WHEN YEAST ATTACK

THE STORY OF BELL’S AND SACCHAROMYCETES CEREVISIAE VAR. DIASTATICUS

Craft Brewers Conference
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Bell’s Brewery
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Disclaimer

This is a story of our journey in discovering a problem and the measures we took to improve it.

We do not know the whole story, but look forward to continuing to shine a light on this problem for the betterment of our industry.
Winter 2013

- Conducting routine 1 month carb check on a batch of Winter White Ale (WW)
- Out of spec high carbonation reading
- Out of spec high ABV
Winter 2013

• Instrument working correctly
• Data mining
  • Carbonation and ABV values at time of package
• Inventory
What now?

• Plated samples
  • UBA, SDA, HLP, NBB, LCSM, WLN
• Sent samples out for sequencing analysis
• Tested more samples for CO2 and ABV
Kevin Verstrepen's Lab
Anecdotes come out

Has this always been a problem?
THE RESULTS ARE IN!
Saccharomyces cerevisiae var. diastaticus
Saccharomyces cerevisiae var. diastaticus

- Contains genes for glucoamylase production
- Can break down starches and dextrins into fermentable sugars
- Can cause phenolic off flavors
- Can increase CO2 and ABV
- Can over-pressurize containers
- Can grow on LCSM, LWYM, and dextrin media
- Can be detected by PCR
- Looks like our house yeast
Lin’s Cupric Sulfate Media (LCSM)

• Had stopped using LCSM for WW Belgian yeast due to high level of growth on plates
• We plated this on LCSM and did see growth, this was sent to Belgium
• Some literature states LCSM is for NON-SACCHAROMYCES wild yeast
We set to work!

- Looked into several batches of WW that had gone to bottle
  - Cold- and warm-stored
- 7/12 batches showed increases in ABV and CO2
- Thus, problem was not completely widespread
Where did it come from?

- Could not conclusively say where the infection came from
- At the time, we did not have great tools for detecting *diastaticus*
- This sent us into a panic and we proposed to hire a trained microbiologist
Winter 2014
#polar vortex
THE PCR! 
Polymerase Chain Reaction
The Details

• Had the PCR, now we needed to find a way to detect *Diastaticus*

• Had to find a kit, incubation media, incubation time, and dilutions

• Create SOPs

• This takes time
PCR DATA

Amplification Curves

Fluorescence (485-530)

M4

PC

NC

Cycles
LIFE LESSONS

• Some tanks are infected and others are not
• We obtained *diastaticus* positive and negative results from the same tank
  • My theory: very low concentration and flocculation
• We test ABV a lot in tank and saw no change
• We don’t know where the infection
REACTION PLAN

• We care about our Beer Lover’s experiences
• Release plan
  • What, where, when?
• Huge increase in lab inventory samples for
Long Term Analysis Plan

• Gather a lot of samples (4 cases/run)
• 2 cases kept cold, 2 cases kept warm
• Test a bottle for CO2 and ABV every two weeks from every batch
• Validate CIPs
• Spot check other brands from the same time frame
Results

Days Post Package

ABV
Results

Days Post Package

Volumes of CO₂ in Package
Results

- Warm stored batches with zero positive PCR hits did not change
- Warm stored batches with vacillating PCR hits did change
  - Some batches went outside of government compliance for ABV
  - Some even increased CO2 beyond glass manufacturer’s spec for pressure
- Cold-store packages were stable, regardless of PCR results
Recovery

• We issued a recovery for beer that had not been sold at the end of the season, this was expensive

• It's always cheaper to fix or stop the problem in house
  • Dr. Luke: A pint of prevention is worth a barrel of cure

• We vowed this would not be a problem in 2015

• What would a recovery or recall do to your brewery?
BELL'S

Winter White Ale
2014
FALL 2015

BRACE YOURSELVES

WHITE

WINTER IS COMING
Preparation for next WW season

• **Goal: have WW be *diastaticus* free**

• Ordered a *diastaticus* type strain

• Started with a new streaked out and purified WW yeast culture

• Moved yeast propagation in house

• Brought Carlsberg flask back into use
We Bought

- -80°C freezer
- Lots of flasks
- More stir plates
- More stir bars
- Accessories for the Carlsberg flask
- A lot more PCR kits
- Media
<table>
<thead>
<tr>
<th>Day</th>
<th>Step</th>
<th>Media / Vessel</th>
<th>QC</th>
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<tbody>
<tr>
<td></td>
<td>Cryogenic Freezer Culture</td>
<td>1.8mL cryotube</td>
<td>Occasional checks for purity and viability</td>
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<tr>
<td></td>
<td>MYPG broth</td>
<td>5mL MYPG broth</td>
<td>Traditional micro/visual observation</td>
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<tr>
<td>Day 1</td>
<td>Working Stock Plate</td>
<td>100 x 15mm MYPG petri dish (MYPGA)</td>
<td>Homogenous colony morphology/ direct microscopy</td>
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<tr>
<td>Day 3</td>
<td>MYPG broth</td>
<td>single 5mL MYPG broth</td>
<td>Traditional micro/visual observation</td>
</tr>
<tr>
<td>Day 5</td>
<td>MYPG broth</td>
<td>multiple 8mL MYPG broth</td>
<td>Traditional micro/visual observation</td>
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<td>Day 7</td>
<td>MYPG broth</td>
<td>50mL MYPG broth on shaker</td>
<td>Traditional micro/visual observation/PCR screen if desired</td>
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<td>Day 9</td>
<td>MYPG broth</td>
<td>500mL MYPG broth</td>
<td>Traditional micro/visual observation/PCR</td>
</tr>
<tr>
<td>Day 11</td>
<td>MYPG broth</td>
<td>1500mL MYPG broth</td>
<td>Traditional micro/visual observation/PCR</td>
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<tr>
<td>Day 13</td>
<td>Carlsberg Flasc</td>
<td>20L wort</td>
<td>Traditional micro/visual observation/PCR</td>
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<td>Day 15</td>
<td>Yeast Prop</td>
<td>7 bbl wort</td>
<td>Traditional micro/visual observation/PCR</td>
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<td>Day 17</td>
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WHAT CAN YOU DO?
What can you do?

- Have a warm (~room temp) and cold-stored inventory
- Know your numbers on the day of package
- Know your specifications for CO2 and ABV in package?
- Decide how often you want to check on your packages
  - 1 month, 3 month, end of shelf life?
- Develop a reaction plan before crisis begins
What can you do?

- Conduct routine maintenance on key instruments
- Establish positive and negative controls and clear-cut reaction plans
  - Keep records, build control charts, and review them
- Operator to operator variation
- Data management
  - Yeast tree data
  - Historical data for trending and further understanding
What can you do?

• Know what tools you have at your disposal
  • Internally
  • Externally: Contract labs, universities

• When in doubt, send it out

• Work with your yeast supplier
  • Check COAs
  • Test your starters
What can you do?

• Take as much control of the process as you can
• Start checking early and frequently
• Do an audit
  • Different yeast strains
If you have found a problem?

- Find a way to verify
- Contain the problem
- Pasteurize
  - Validate pasteurization

- Sterile filter
  - Validate
  - Dump 😞
  - Validate your CIPs
  - Plating rinse water
  - ATP
THE LOOK I GIVE
WHEN I'M COMPLETELY OVERWHELMED
KEEP SHARING YOUR STORY
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THANK YOU!
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