When Good Bugs Go Bad
Detection of Beer Spoiling Microorganisms in a Mixed Fermentation Environment
Jack’s Abby Brewing
The Beginning

- **Established 2011**
  - Volunteer staff
  - 5,000 sq feet
  - 100 BBLs (3,100 gallons)
  - Pouring samples only
  - Self distributed

- **Expanded 2016**
  - 25 Production employees
  - 65,000 sq feet
  - 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
  ○ 60 sq feet → 250 sq feet
  ○ Microscope and flow hood → Fully outfitted analytical lab
  ○ 1 employee → 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

Lactic 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 → >90% lactic acid/lactate production
    - Hetero → >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. clausenii*
  - 90% carbon utilization → 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
Detection Methods
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
  - Observe for turbidity, gas production, signs of life
Detection Methods

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**
Detection Methods

Bacteria

Wallerstein Laboratories Differential Medium

● Cycloheximide
  ○ Prevents growth of wild yeast
● Brom cresol Green
  ○ pH indicator
● Enumeration of bacteria
● Can grow all spoilage organisms, plus sentinel organisms
Detection Methods

Bacteria

NBB Broth

- Selective media for lactic acid bacteria
- Based on color change
- Limited information about ingredients
Detection Methods
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
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
  ○ 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
Tracking Issues
Mapping the beer

- Largest concern is contaminated yeast slurry
- Trace yeast throughout brewery
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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