Flavor and Freshness

Micro in the Growing Brewery:
A Case Study of Mash & Grind

The Bruery: Orange County, CA

ABV: 13.7%
AE: 1.9
pH: 4.6
BU: 35

Mash & Grind Process

Stainless Steel Fermented Mash

Transferred for Barrel Aging – 8mo to 1yr

Whole Coffee Beans Ground Through a Commercial Coffee Grinder

Coffee Grounds are Collected into a Bucket Lined with a Mesh Bag

Mesh Bag is Cinched and Transferred to the Bright Tank

Barrel Aged Beer is Racked on Top of Ingredients

No Filtration, No Pasteurization, No Sterile Filtration

Beer is Monitored for Flavor and Then Packaged

The Original Micro Quality Program

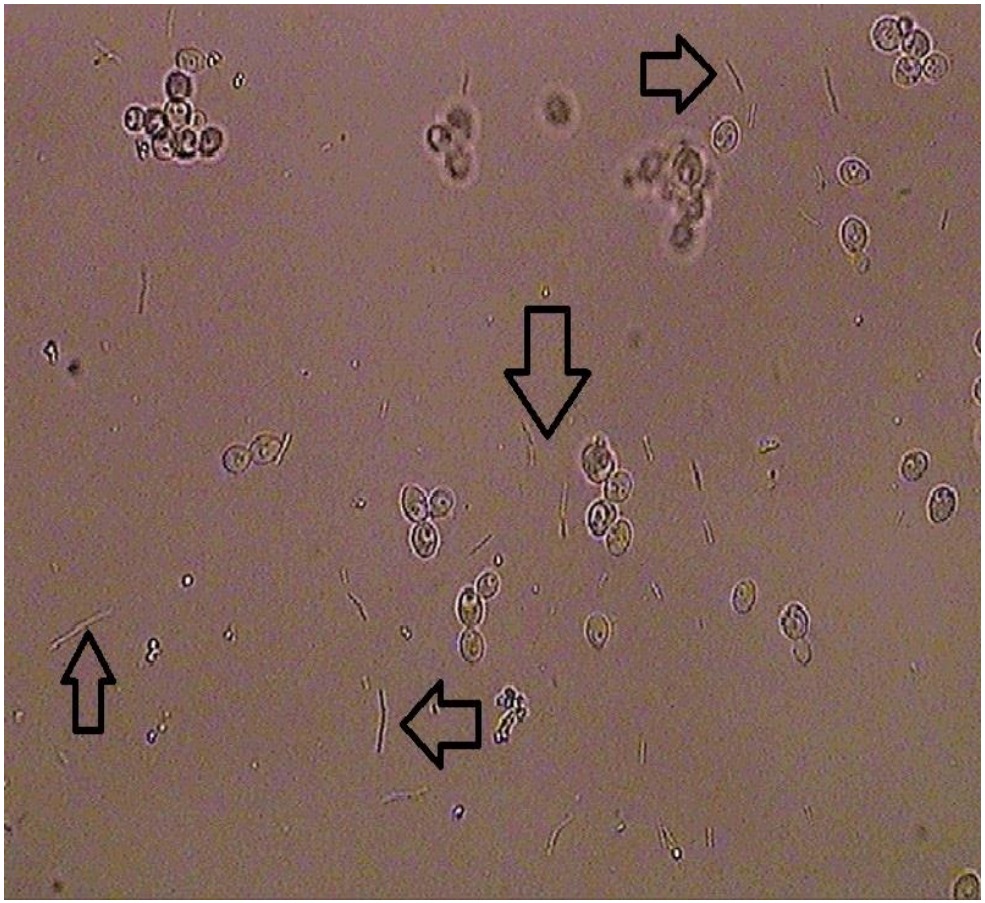
- Stainless Steel Fermentations, Bright Beer, Bottles & Kegs
  - Anaerobic Incubation on BMB with cyclohexlimide @ 28°C
  - Aerobic Incubation on mUBA with cyclohexlimide @ 28°C
- Barrel Aged Beer
  - Anaerobic Incubation on BMB with cyclohexlimide @ 28°C

The Micro Quality - Original Plan

- ✓ **Propagation Checks**
  - ✓ Cell Counts, Viability, pH, Gravity
- ✓ **Primary Fermentation Checks**
  - ✓ ATP testing on CIP rinse water
  - ✓ Fermenter Full
  - ✓ End of Fermentation
  - ✓ pH monitoring
- ✓ **Finished Aging Barrel Aged Beer Checks**
  - ✓ Micro-filtration plates on every barrel
  - ✓ pH on every barrel
- • **Bright Beer Checks**
  - ✓ ATP testing on CIP rinse water
  - X Finished Beer plates
- X **Bottled Beer Checks**

Lactobacillus Among the Yeast

- Bright Tank and Bottles Anaerobic Plates **did not** have Distinct Colony Growth
- Microscopic Examination **did** Reveal Potential Lactobacillus



The Result: Sour Mash & Grind

- With in 2 Weeks the Flavor Changed in the Following Ways:
  - pH Dropped from 4.6 to 3.9
  - L-lactic acid content increased from 0 g/L to 1.6g/L
  - Overall not Drinkable

So What Now? - Corrective Action Plan

- In the Growing Brewery Production Demands Often Out Grow Process Procedures

  Processes To Consider:

  - Why Did We Not See the Beer Spoiler in the Barrel Micro Plates?
  - Barrel Treatment and Handling
  - Barrel Filling
  - Ingredient Handling
  - Processing Equipment SOPs
  - Treatment of Processed Ingredients
  - Final Checks on Finished Beer

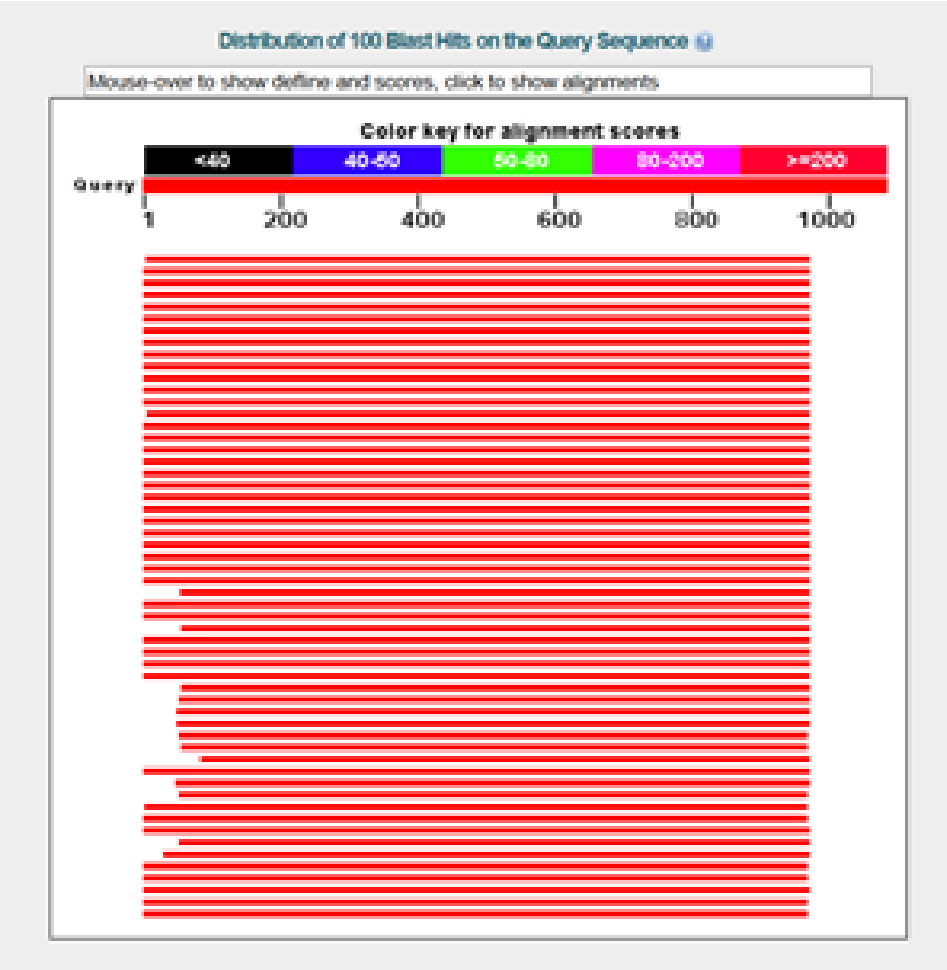
Growing the Problem

- **Barrel Testing Did Not Reveal the Microbes Until the Beer was Racked into the Bright Tank**
  - What Type of Spoiler Was I Combating?
  - Why is it Important to Know ?
    - Determine Mediums to Use, Incubation Times and Temperatures
    - Narrowing Down Areas to Troubleshoot

DNA Sequencing

- Isolate the bacteria
  - Can take several iterations of plate streaking
- 3rd party GeneWiz
  - Very Affordable
  - 16s rRNA bacteria sequencing
- BLAST Search

  http://blast.ncbi.nlm.nih.gov/Blast.cgi



DNA Forward & Reverse

Sequences producing significant alignments:							
Select for downloading or viewing reports	Description	Max score	Total score	Query cover	E value	Ident	Accession
1Select seq ref|NR_117073.1|	Lactobacillus acetotolerans strain JCM 3825 16S ribosomal RNA gene, partial sequence	1796	1796	100%	0.0	98%	NR_117073.1
2Select seq ref|NR_112683.1|	Lactobacillus acetotolerans strain JCM 3825 16S ribosomal RNA gene, partial sequence	1796	1796	100%	0.0	98%	NR_112683.1
3Select seq ref|NR_044699.2|	Lactobacillus acetotolerans strain DSM 20749 16S ribosomal RNA gene, complete sequence	1760	1760	100%	0.0	97%	NR_044699.2
4Select seq ref|NR_117071.1|	Lactobacillus intestinalis strain TH4 16S ribosomal RNA gene, partial sequence	1587	1587	94%	0.0	96%	NR_117071.1

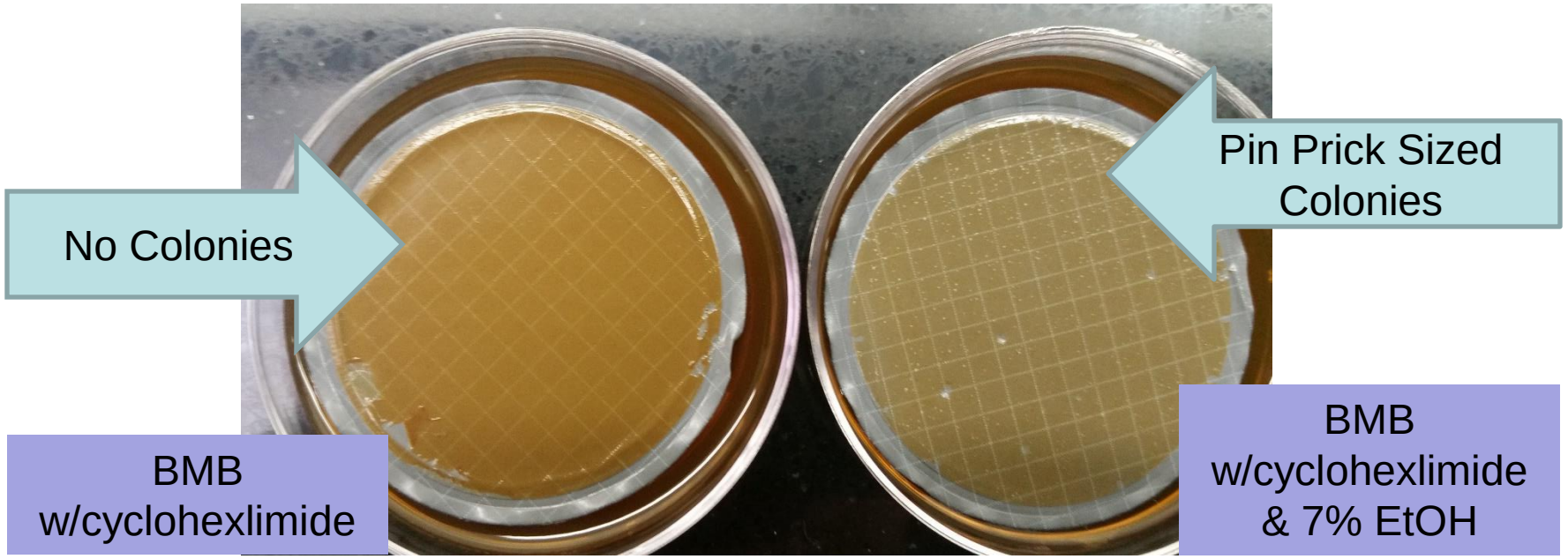
Not Your Typical Beer Spoiler

- *Lactobacillus Acetotolerans*
  - Light turbidity and acetic acid production
  - Vinegar loving, growth potential as low as 3.2 pH
  - Found in rice vinegar
  - Facultative Anaerobe
  - Gram Variable
  - Catalase negative

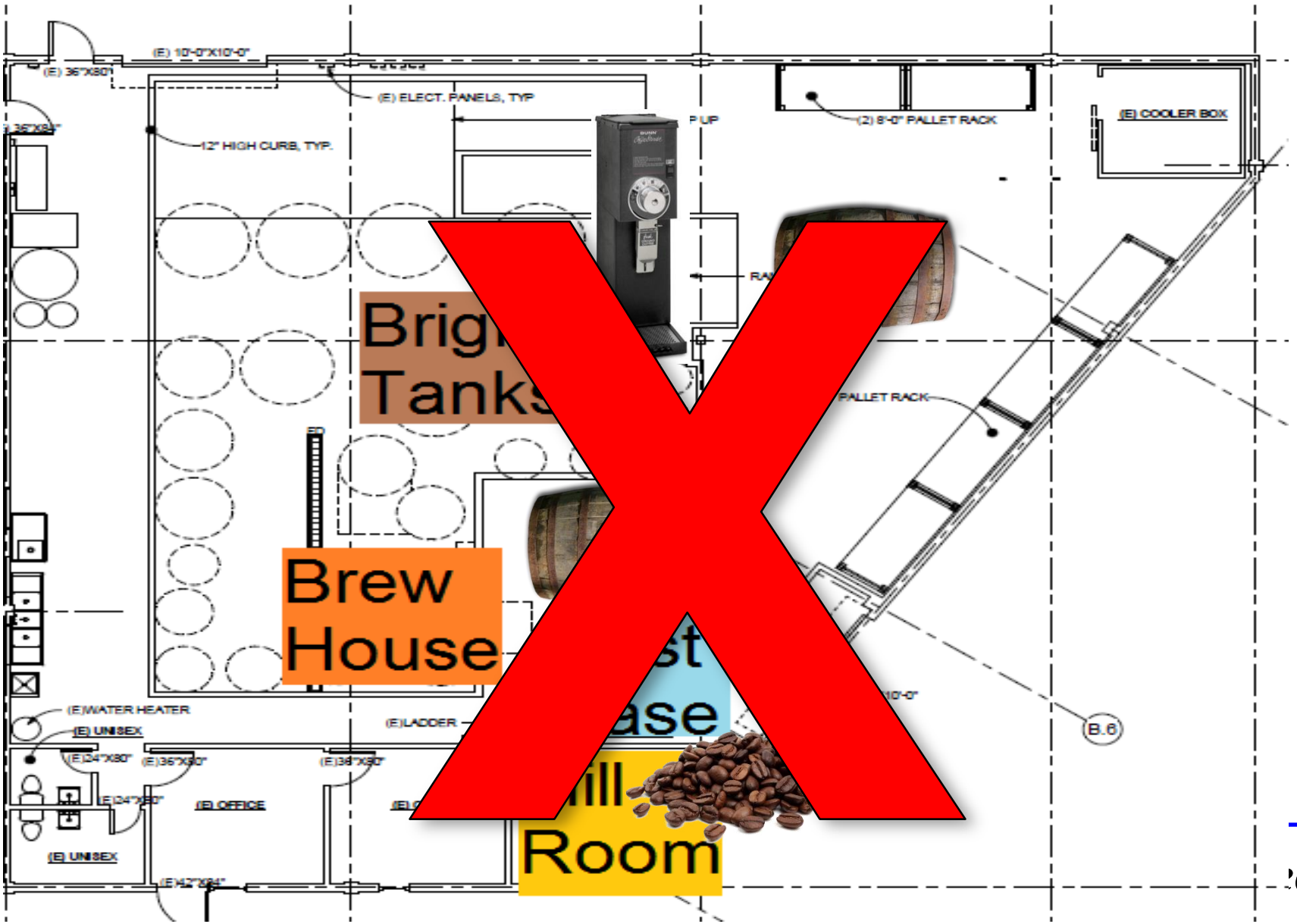
- *Lactobacillus Homohiochii*
  - Light turbidity and acidification
  - Sake spoiler, growth potential at 25% ABV
  - Found on rice
  - Facultative Anaerobe
  - Gram Variable
  - Catalase negative Anaerobic Conditions, Catalase positive Aerobic Conditions

Supplemented Medium = Better Results

- Growing Lactobacillus Homohiochii
  - BMB cyclohexlimide plates supplemented with 7% EtOH
- Growing Lactobacillus Acetotolerans
  - BMB cyclohexlimide plates pH adjusted to 3.5



Where is it Coming From?



Rice Was the Common Denominator

Rice Hulls

Rice Flakes

Second Try: Mash & Grind V2

The 1st batch of Mash & Grind was not distributed.
However, There Was a Strong Need to Release This Beer

- Making Mash & Grind Version 2 Free of Beer Spoilers Required:
  - Micro Testing Another Set of Barrels
  - Standard Operating Procedures:
    - Ingredients – Storage & Handling
    - Processing Equipment - Use and Maintenance
    - Adding Ingredients to the Bright Tank
    - How to Treat Ingredients Before Additions are Made to the Bright Tank

New Set of Barrels for V2

- New Micro Testing Plating Regime Yields:
  - 3 Barrels were Kicked Out of the Blend due to Detection of *L. homohiochii*
  - Originally Kicked Out NO Barrels

The Grind: Ingredient Handling

- Process Equipment

- Post Fermentation Ingredient Storage and Handling

Ingredient Treatment

- Options for Post-Fermentation Additions:
  - Heat Sterilizing
  - Chemical Treatment – Sanitizing agent
  - Ozone
- Quick movement of ingredients
- Positive Pressure

The Pay Off:

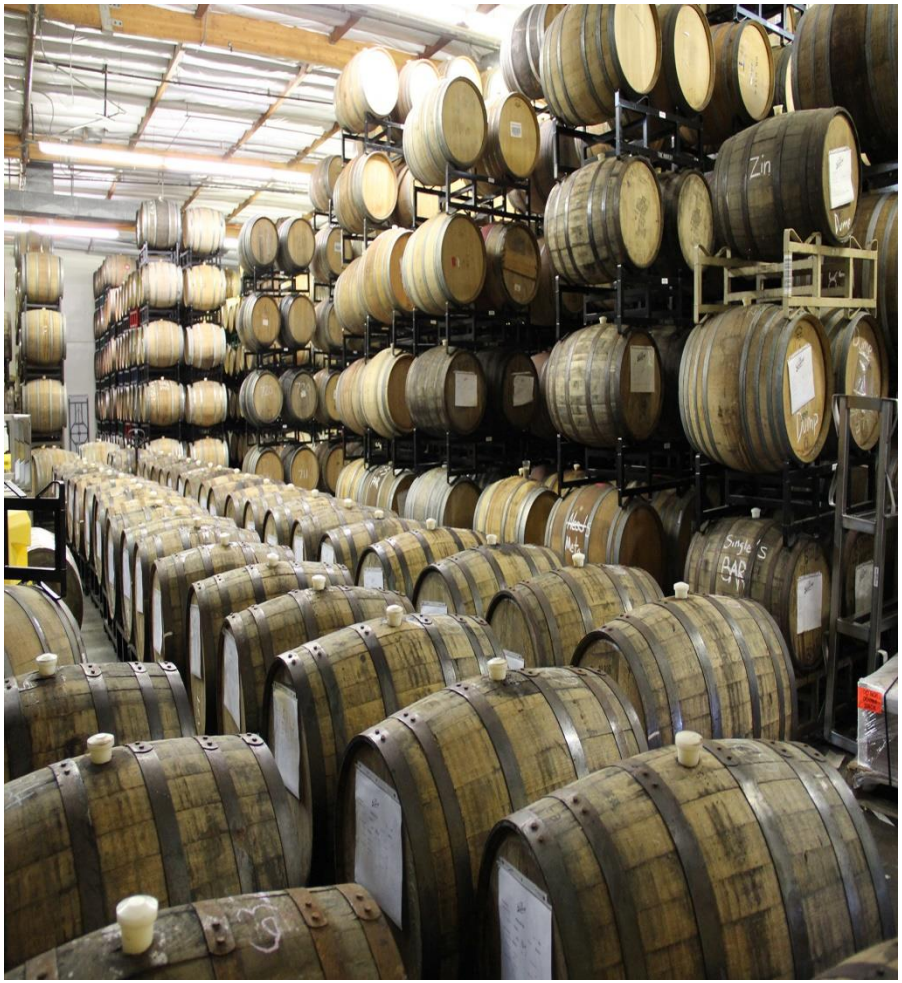
Clean Flavor Fresh
Mash & Grind

Additional Lessons Learned:

- More SOPs to Follow:
  - New to Us Barrel Treatment
  - Barrel Filling (Barreling Out)
  - Barrel Racking
  - More Lab Testing Barreled Beer Testing at 2-3 Months

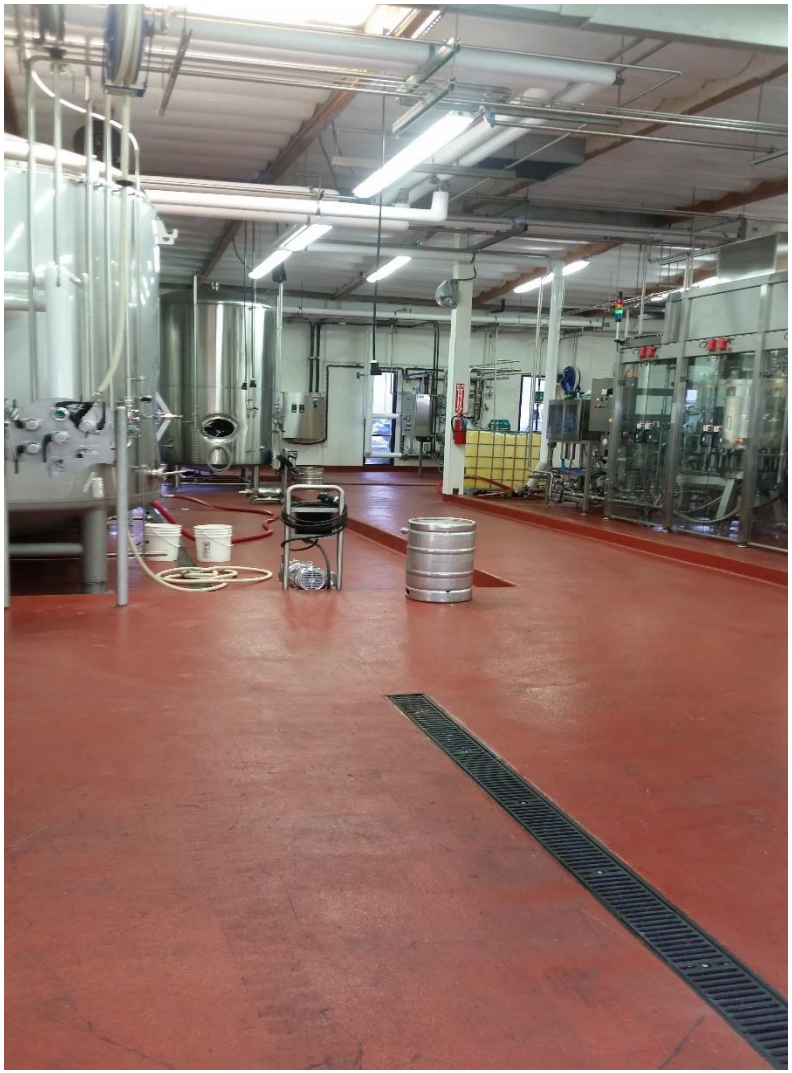
New To Us Barrels

- Not All Barrels are Created Equal
  - Pressure Washing
  - Keeping the Barrels Moist
  - Ozone With In a 24hr Window of Being Filled



Barrel Filling

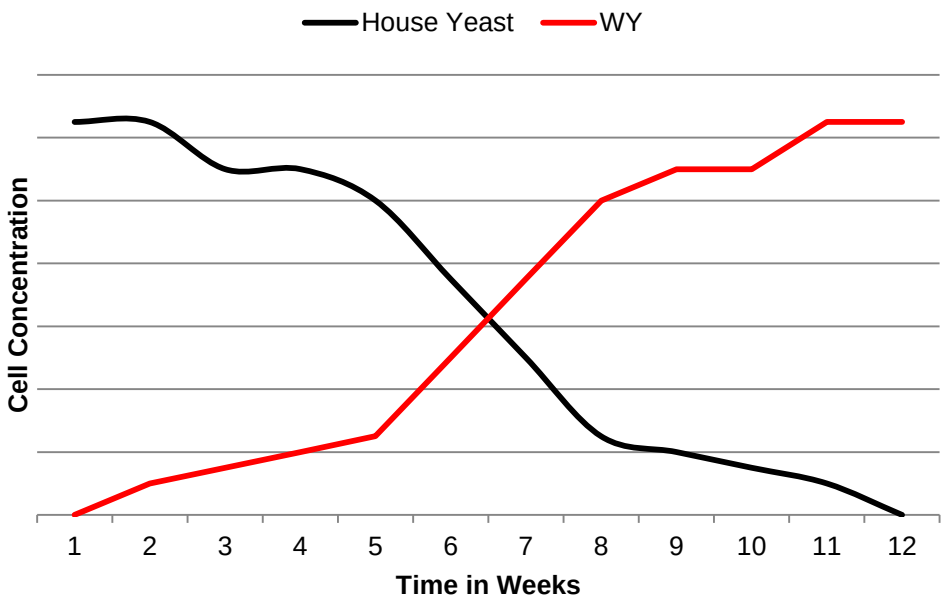
- Location, Location, Location
  - New Packaging Hall



Barrel Racking

- Leaving Behind The Sediment

Barrel Aging: 1st Few Months



- Test every single barrel at 2-3 months
  - Micro filtration plating
    - BMB with cyclohexlimide only
  - pH

What to Do With Wild Yeast

- Important, Why?
  - Avoiding gushing
  - Off-Flavor Development Long Term
- What we Test For
  - CO_2 - Fermentation tubes
  - Survival - Anaerobic force attenuation

Mash & Grind: New Process

Propagation & Fermentation Monitoring

Stainless Steel Fermented Mash

Micro Testing at 2-3 Months

Transferred for Barrel Aging – 8mo to 1yr

Micro Testing Before Racking

Barrel Aged Beer is Racked on Top of Ingredients

Quick Movement of Ingredients to a BBT under Slight Positive Pressure

QC on Finished Beer

Beer is Monitored for Flavor and Then Packaged

Ingredient Handling and Containment

Whole Coffee Beans Ground Through a Commercial Coffee Grinder

Process Equipment Use & Maintenance

Coffee Grounds are Collected into a Bucket Lined with a Mesh Bag

Use of a Sanitizing Agent

Mesh Bag is Cinched and Transferred to the Bright Tank

Avoid the Sour Mash & Grind Experience

- Pro-Active Approach
  - Space Requirements & Layout
  - Understand the Ingredients in Use
  - Anticipate Process Loop Holes
  - Plating Methods
  - Get Quality Involved Early

If You Find Yourself in a Micro Dilemma

- Dig Deep
  - Understand what microflora you are combating
  - Pick Apart the Process, Find its Weaknesses
  - Get a Quality Perspective
  - Ask for Help

Thank You!

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References

- **Entani, Etsuzo, Masaand, Hiroshi and Suzuk, Ken-Ichiro.** 1986. Lactobacillus acetotolerans, a New Species from Fermented Vinegar Broth Nakano Biochemical Research Institute, Nakano Vinegar Co., Ltd., Handa-shi, Aichi 475,' and Japan Collection of Microorganisms, RIKEN, Wako-shi, Saitama 351 -01 ,2 Japan
- **Suzuki, Koji, Asano, Shizuka, Iijima, Kazumaru and Kitamoto, Katsuhiko.** 2008. Sake and Beer Spoilage Lactic Acid Bacteria – A Review, J. Inst. Brew. 114(3), 209–223.