patagonia Brewing Experience

Sensory information was gathered through a BJCP-sanctioned competition judged by Certified, National, and Grand Master judges.

Mean score of 27 +/- 6 points and ranged from 13.5 to 39.
Judging Observations/Comments

Grape or white wine aroma and flavor, apple and pear esters, and an artificial berry flavor at times

Phenols to varying degrees

Lots of sulfur during fermentation, can stick around in beer

Biggest criticism was under-attenuation

“clean, crisp, inviting”, “sock funk”, “sour baby puke or rancid feet”, “this beer/yeast combo was not a pleasant experience”

Works well with crystal and lightly roasted malts, pale beers, citrus American hops, Belgian-style

Beware of dark roasted malts, spicy hops
## Winning Recipes

### Eubayanus Brown Porter

**Chris Clair**

- **Batch Size (Gal):** 5.50
- **Original Gravity:** 1.048
- **Final Gravity:** 1.020
- **Anticipated SRM:** 28.4
- **Anticipated IBU:** 29.2
- **Brewhouse Efficiency:** 70%
- **Wort Boil Time:** 90 min
- **Saccharification Rest Temp:** 152°F Time: 60 min
- **Sparge Temp:** 170°F Time: 60 min

**Malts**

- 8.00 lbs. Maris Otter
- 1.00 lbs. Brown Malt
- 0.38 lbs. Chocolate Malt
- 0.38 lbs. Pale Chocolate Malt
- 0.25 lbs. Crystal 120L
- 0.25 lbs. Crystal 60L

**Hops**

- 1.00 oz. Glacier (5.70%) 60 min
- 1.00 oz. Glacier (5.7%) 10 min

Fermented 65°F 6 days, racked and 55°F for 7 days

### Citra IPL/APA

**Steve Groff**

- **Batch Size (Gal):** 7.50
- **Original Gravity:** 1.055
- **Final Gravity:** 1.018
- **Anticipated SRM:** 4.77
- **Anticipated IBU:** 52
- **Brewhouse Efficiency:** 67%
- **Wort Boil Time:** 90 min
- **Protein Rest Temp:** 135°F Time: 20 min
- **Saccharification Rest Temp:** 148°F Time: 40 min
- **Sparge Temp:** 170°F Time: 60 min

**Malts**

- 6.00 lbs. Pilsner Malt
- 10.00 lbs. Vienna Malt
- 3.75 oz. Acidulated Malt

**Hops**

- 1.75 oz. Citra (11%) 30 min
- 1.25 oz. Citra (11%) 10 min
- 1.25 oz. Citra (11%) flameout

Fermented for 15 days at 50°F (India Pale Lager) with a 2-day diacetyl rest at 65°F or 62°F (American Pale Ale)
Recommendations

Keep it simple

Mash low, but not too long
- 145-150°F
- 60-90 min

Keep the roasted malt restrained
- roasted barley clashes with the phenols
- chocolate and Carafa work well

Test different fermentation temperatures
- nuances in ester and phenol production
- Citra IPL (10 °C/50°F) and Citra APA (15.5°C/60°F) had 6-point difference

Add some sugar to dry it out

Choose your hops wisely
- Compensate by increasing the IBUs
- Try citrusy hops, be careful of spicy/herbal

Add another yeast
- *Brettanomyces* flavors pair well with *S. eubayanus* esters and phenols
- A clean *S. cerevisiae* strain can be used to dry out beer without affecting flavor
What to Brew with *S. eubayanus*

Split batch four ways

1. Wyeast 1056
   - Clean with some malt and subtle hops
2. *S. eubayanus*
   - 64% attenuation
   - Yeast dominates, slight malt and hops
3. + sucrose (0.005 points)
   - 68% attenuation
   - Cleaner than #2, less phenolic, more “refined”
   - Preference between 2 & 3 split
4. + *Brettanomyces bruxellensis* (WLP650)
   - 77% attenuation (still slowly fermenting)
   - Work well together, can taste qualities of both yeast

Eubayanus Pale Ale

97.5% Pilsner Malt; 2.5% Crystal 120
- 64 °C (152 °F), 1.5 hour

Hops: 42 IBU, 0.79 BU:GU
- Amarillo Hops
  - 26 IBU @ 60 min, 9.4 IBU @ 10 min, 2.6 IBU @ 5 min
- Azacca Hops
  - 3.5 IBU @ 5 min

1 oz/11 gallons of Amarillo and Azacca steeped for 20 min at flameout

Fermented 7 °C (45 °F), 4 weeks, racked and lagered

SG: 1.052
FG: 1.022
58% attenuation
ABV: 4%
The Future of *S. eubayanus*
Commercial use of *S. eubayanus*

Great for homebrewing, but foresee problems at a commercial scale

Heineken licensed for 195k € + royalties

“When we started working with it, it just died on the spot...” Willem van Waesberghe, master brewer at Heineken

H41 released March 2016 in the Netherlands and Italy

“The new lager has a fuller taste, with spicy notes balanced by subtle fruity hints.”

https://www.youtube.com/watch?v=8hoV2JMgtL4
Yeast Breeding

Workaround for GM brewing yeast
Several groups created novel lager strains by crossing *S. cerevisiae* and *S. eubayanus* 1,2,3,4

Select clones for whatever phenotype you want (attenuation, cold tolerance, fruitiness, phenolics, flocculation, etc)

Difficult getting rid of all phenolic flavor/aroma

Thank You

Brett Baker & Krogerus Kristoffer
White Labs
BUZZ; Stoney Creek Homebrewers (Bryon Martinez); Keystone Hops (Andy Hejl)
Judges (Chris Clair, Dave Houseman, Dave Manning, Bryon Martinez, Bill McGeeney, Mark Prior)
Competition Sponsors - The Yeast Bay and Northern Brewer

www.shantybrewery.com for updates and other musings

NOTE: *S. eubayanus* is not commercially available, and may be obtained with a research license through the Portuguese Yeast Culture Collection (PYCC 6148).