

When Good Bugs Go Bad

Detection of Beer Spoiling Microorganisms in a Mixed Fermentation Environment

Kate Steblenko

Jack's Abby Brewing

The Beginning

• Established 2011

- Volunteer staff
- 5,000 sq feet
- 100 BBLs (3,100 gallons)
- Pouring samples only
- \circ Self distributed

• Expanded 2016

- 25 Production employees
- o 65,000 sq feet
- o 20,000 BBLs (620,000 gallons)
- Restaurant opened
- Distribution across New England





Jack's Abby Brewing

Expanding

• Growth 2017

- 30 Production employees
- 120,000 sq feet
- 45,000 BBLs (1,395,000 gallons)
- Springdale Opened

Lab Growth

- \circ 60 sq feet \rightarrow 250 sq feet
- \circ Microscope and flow hood \rightarrow Fully outfitted analytical lab
- \circ 1 employee \rightarrow 3 employees





Sour Beer Culture

The next big thing in beer

• Gaining popularity in craft beer world

- Yeast variety
- Barrel Ageing
- Dedicated festivals
- Sour focused breweries

• Historical styles and new innovation

- Not always intentional
- Product of technology
- Modern "control"





Souring Cultures

Lacid Acid Producing Bacteria

Sour Cultures

Lactic Acid Bacteria

• Lactobacillus species

- L. buchneri, L. brevis, L. delbrueckii, L. rhamnosus and L. plantarum
- Heterofermentative vs homofermentative
 - Homo \rightarrow >90% lactic acid/lactate production
 - Hetero \rightarrow >50% lactic acid/lactate production
 - Acetic Acid
 - Ethanol
- Sugar utilization differs between species
- Creating blends that work together
- Secondary metabolites
 - Esters
 - Fusel alcohols
 - Diacetyl?



Sour Cultures

Lactic Acid Bacteria

Pediococcus species

- P. damnosus, P. claussenii
- \circ 90% carbon utilization \rightarrow Lactic acid/lactate
- Less variation between species
- Alpha/Beta-glucosidase activity
- Secondary metabolites
 - Diacetyl



https://phdinbeer.com/2015/01/30/beer-microbiology-what-is-a-pellicle/



https://homebrew.stackexchange.com/questions/9569/is-this-lactobacillus



Sour Cultures

Wild Yeast

• Brettanomyces species

- Not souring agents
- Funk factor
 - Barnyard
 - Horse blanket
 - Tropical fruit
 - Sweaty
 - Cheese
- Metabolizes byproducts of Lactobacillus and Pediococcus
 - Mutually beneficial relationship
- Can ferment many types of sugar
 - Beta-glucosidase





Detection Methods Simple and Advanced

Wort Stability

Wort before yeast pitching is extremely susceptible to contamination

- Sugar water at pH 5.5 = perfect growth conditions
- Heat exchanger has the most beer contact surface on cold side
- Difficult to manage multiple medias for large scale detection
- Solution Wort Stability Test
 - Collect a sterile sample of wort
 - Allow to sit warm (~70F) for 3 days
 - \circ Observe for turbidity, gas production, signs of life



Bacteria

Hsu's *Lactobacillus* and *Pediococcus* Medium

- Cycloheximide
 - Prevents growth of wild yeast
- Sodium mercaptoacetate
 - Oxygen scavenger
- Cultures grow in comets or snowballs
- Easy to use, no need for an anaerobic incubator
- Detection more than enumeration





Bacteria

Wallerstein Laboratories Differential Medium

• Cycloheximide

Prevents growth of wild yeast

Bromcresol Green

o pH indicator

- Enumeration of bacteria
- Can grow all spoilage organisms, plus sentinel organisms





Bacteria

NBB Broth

- Selective media for lactic acid bacteria
- Based on color change
- Limited information about ingredients





Wild Yeast

Lin's Cupric Sulfate Medium

- Copper Sulfate
- Ammonium Chloride
 - Together suppress culture yeast growth, allowing wild yeast to be seen
- Yeast slurry pureness
- Prevent over attenuation in package





PCR Detection of Spoilers

Rapid detection for same day results

- Invisible Sentinel
- PCR detection of hop resistance
 - horA and horC genes
 - Plasmid encoded
 - Horizontal gene transfer
 - Detection at 10 cells / mL
 - Performed on every single batch of beer in brite tank before package and every single canned batch
- Confirmation of shelf stability before shipping
- Prevents recalls
- Maintain brand image



BrewPal Detection Methods

Cassette System > Gel Electrophoresis



PAL SCORE[™] REFERENCE SHEET



Cassette Analysis

The most stressful part of my day





Developing Sample Points

Evolving with Growth

Sampling Methods

Choosing our points

- New brewery, new techniques, new technologies
- Every new beer contact surface on cold side
 - Heat exchanger
 - Fermentation Vessel
 - Hoses
 - Clamps
 - Gaskets
 - Hard piping
 - Dry hop tank
 - Hops
 - Air
 - Brite tank
 - Centrifuge
 - Final Package
 - Filler



Sampling Methods

Testing the steel

• Production team members check cleaning regimens

ATP Luminometer

- Enzymatic detection
- Results are relative
- Organic soil
- Verification of cleaning

Also used for water testing

- Differences across brewery faucets
- Rinse water



Media Choice

Which samples on which media

• Day 1

- \circ Wild Yeast
 - LCSM swab ~1 million cells
- Beer Spoilers
 - HLP 1 mL
 - WLD 5 mL
 - NBB 500 uL
- Day 8
 - Wild Yeast
 - LCSM swab ~1 million cells
 - Beer Spoilers
 - HLP 1 mL
 - WLD 5 mL
 - NBB 500 uL



Media Choice

Which samples on which media

• Post Dry Hop

- Wild Yeast
 - LCSM swab ~1 million cells
- Beer Spoilers
 - HLP 1 mL
 - WLD 5 mL
 - NBB 500 uL

• Brite Tank

- Beer Spoilers
 - HLP 1 mL
 - WLD filter 30+ mL
 - NBB 500 uL
- BrewPal PCR



Media Choice

Which samples on which media

• Final Product - Can

- Beer Spoilers
 - HLP 1 mL
 - WLD filter 30+ mL
 - NBB 500 uL
- BrewPal PCR

• Final Product - Keg

- Beer Spoilers
 - HLP 1 mL
 - WLD filter 30+ mL
 - NBB 500 uL



Don't Panic

Interpreting Media

- Something is bound to grow eventually
- Legitimate Concern vs Human Error
 - Multiple media types must have growth
 - Growth must be repeatable
 - Similar bacterial load seen across media
 - BrewPal colony check
 - Gram Stain of colony
 - Catalase test
 - Oxidase test

Process audit

 Sentinel microbes indicate a gap in our cleaning or testing process







Why it's all worth it

A whole new world of beer





Questions?

Please use the microphone indicated so our recording includes audio of your question

For further information, please contact

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