Cultured in Yeast

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Owner/Brewer

BRETTANOMYCES PROJECT

The Brettanomyces Project

Heriot-Watt University, Institute of Brewing and Distilling, Edinburgh Scotland

M.Sc. Brewing and Distilling:

> Masters dissertation: "Pure Culture Fermentation Characteristics of Brettanomyces Yeast Species and Their Use in the Brewing Industry"

Open source research aimed at increasing the knowledge and promoting Brettanomyces yeast.

Research included:

- Lab culturing and storage media
- Propagation
- Primary Fermentation
  - Pitching rate
  - Attenuation
  - Flavor/Aroma compounds

BRETTANOMYCES PROJECT

http://www.brettanomycesproject.com

Brettanomyces/Dekkera

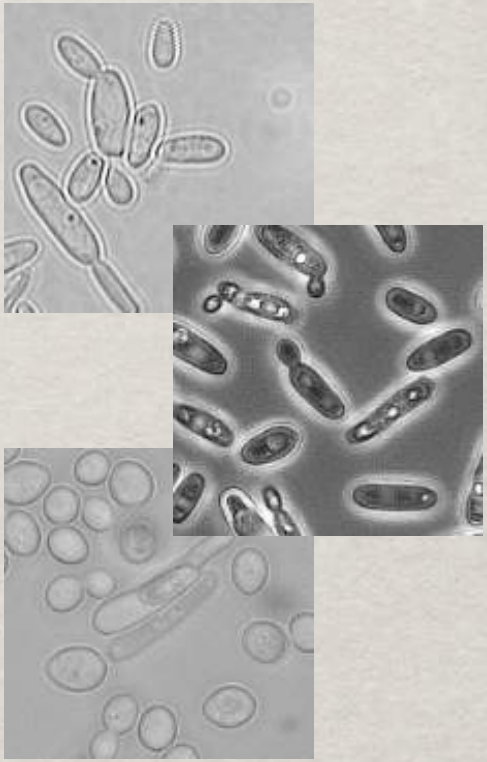
Same organism, nomenclature is more or less interchangeable...

Brettanomyces - the asexual reproducing (budding) form, known as an anamorph

Dekkera - the sexual reproducing form, known as a teleomorph

Original nomenclature:

- Brettanomyces bruxellensis
- Brettanomyces lambicus
- Brettanomyces intermedius
- Brettanomyces custersii
- Brettanomyces claussenii
- Brettanomyces anomalus



Initial Characterization

The earliest published account came from a paper presented to the Institute of Brewing in 1904 by N. Hjelte Claussen.

- He proposed the yeast be called Brettanomyces (British Brewing Fungus), as it was responsible for the secondary fermentation and development of characteristic flavors and aromas of the finest English stock ales.

In 1940 the first systematic investigation was presented by M.T.J. Custers, and his findings on 17 strains of Brettanomyces.

- At that time Custers believed Brettanomyces yeasts were only found in English and Belgian beers,
- We now know it is a yeast that is found around the world and in every known wine making region. NOT a Belgian yeast.. More English if anything..

Custers Thesis

Fermentation of glucose to ethanol occurred more rapidly under aerobic conditions then anaerobic conditions, Custers termed this “negative Pasteur effect”.

This was by no means a complete fermentation by a brewers standards, only a measure of how quickly fermentation or metabolism started and continued under the studies conditions.

Considerable amounts of acetic acid produced during aerobic conditions, while no appreciable amounts were formed during anaerobic conditions.

Custers believed cells slowly became adapted to anaerobic conditions eventually resulting in a normal anaerobic fermentation.

Enzyme Activity

Two unique and important enzymes.

α-glucosidase

- Enzyme capable of hydrolyzing (breaking down) wort dextrins with up to 9 degrees of polymerization, a malto-oligosaccharide containing 9 glucose molecules.
- Enzyme functions by cleaving off a glucose molecule forming the next lower malto-oligosaccharide.
- Both extracellular and intracellular forms of the enzyme are produced by Brettanomyces yeasts.
- Enzyme responsible for the over attenuation observed in Lambic and Sour beers.

Enzyme Activity

β-glucosidase

- Enzyme capable of hydrolyzing multiple sugars and glycosidic compounds including;
  Lactose - milk sugar
- Cellobiose - sugar from cellulose in wood/plant material
- Glycosides - present in various Hops, Fruit and Spices
- Early studies conducted at Guiness in Dublin found strains of Brettanomyces claussenii were able to ferment lactose and cellobiose.
- Research into ethanol production found a Brettanomyces custersii strain that was capable of fermenting cellobiose to ethanol, and exhibited a higher utilization of cellobiose then other Brettanomyces claussenii strains used in the study.
- Present at varying levels in only certain Brettanomyces species.

Flavor & Aroma Compounds

Most significant aspect of Brettanomyces yeasts is their ability to influence the flavor and aroma of beer.

- Many organoleptic descriptors exist including; clove, spicy, horsey, barnyard, smokey, medicinal, band-aide, metallic, cracker biscuit, goat-like, cat piss, apple, floral, tropical fruit and citrus.

- Research from the Wine and Brewing industries have explained a few of these compounds

- Precursor compounds come from raw materials with their production emphasized or limited through brewing techniques.

- Ability to produce various aromatic or flavor compounds varies greatly between strains. Strain Dependent!

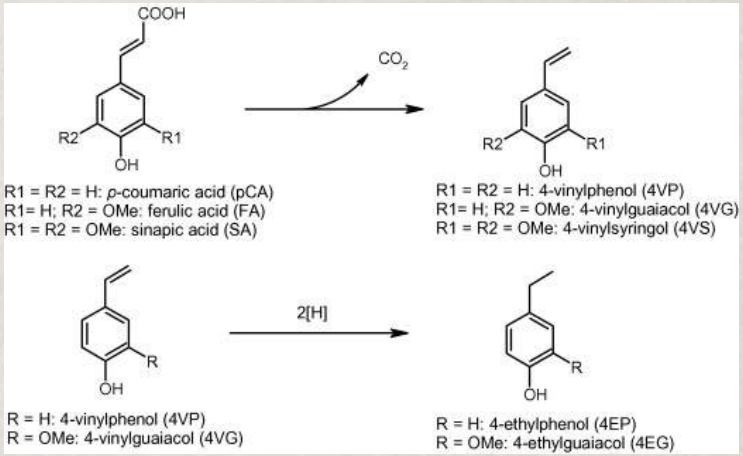
Volatile Phenolics

Responsible for some of the most recognized aromatic characteristics associated with *Brettanomyces*

- 4-vinylguiacol – Clove like
- 4-ethylguiacol – Spicy, Clove

- 4-vinylphenol – Phenol, Plastic, Smokey
- 4-ethylphenol – Spicy, Smoky, Horsy

- 4-vinylcatechol – Plastic, Bitter, Smoky
- 4-ethylcatechol – Band-aide, Medicinal, Barnyard



Appear to have a synergistic or additive effect making their presence observable while the actual compound levels are below recognized threshold levels.

The mechanism responsible for the metabolism of these volatile phenolics is a two enzyme system.

- First is a phenolic (cinnamic) acid decarboxylase,
  - Responsible for the decarboxilation of hydroxycinnamic acids into the respective 4-vinyl derivative

  Present in both *Brettanomyces* and various brewers yeast strains
- Second, a vinyl phenol reductase responsible for the reduction of the 4-vinyl derivative into its respective 4-ethyl derivate.
  - The vinyl reductase enzyme is unique to *Brettanomyces* yeasts

Ester Production

Esterases present in *Brettanomyces* species have ester synthesizing activity with an increase of the following esters observed during the period *Brettanomyces* species dominate as active yeasts in Lambic and Sour beers.

- Ethyl acetate - Fruity, solventy
- Ethyl lactate - Fruity, buttery
- Phenethyl acetate - Rose flower like

-The esterases decrease any isoamyl acetate present.

-High concentrations of C_8 to C_{12} fatty acids accumulate in Lambic as well during the same period.

-These Fatty acids then become esterfied into their respective ethyl esters.

-Tropical fruit/Pineapple like aromas produced by *Brettanomyces* yeasts.

-Unknown which compounds are responsible for these flavors or aromas

-Some people have suggested ethyl lactate, others ethyl butyrate.

-No studies previously looked at a range of esters produced by pure cultures of *Brettanomyces* yeasts because up until recently it has almost exclusively been considered a spoilage organism.

Important Aroma & Flavors

Table 1. Quantitative analysis of 16 standard fermentation compounds analysed from pure culture fermentations conducted with eight strains of *Brettanomyces* yeast. Fermentations were inoculated at a pitching rate of 12×10^6.

Compound	Threshold (mg/l)	Strains							
		WLP645	WLP650	WLP653	CMY001	BSI-Drie	WY5112	WY5526	WY5151
Acetaldehyde	10	1.37	1.21	1.25	1.26	1.98	0.80	1.05	1.16
Ethyl Acetate	30	1.26	3.62	12.25	16.76	35.80*	1.88	8.38	2.68
Ethyl Lactate	250	0.28	0.18	1.81	1.44	1.29	1.72	0.75	0.59
Isobutyl Acetate	1.6	ND	ND	ND	ND	ND	ND	ND	ND
Ethyl Butyrate	0.4	ND	ND	0.04	0.06	0.08	ND	0.05	ND
Isoamyl Acetate	1.6	ND	ND	ND	ND	ND	ND	ND	ND
Ethyl Caproate	0.21	ND	0.16	0.25*	0.39*	0.36*	0.11	0.29*	0.04
Ethyl Caprylate	0.9	0.08	1.65*	4.13*	3.35*	3.00*	0.72	1.34*	0.22
n-Propanol	800	0.59	2.57	4.14	3.72	6.56	0.88	3.01	1.12
Isobutanol	200	0.70	3.83	2.32	7.04	8.00	2.46	3.16	1.63
2-Methylbutanol	65	0.27	5.22	1.09	2.67	2.61	1.31	1.49	0.60
3-Methylbutanol	70	0.91	5.13	3.82	6.71	8.62	2.15	5.39	2.14
4-Vinylphenol	**	ND	ND	ND	ND	ND	ND	ND	ND
4-Vinylguaiacol	0.3	0.0397	0.0529	0.0244	0.0390	0.0458	0.0400	0.0511	0.0849
Diacetyl	0.15	0.012	0.024	0.220*	0.029	0.029	0.056	0.051	0.038
2,3-Pentanedione	0.9	0.003	0.004	0.018	0.004	0.004	0.009	0.003	0.007

ND, Not Detectable; *, Indicates levels at or above threshold; **, No conclusive data available. Fermentations were conducted in duplicate with samples of those fermentations analysed in duplicate. Data shown is the average of all figures attained.

Threshold	**Low / Not Produced**
Ethyl acetate (*only one strain)	Ethyl acetate
Ethyl caproate - C_6 fatty acid ester	Isoamyl acetate
Sweet, Fruity, Pineapple	Higher Alcohols
Ethyl caprylate - C_8 fatty acid ester	Diacetyl
Musty, Pineapple, Fruity, Waxy	Acetaldehyde

Wild vs. Sour

- Brettanomyces does NOT equal sour!
- Historically a “Wild” yeast, similar to the historical terms of Ale and Lager
- Beers which use Brettanomyces can be talked about as a Wild beer given the historical significance of Brettanomyces.
- Without bacteria present the beer can be Wild but is not sour.
- Bacteria and Brettanomyces/wild yeast is also a Wild Beer but the adjective to describe is “Sour”. A more precise term for longer aged more tart Wild beers.

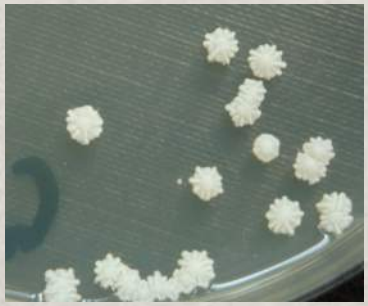
Brewing with Brettanomyces

- Things to take into consideration
  - Fermentation type
    - Primary fermentation
    - Hybrid/Mixed fermentation
    - Secondary fermentation
    - Bottle conditioning
  - Raw Materials
    - Malt / Adjuncts
    - Hops
    - Fruit
  - Brewing Technique
    - To rest or not to rest
    - Sour mashing/wort
    - Rest temp and time
    - Mash thickness
    - Sparge temp

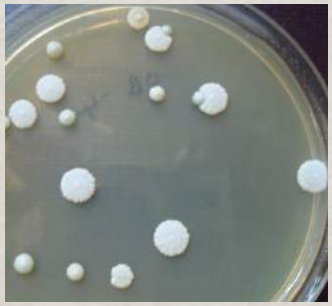
Quality Control

- Use separately marked designated equipment
  - As you retire equipment use it as *Brettanomyces* and sour brewing equipment
- *Brettanomyces* is not a super organism, it is a yeast and can be cleaned and removed like yeast. Heat killing or sterilizing will do the trick
- Recommended media agars

MYPG (Malt extract) agar



WLN agar



$CuSO_4$ agar

Propagation

- I would highly recommend propagating up an active starter if using *Brettanomyces* yeast for primary fermentation.
  - Proper cell counts needed for primary
  - Proper cell physiology will lead to a healthy active fermentation
  - Higher viability and vitality

- Same techniques used to grow *Saccharomyces* can be used with *Brettanomyces*, only more time is needed.
  - 24 hour lag phase
  - 3 days of exponential growth
  - 1-2 day lag or slowed growth
  - 2-3 days of near exponential growth
  - Generally took 7-8 days to reach stationary phase

- **Equipment needed**
  - 1 litre Erlenmeyer flask
  - Sterile breathable foam/film to cover flask (still need the exchange of O_2 and CO_2)
  - Warm environment (80° F is nice)
  - Stir plate will maximize cell counts (give a good swirl daily or often if you don't have a stir plate)
  - Lightly hopped wort of 12°Plato or 1.048 Gravity

Batch Culture Growth Curve

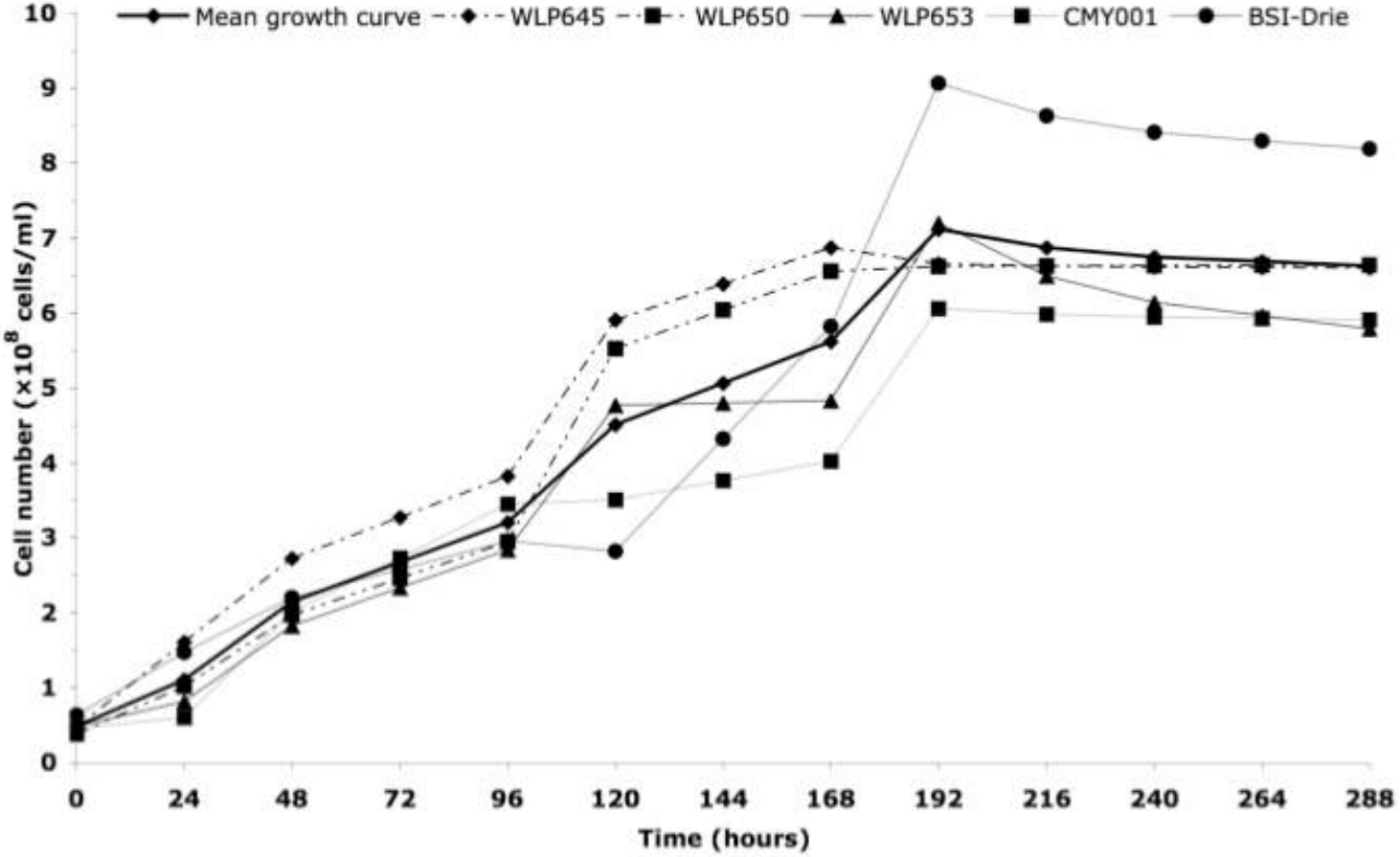
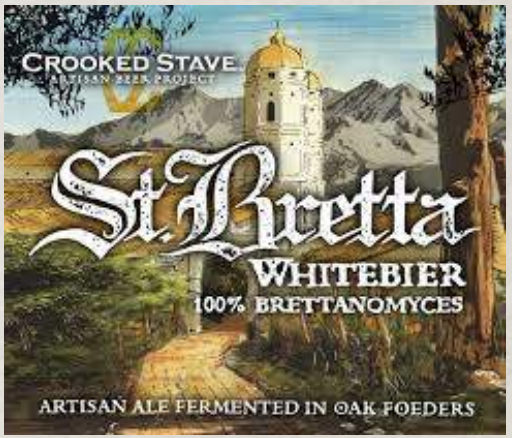
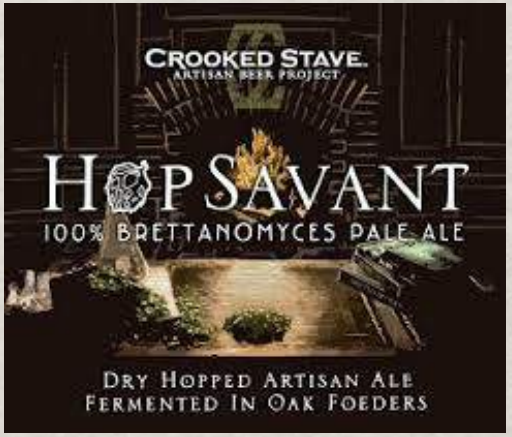


Figure 1. Growth curve for five strains of *Brettanomyces* during semi-aerobic batch culture. Cultures were grown in 500ml of wort substrate over a 288-hour period at 28°C with 80-rpm agitation. Viability was taken into account to reflect the actual cell number.

Production Beers
100% Brettanomyces



St. Bretta - Citrus WildBier



HopSavant - Hoppy Pale

Primary Fermentation

- I knockout at 68°F (20°C) and let fermentation ramp up to 72°F (22°C)
  - I prefer to keep the fermentation around 70°F (20-22°C) for a stable sustained fermentation

- I like to heavily oxygenate at knockout - Always oxygenate!
  - Estimated dissolved oxygen of 12-15 pmm
  - Increases O_2 and increases acetic acid production
  - I like a slight acetic character as at 45-150 ppm acetic is a soft and sweet acid, very complimentary to the beer

- Pitching rate of 1×10^6 cell/ml/°Plato
  - This is the standard rule of thumb pitching rate for ale yeast

- 14°Plato (1.056) beer attenuates down to 2.5°P (1.010) in 7-10 days

- Faster fermentation time when re-pitching with successive generations
- Greater ability to ferment high gravity beers
  - 22°P beer attenuated down to 4°P in 10 days

Production Beers
Barrel-aged Saison

Vieille - Artisanal Saison

Surette - Provision Saison

Brewing Techniques

- Single infusion mash - not sure there is anything to be gained by a step mash.
  - Ferulic acid - pre-curser for 4-vinylguiacol and 4-ethylguaicol
  - Want spicy, smokey, phenolic characteristics?
    - Ferulic acid rest at 43-44°C (109-111 °F) enzymes release ferulic acid and other hydroxycinnamic acids into the mash

- Mash Temp - 65-67°C (150-153°F) adequate conversion with highly modified malts, large starch granule gelatinization.

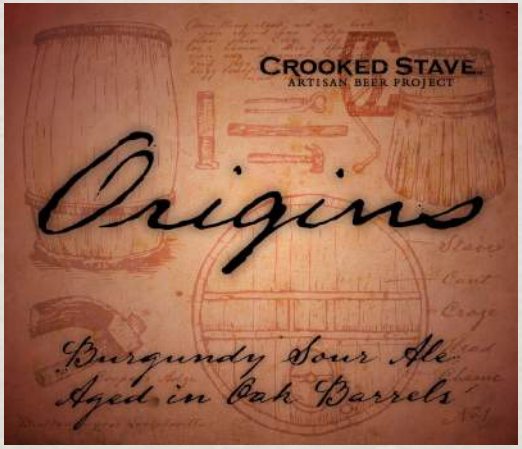
- Mash rest - 30 minutes, enough time for conversion.
  - Re-circ (vorlauf) till solids are cleared

- Mash thickness - I like 2.5:1 liquor to grist ratio (Liters:Kilograms)
  - Too high promotes dryness.

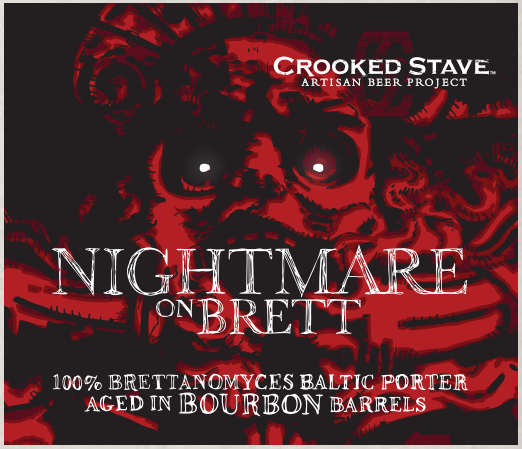
- Sparging - To hot of a sparge temp extracts husk and tannin flavors as well as over sparging.
  - Creates astringency and the possibility for phenolic pre-cursers to be leached into the wort
  - 168°F (75°C) will do the job
  - Final running depends on the beer but don't go below 2-3°P

Production Beers
American Sour/Wild

L'Brett d'Or
Golden Sour



Origins
Burgundy Sour



Nightmare on Brett
Dark Sour

Barrel Cellar Blending

- Bulk beer ages in larger Foeders
- Beers are blended from smaller wine casks
- Cascading system from Brew to Foeders to Barrels to blending and packaging

Bottle Conditoning

- Popular technique to get the Brett character by adding the yeast in the bottling bucket. Need extra sets of all gaskets and porous material on the bottle filler.
  - Best practice is to have a second bottling unit, unless you don't mind all your beers possibly having Brett.

- Can be used to put the final touch on a beer and allow for a light integrated *Brettanomyces* character instead of a Brett bomb.
    - See what 100,000 cells/ml can do to a beer in bottle conditioning.
    - Time to reach the desired character will be strain dependent. New Belgium is typically two weeks, others take 3 months.
    - Careful over time as autolysis occurs in *Saccharomyces,* trehelose is released which is a fermentable sugar for *Brettanomyces* and further carbonation will occur.
    - It has also been suggested that unfiltered beers where there is a large portion of *Saccharomyces* in contact with *Brettanomyces* will produce goaty aromas due to caproic, caprylic and capric acids released from the cell wall of *Saccharomyces*.
        - *Brettanomyces* could turns those acids into esters then over time.
        - Would need to sit on the beer after bottling to develop these esters.

  - Good practice is to let the beer drop bright or filter before secondary unless you want these aromas and flavors.

Questions

Cheers!