Oxygen Levels Effect on Bacteria in Kombucha

Skyleigh James and Elizabeth Rosen
Biology 222: Microbiology

Abstract

For this project we focused on the affect's oxygen levels have on the diversity and concentration of kombucha. We did this by bottling up kombucha and then opening a new one each week. With each bottle we then plated, performed PCR, Gel Electrophoresis, and then sent the data off for sequencing. Our results showed that more bacteria types were present in week two. The concentrations increased with lower oxygen levels. This shows that our hypothesis was refuted, meaning that lack of oxygen increased bacteria's diversity and concentration. This research is important to recognize how

Introduction

Our experiment is on the effect oxygen has on the bacteria in kombucha. To research this, we controlled oxygen levels, as it is our independent variable, and measured the amount and diversity as our dependent. By removing oxygen, aerobic respiration cannot take place, leading to fermentation and therefore, alcohol lactic acid, carbon dioxide, and ethanol are produced as the primary final products (Bishop, 2022). The bacteria that requires oxygen will slowly die off, because they only get energy from fermentation. Due to this, we hypothesize that if there are lower oxygen levels, then there will be less diversity in the bacteria.

Methods

Materials & Location

For our experiment, we selected the Senergy Ginger Kombucha brand. We also used nystatin plates when growing our colonies, as well as we used equipment for PCR and Gel Electrophoresis.

Procedure

When beginning our experiment, we first bottled kombucha in an air-tight container. After that, we then plated the controlled, freshly opened kombucha. To do this, we put 200 micro liters of a 1/10 dilution onto nystatin plates. Next, we incubated the plates at 37 degrees for 48 hours, and then extracted DNA from six different colonies to do PCR. We then preformed gel electrophoresis to send the results off for sequencing. While doing all of this, we opened our next tube of kombucha after it had been sitting fermenting for a week. We repeated the steps for plating and incubation. We then compared the colonies that grew from week two to our control and selected the most abundant or different to use for PCR.

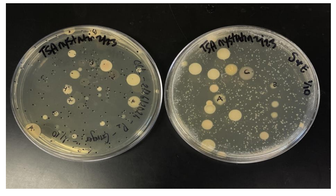

Figure 1. On the left: week one plate, right: week two

Types of Bacteria Found

WEEK 1 167 countable colony forming units	WEEK 2 680 countable colony forming units
Heyndrickxia coagulan (formerly *Bacillus coagulans*) 99.38% match	*Heyndrickxia coagulan* (formerly *Bacillus coagulans*) 99.38% match
Peribacillus frigoritolerans 99.10% match	*Peribacillus frigoritolerans* 99.39% match
Staphylococcus sp. 99.54% match	*Cytobacillus firmus* 99.88% match

Table 1. After receiving the DNA sequences, we translated the code to determine the different species of bacteria.

Countable Colony Forming Units

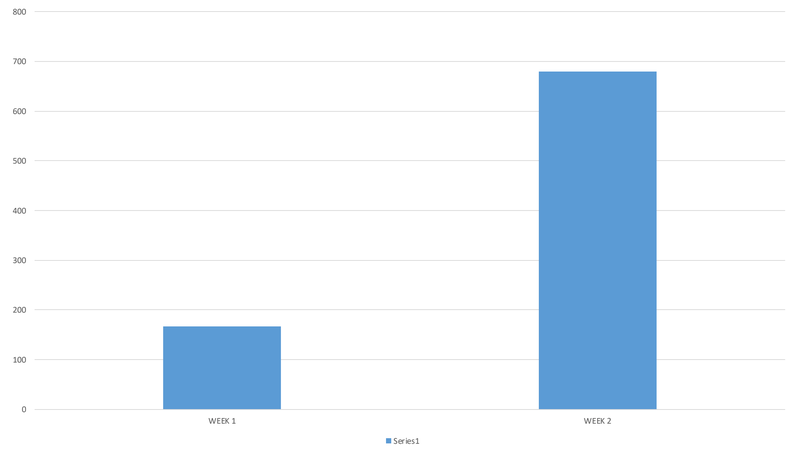

Figure 2. This graph shows how many countable colonies were formed on the week one and week two plates.

Discussion

Our results from plating and sequencing showed that the kombucha with less oxygen had more colonies and was more diverse because it contained the bacteria Cytobacillus firmus plus the original bacteria from week one.

In one article, *Does Kombucha Need Oxygen to Ferment*, it discusses how the scoby requires oxygen to continue to produce beneficial compounds via aerobic respiration. Many articles like this one aligned with our hypothesis that an increase in oxygen levels would increase the concentration and diversity of bacteria in kombucha, but our results showed otherwise. This could have occurred for many different reasons. First off, we assumed that there was oxygen in the bottle to begin with before we broke the seal. One possibility could have been that the manufacturer used a sealing method that removes the oxygen from the bottles at the start. Another article titled *How Much Alcohol Is in Kombucha* goes over how limiting oxygen levels increases alcohol content of the kombucha because more fermentation takes place. This reason is why it is important to understand the affects oxygen levels have on kombucha, so we can control the desired alcohol ratio.

Our major limitation during this study was time. So, if this experiment is repeated in the future, it would be best to bottle more tubes of kombucha at the start and let them rest for longer periods of time. We were only able to let our kombucha sit for one week, so we were unable to test a broader range. Another aspect that we would change for the future is how we plated. The 1/10th dilution worked well however, we would suggest plating multiple of each week, and averaging the numbers of colonies in order to achieve a more accurate count. The last aspect we would alter would be finding a device or tool to measure the amount of oxygen to ensure our independent variable was changing.

Acknowledgements

We would like to thank the lab technicians for helping us with our experimental design, supplying us with the materials needed for our procedure, and assisting us outside of lab when we needed extra time. Our whole experiment would also not have been possible without Caleb, Charlotte, and Linh (CCL), who lent us one of their plates to serve as our control group when ours had no growth after incubation.

References/ Work Cited

Bishop, P., Pitts, E. R., Budner, D., & Thompson-Witrick, K. A. (2022). Kombucha: Biochemical and microbiological impacts on the chemical and flavor profile. *Food Chemistry Advances, 1*, 100025. doi:10.1016/j.focha.2022.100025

Does Kombucha Need Oxygen To Ferment? (2023, May 31). Atlas Scientific. https://atlas-scientific.com/blog/does-kombucha-need-oxygen/#:~:text=Kombucha%20needs%20oxygen%20to%20start

How Much Alcohol Is in Kombucha? (2022, June 15). Revolution Fermentation. https://revolutionfermentation.com/en/blogs/kombucha/how-much-alcohol-kombucha/#:~:text=Acetic%20bacteria%20need%20oxygen%20to