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# Get Hard With Boochcraft

KombuchaKon 2020





## You all know Kombucha...

Kombucha tea is a slightly sweet/acidic fermented beverage made with an aqueous extract of tea leaves (fermented) utilizing a symbiotic culture of bacteria and yeast (SCOBY or pellicle). Kombucha typically has a pH range of 2.5 to 3.5. The fermentation produces a beverage with some natural carbonation, organic acids, nutrients in natural form and trace amounts of alcohol. Variations in the base solution and optional ingredients, as well as further possible fermentation processes provide a variety of related similar products with different flavor profiles. Sanctioned variations are listed in this standard.



**So...**

**Let's talk about how to get it  
hard!**

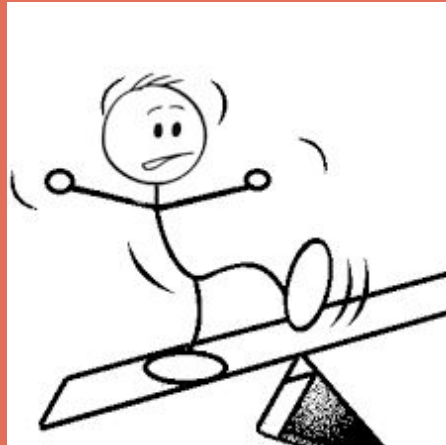
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# How do you get it harder?

- Kombucha is all about balance
- You need to disrupt that balance, but calculated at the proper moment
  - I.e. add yeast to your ferment!
- But you can't add just any yeast at any time...



# When do you throw off the balance?



# Throwing off that balance

1. Primary Kombucha
  - a. Brew it like you do now!



# Throwing off that balance

1. Primary Kombucha
  - a. Brew it like you do now!



2. Then we throw off that balance!
  - a. Switch to a beer/wine-like ferment



**Does it matter when you throw off the balance?**

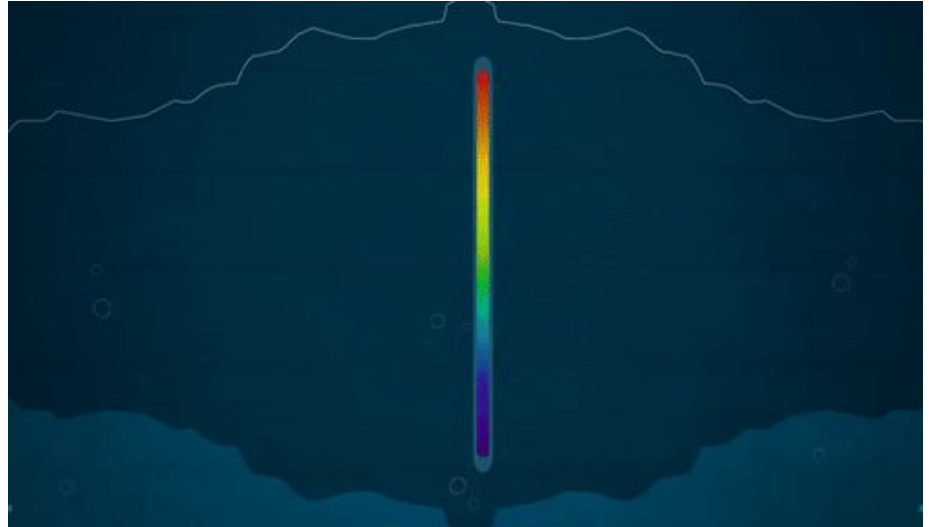
Yes!



# Does it matter when you throw off the balance?

Yes!

Acidity Levels



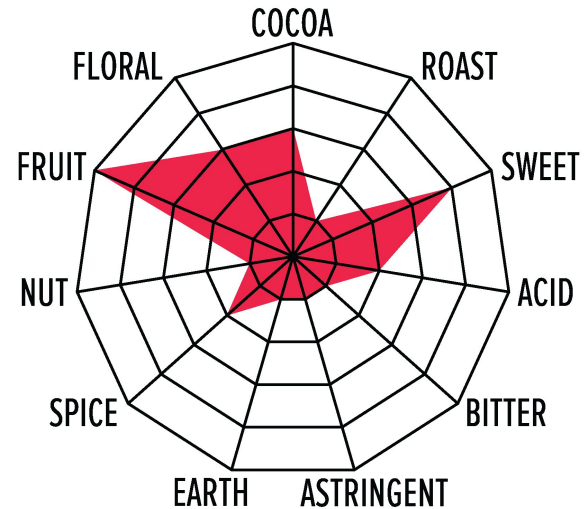


# Does it matter when you throw off the balance?

Yes!

Acidity Levels

Flavor profile



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# Does it matter when you throw off the balance?

Yes!

Acidity Levels

Flavor profile

Bacterial growth



# With high ABV, It's all about that yeast... Mostly

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# Brewing Fermentation Considerations

1. Sugars
2. Sugar Amounts
3. pH
4. Ethanol Production/Tolerance
5. Flavor/Aroma Compounds
6. Flocculation
7. Temperature Range
8. Biomass/Pitch Rate
9. Yeast Strain
10. Time
11. Yeast Activity and Phases
12. Yeast Generations
13. Yeast Form- I.e. Dried or Liquid
14. Final Product
15. Yeast Health
16. Oxygen Levels
17. Desired ABV
18. Yeast health
19. AND more...



# Brewing Fermentation Considerations

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*These all apply to High Alcohol Kombucha as well!*

# It's kind of a lot...



**It's kind of a lot...**



**... But it has been done  
before!**

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**So let's talk about a couple  
of these considerations**

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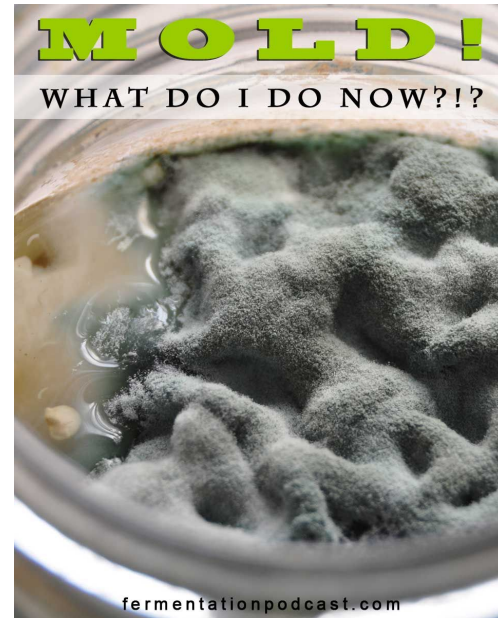
**Check that acidity... pH is  
important**





## Check that acidity... pH

- For Mold Growth
  - Below 4



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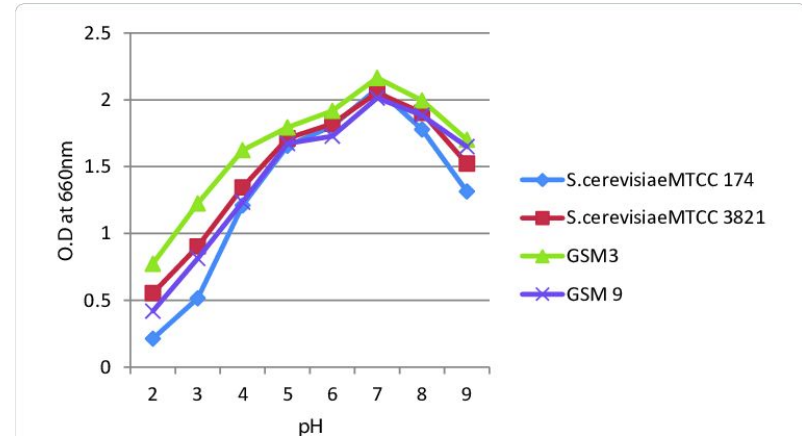
## Check that acidity... pH

- For Mold Growth
  - Below 4
- For Beer/Wine
  - 3-4
- For Kombucha
  - 2.5-3.5



## Check that acidity... pH

- For Mold Growth
  - Below 4
- For Beer/Wine
  - 3-4
- For Kombucha
  - 2.5-3.5
- For Getting Hard



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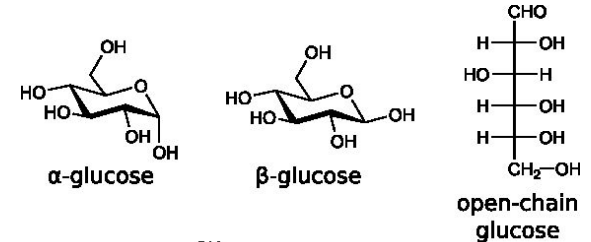
**Give me that Sweet Stuff!...**



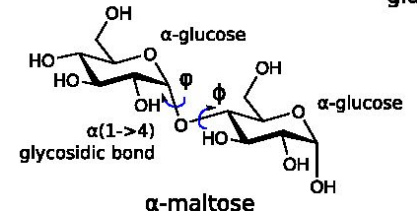
# Give me that Sweet Stuff!...

## Starting with Sugar

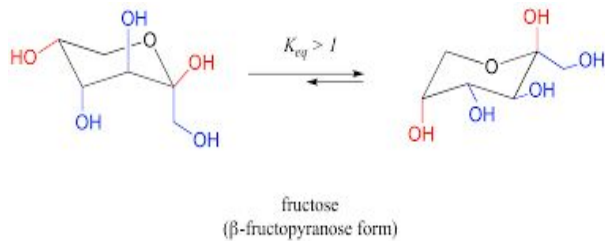
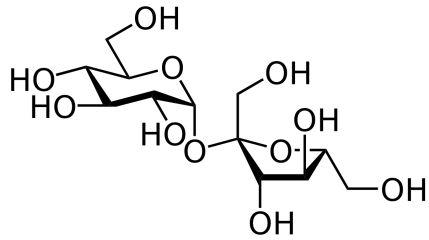
- Which types of Sugars?



- How much sugar?



- When do I add it?



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## Which types of Sugars?

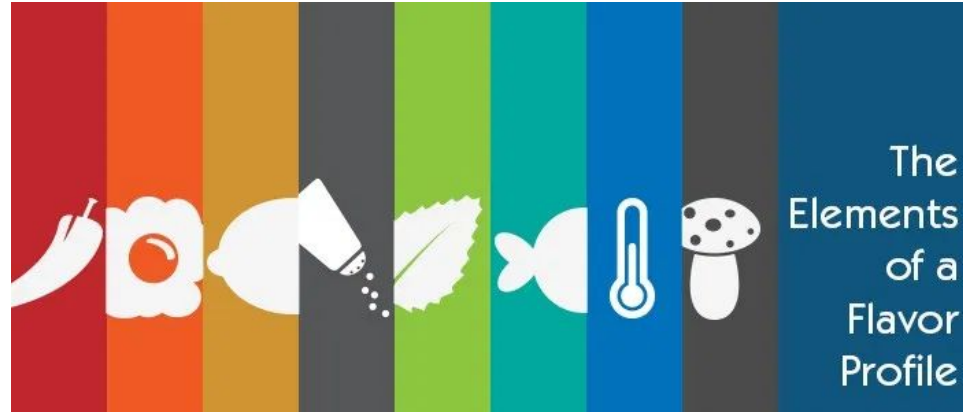
- Think about your style
  - Kombucha or Jun



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## Which types of Sugars?

- Think about your style
  - Kombucha or Jun
- Think about your end flavor goal



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## Which types of Sugars?

- Think about your style
  - Kombucha or Jun
- Think about your end flavor goal
- Think about your yeast





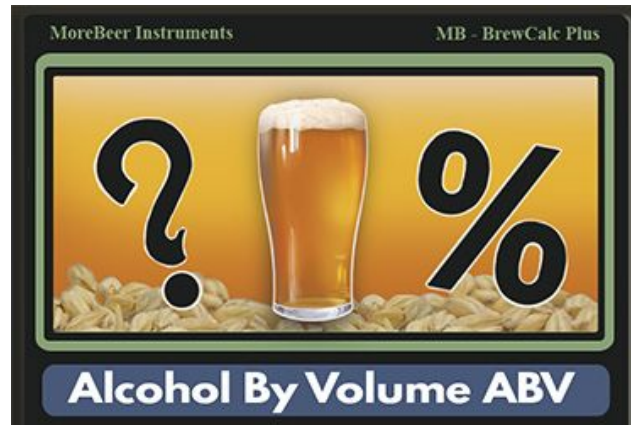
# How much sugar?

This can make or break a ferment!!... Why?

# How much sugar?

This can make or break a ferment!!... Why?

For starters, ABV and compliance



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How much sugar?





## How much sugar?



## How much sugar?

- What ABV do you want to produce?
- Different Yeast Strains
  - Low (2-5%)
  - Medium (5-10%)
  - High (10-15%)



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## And when do I add it?

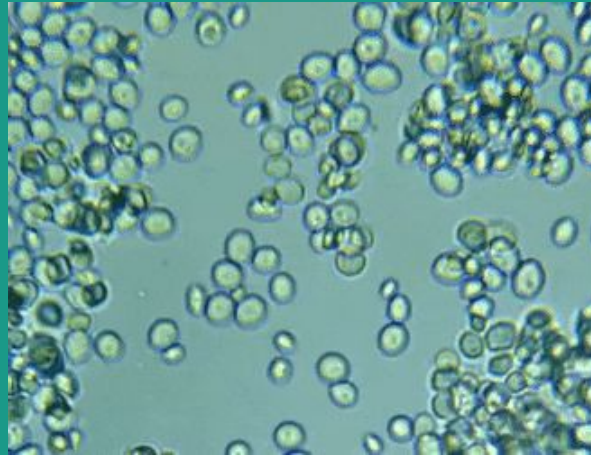
When you're ready to get  
hard!

But remember you may  
have some sugar left over  
from primary



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# Biomass... How much yeast you add



# Biomass

- Amount of yeast pitched per unit measure
- Determines speed of fermentation and flavor characteristics
  - Higher pitching rate = “Cleaner”
  - Lower pitching rate = Esters



# Biomass

- Getting the correct amount is important
  - Underpitching
    - Increased yeast characteristics but also flaws
  - Over Pitching
    - Reduce esters but also Autolysis
  - Changes in body, aromatics etc.





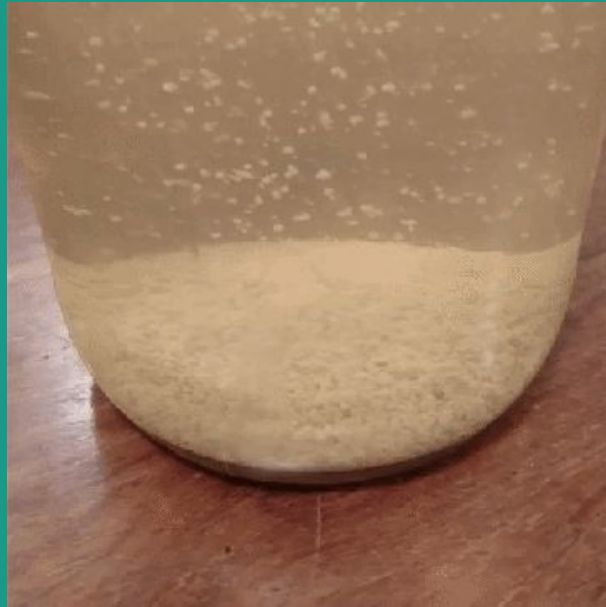
# Biomass

- Getting it right:
  - Manufacturer recommendations
  - Trial and error
    - SENSORY!
      - Think about the flavor profile you are looking for



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# Flocculation Rate... Yeast crashing out



# Flocculation

- Flocculation is a two-step particle aggregation process in which a large number of small particles form a small number large flocs
  - Wild Yeast vs. Brewers Yeast

Raw water (pond)



Flocculation



Precipitation



- Mettler-Toledo International Inc. all rights reserved. "Flocculation." *Theory and Background*, 5 Aug. 2020, [www.mt.com/us/en/home/applications/L1\\_AutoChem\\_Applications/L2\\_ParticleProcessing/Formulation\\_Flocculation.html](http://www.mt.com/us/en/home/applications/L1_AutoChem_Applications/L2_ParticleProcessing/Formulation_Flocculation.html).
- "Attenuation and Flocculation." Attenuation and Flocculation | White Labs, [www.whitelabs.com/resources/attenuation-and-flocculation](http://www.whitelabs.com/resources/attenuation-and-flocculation).

# Flocculation

- How much do you want?
  - Why it matters
    - Flavor
    - Clarity
    - Refermentation Potential

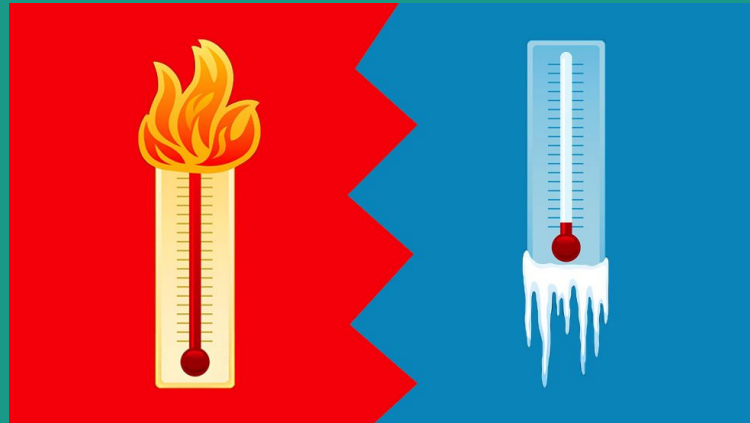


- Mettler-Toledo International Inc. all rights reserved. "Flocculation." *Theory and Background*, 5 Aug. 2020, [www.mt.com/us/en/home/applications/L1\\_AutoChem\\_Applications/L2\\_ParticleProcessing/Formulation\\_Flocculation.html](http://www.mt.com/us/en/home/applications/L1_AutoChem_Applications/L2_ParticleProcessing/Formulation_Flocculation.html).
- "Attenuation and Flocculation." Attenuation and Flocculation | White Labs, [www.whitelabs.com/resources/attenuation-and-flocculation](http://www.whitelabs.com/resources/attenuation-and-flocculation).

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# Temperature Range...

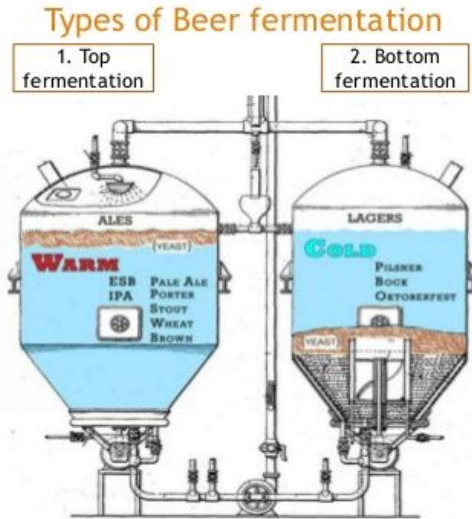
## How low can you go? Or how high



# Temperature Range- How low can you go? Or how high

- What is your equipment capable of?
- Keep within the good range

- Use ale or 'top-fermenting' yeast  
*Saccharomyces cerevisiae*
- Warmer temp: 65 - 75°F
- Yeast and foam found at the top of the medium is removed
- Second crop that is produced by the end of fermentation is harvested since the yeast is pure.
- Types of beer produced: Ale, porter, stout...



- Use lager or 'bottom-fermenting' yeast  
*Saccharomyces uvarum*
- Cooler temp: 45 - 55°F
- Settled yeast is decanted from the unconditioned beer.
- It is manually collected from middle layer of the sediment due to its purity.
- Types of beer produced: Lager, pilsner...



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**All this to say... Your yeast is kind  
of a big deal**

**OK, but... What about  
the white elephant in  
the room...**

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**Doesn't the ethanol  
kill the bacteria?**

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**Let's Talk It Through!**

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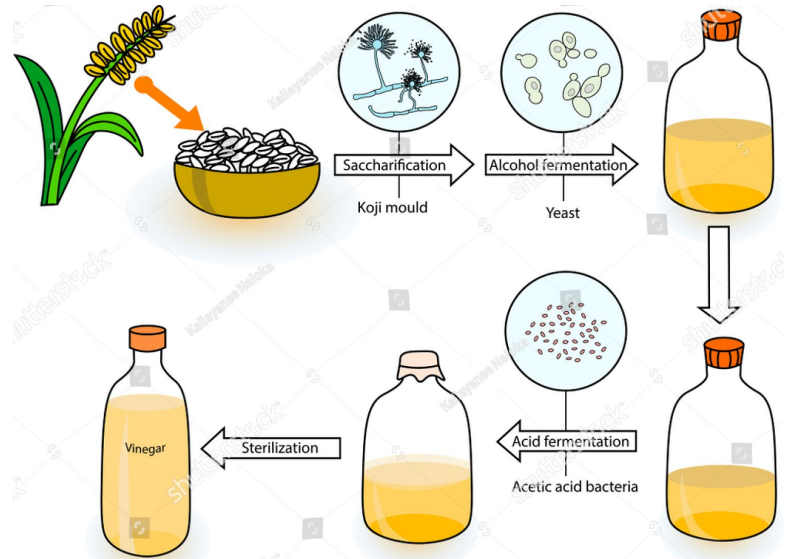
**First...**

**What Do Other Industries Think?**

# First...

## What Do Other Industries Think?

- Vinegar
  - 9-12% ideal range for production\*\*\*



Kamozawa , Aki, and H. Alexander Talbot. "Making Vinegar at Home." *Popular Science*, 8 Dec. 2008, [www.popsci.com/diy/article/2008-12/making-vinegar-home/](http://www.popsci.com/diy/article/2008-12/making-vinegar-home/).\*

Moustafa, Ahmad. "Salad Oil, Mayonnaise, and Salad Dressings." *Practical Handbook of Soybean Processing and Utilization*, 1995, pp. 314–338.,

doi:10.1016/b978-0-935315-63-9.50022-x.\*\*

# First...

## What Do Other Industries Think?

- Wine- Spoilage Organisms
  - 5.5-16% ABV



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**Yah, yah... But what about  
lactobacillus?**



**Still..**

**What Do Other Industries Think?**

# Still..

## What Do Other Industries Think?

- Beer- 3-13%
  - Spoilage

AND

- Sour Beers- ABV 3-7%



# Ethanol Tolerance of Lactic Acid Bacteria, Including Relevance of the Exopolysaccharide Gene *gtf*

Vanessa Pittet, Kendra Morrow, and Barry Ziola,<sup>1</sup> *Department of Pathology and Laboratory Medicine, University of Saskatchewan, Royal University Hospital, Saskatoon, SK, Canada*

## ABSTRACT

J. Am. Soc. Brew. Chem. 69(1):57-61, 2011

Lactic acid bacteria (LAB) are generally ethanol-tolerant organisms that have a higher resistance to ethanol than most bacteria. However, little is known with regard to the role ethanol tolerance plays in beer spoilage. Various stresses found in beer need to be overcome for an organism to be able to grow and cause spoilage. Because of this, a broad range of beer-spoilage abilities is found in LAB, and no conservation of this phenotype exists within species. As such, it is very difficult to accurately predict when a contaminating LAB would be able to spoil beer. Analysis of LAB ethanol tolerance was performed to determine whether a predictive factor could be found for the ability to grow in beer. Minimum inhibitory and bactericidal concentrations were determined for 61 LAB that were also analyzed for their ability to spoil beer. No significant correlation was found between ethanol tolerance and ability to spoil beer because ethanol tolerance was essentially conserved within species. In addition, 153 LAB isolates were screened for the glucosyltransferase gene *gtf*, which is responsible for exopolysaccharide (EPS) production, to determine whether the presence of the gene was correlated with the ability to spoil beer or to tolerate high ethanol concentrations. The *gtf* gene was found in only six isolates, and no difference in beer-spoilage ability was found between ropy and nonropy isolates. Further, ethanol tolerance of EPS-producing variants was comparable with their nonropy counterparts. The results of this study show that ethanol tolerance does not play a discriminating role in LAB beer spoilage and that the presence of the *gtf* gene does not provide a selective advantage for ethanol tolerance or beer spoilage.

Keywords: Beer spoilage, Ethanol tolerance, Exopolysaccharide production, Glucosyltransferase, Lactic acid bacteria

entre viscosa y no-viscosa aislamientos. Además, la tolerancia de etanol de variantes EPS-producción era comparable con su no-viscosa homólogo. Los resultados de este estudio muestran que la tolerancia al etanol no juega un papel discriminación en el deterioro de cerveza por LAB y que la presencia del gen *gtf* no proporciona una ventaja selectiva para la tolerancia a etanol o deterioro de la cerveza.

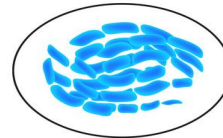
Palabras claves: Bacterias del ácido láctico, Deterioro de la cerveza, Glucosiltransferasa, Producción de exopolisacáridos, Tolerancia del etanol

Lactic acid bacteria (LAB), including bacteria belonging to the genera *Lactobacillus*, *Leuconostoc*, *Oenococcus*, and *Pediococcus*, are generally ethanol-tolerant organisms (4,6,12,14,15). It is not surprising, therefore, that LAB are either used in or cause contamination problems for the alcoholic beverage and fuel ethanol industries (5,13,22,23,26,29). In the context of breweries, the presence of LAB can be extremely detrimental, with *Lactobacillus* and *Pediococcus* organisms being the most common beer spoilers. Growth in beer requires adaptation, tolerance, or resistance to various factors present in its harsh environment, such as the antimicrobial compounds ethanol and hops, high CO<sub>2</sub>, low pH, and very little oxygen and available nutrients (24). Interestingly, the ability to grow in beer is not a species-conserved phenotype (16), i.e., isolates of the same species show different beer-spoilage abilities. Because of this, it is difficult to accurately identify whether a contaminating organism will be problematic, because no perfect spoilage prediction method is currently available. Based on the factors impeding growth in beer and ongoing research on hops resistance (1, 2,25,30), the focus of this study was the assessment of LAB ethanol tolerance from the perspective of the ability to predict beer spoil-

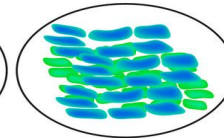
# What they did and why

- Looked at ethanol tolerance in relation to LAB spoiling beer
- Goal- Help better predict when an LAB would spoil beer
  - Basically could they predict the spoilage based on traits

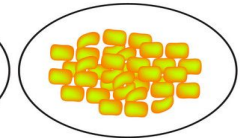
## LACTOBACILLUS TYPES



Lactobacillus acidophilus



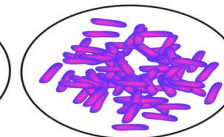
Lactobacillus rhamnosus



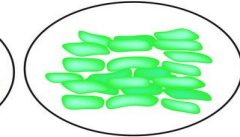
Lactococcus lactis



Lactobacillus plantarum



Lactobacillus casei



Lactobacillus paracasei



What they found...





## What they found...

- LAB are spoilers
  - More so that the *gtf* gene did not lead to more or an ability to spoil beer, they all can!



# Lacto Ethanol Tolerance

<b>Strain</b>	<b>Minimum Inhibitory Concentration</b>	<b>Minimum Bactericidal Concentration</b>
Lactobacillus brevis	8-15	12-20
Lactobacillus casei	15	25
Lactobacillus delbrueckii	6-10	8-10
Lactobacillus fermentum	10-12	15-20
Lactobacillus hilgardii	12	20
Lactobacillus plantarum	12-20	20
Lactobacillus reuteri	10	15
Lactobacillus rhamnosus	12	20

**Luckily for us, bacteria  
are hearty little bugs**

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**Thank you!**



# Questions?

Kyle Oliver

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