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Background Information

Kombucha has made a recent media surge to being a health "super drink"

Kombucha is tea fermented with a SCOBY, which is yeast and bacteria layered in a cellulose matrix (4)

Kombucha often contains bacterial genera *Acetobacter* and *Gluconobacter* and the yeast genus *Saccharomyces* (5)

The main composition of finished kombucha is acetic, lactic, gluconic and glucuronic acids; as well as ethanol and glycerol (2)

Kombucha has been consumed since the Tsin dynasty (220BC) in China (4)

Kombucha made with various types of teas showed a difference in the microbial profile of the beverage (3)

Kombucha fermented and held at various temperatures while fermenting has been shown to have differing microbial profiles (1)

Kombucha, a fermented tea made with a symbiotic colony of bacteria and yeast (SCOBY), has been touted as a health beverage for years. Previously, the health benefits attributed to kombucha have been primarily anecdotal; however, as kombucha consumption has increased, scientists are beginning to question what causes the supposed benefits, with many researchers attributing the advantages to kombucha's microbial ecosystem. Previous research examining kombucha produced with different types of tea demonstrated many variations in the microbiome. Therefore, manipulating the amount of tea used and the tea brewing methods may affect the microbiome of kombucha as well. This study was conducted to determine how different kombucha production techniques – tea concentration and steeping temperature – alter the kombucha's microbiome. For this project, kombucha was made using varying concentrations of black tea and a range of brewing temperatures to determine if either had an effect on the microbial profile. The teas were fermented to the desired end-point and were then plated on selective agars to identify and quantify the types of microbes present. The results of this study will provide further insight into how different production practices affect the microbial profile of kombucha and how that might translate to the wellbeing of the consumer.

Methods

Tea was brewed for 24 hours at varying temperatures and concentrations

Tea bags were removed and the pH of the beginning tea was measured

A SCOBY was placed in the tea and the jar was covered with cheesecloth

Every Monday, Wednesday, and Friday pH readings were taken of the ferment

After 16 days, the kombucha was diluted to 10^{-4}

Dilutions of 10^{-3} and 10^{-4} were plated on YGC and MRS agar

Plates were incubated at 30°C for 5 days.

Colonies were counted and analyzed

The experiment was run in triplicate

Hypotheses

Null: The brewing temperature and tea concentration will have no effect on the microbial profile of the kombucha made.

Alternate: Both the brewing temperature and the tea concentration will have an effect on the microbial profile of the kombucha.

Future Research

Determining if the brand of SCOBY has an effect on the microbial profile

Determining how many acetic acid bacteria are in the beverage when the parameters are changed

Determining if a sugar concentration difference has an effect on the microbial profile

Resources

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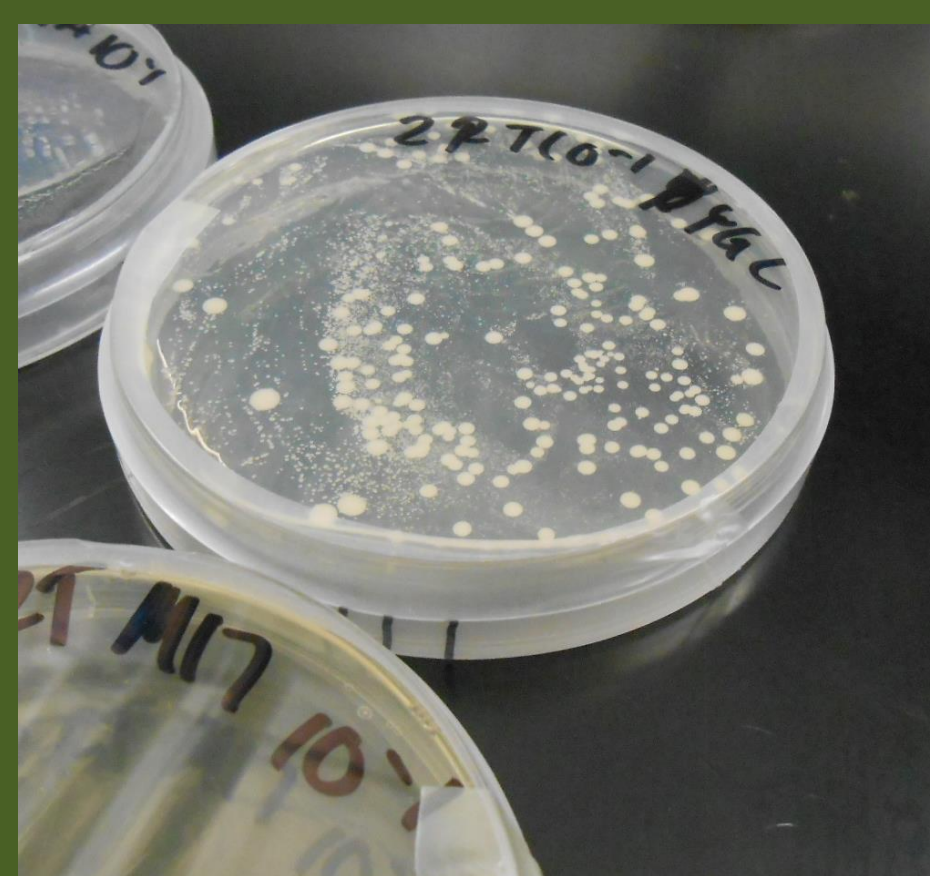
Results

pH of the kombucha decreased over the course of the ferment

Hotter brewing temperature often resulted in a higher ending pH

pH was directly related to tea concentration

The 2X tea concentration when brewed in boiling water had the highest yeast populations



Picture 1: Yeast colonies growing on YGC agar.



Picture 2: Kombucha brewing

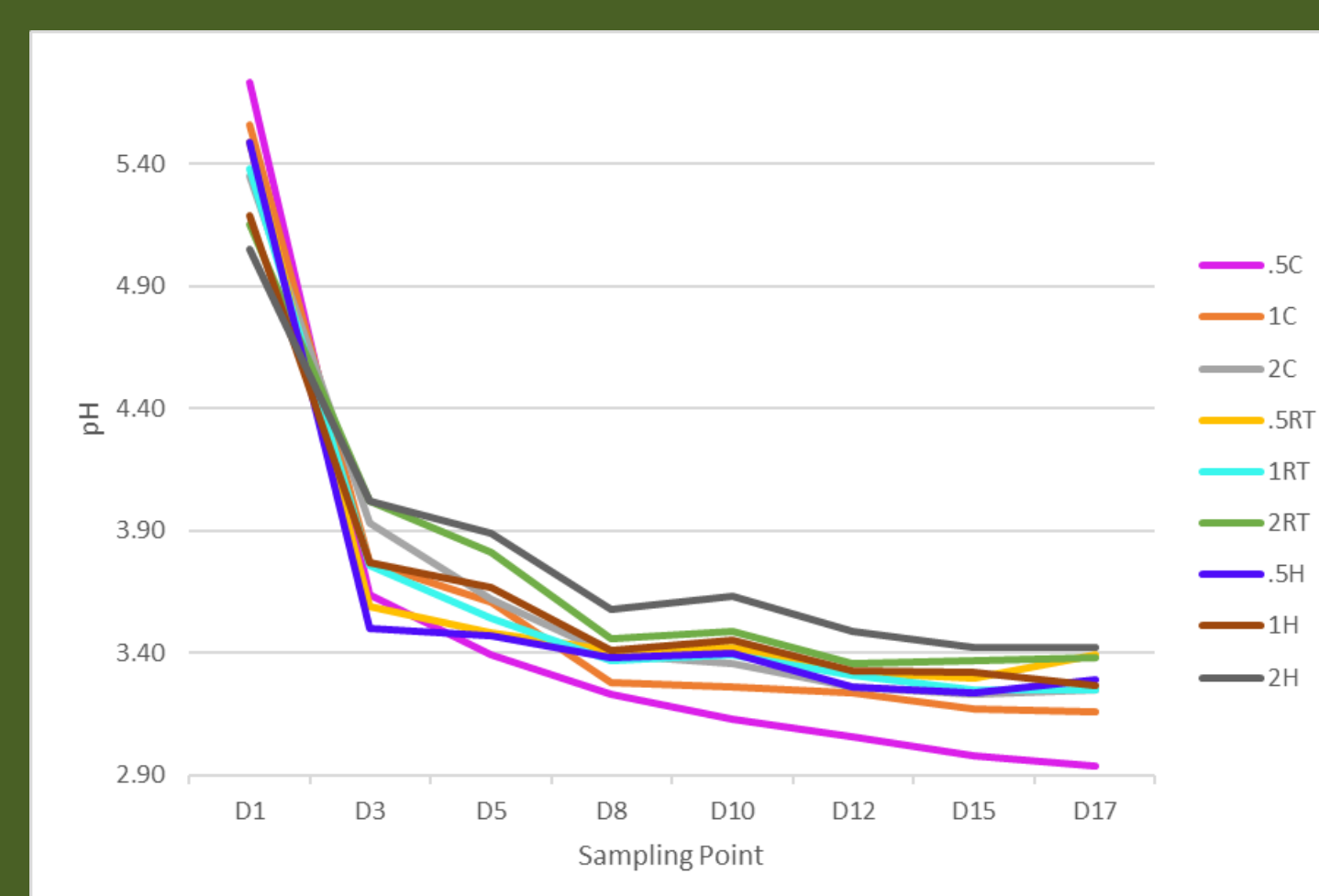
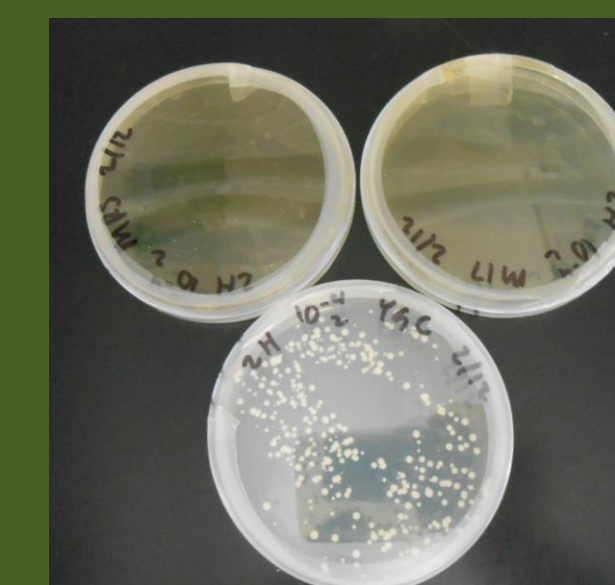
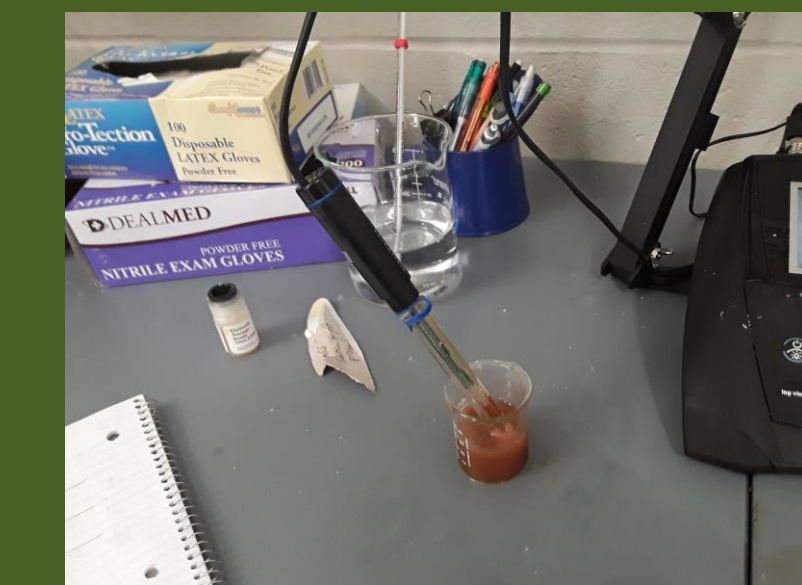


Figure 1: pH data the kombucha fermentation. The .5, 1, and 2 correlate to the concentration of tea used; the C, RT, and H correspond to the brewing temperature. For instance, .5C means half tea concentration brewed at a cold temperature



Picture 3: Microbial growth on plates



Picture 4: pH testing of kombucha



Picture 5: Stacks of plates growing microbes

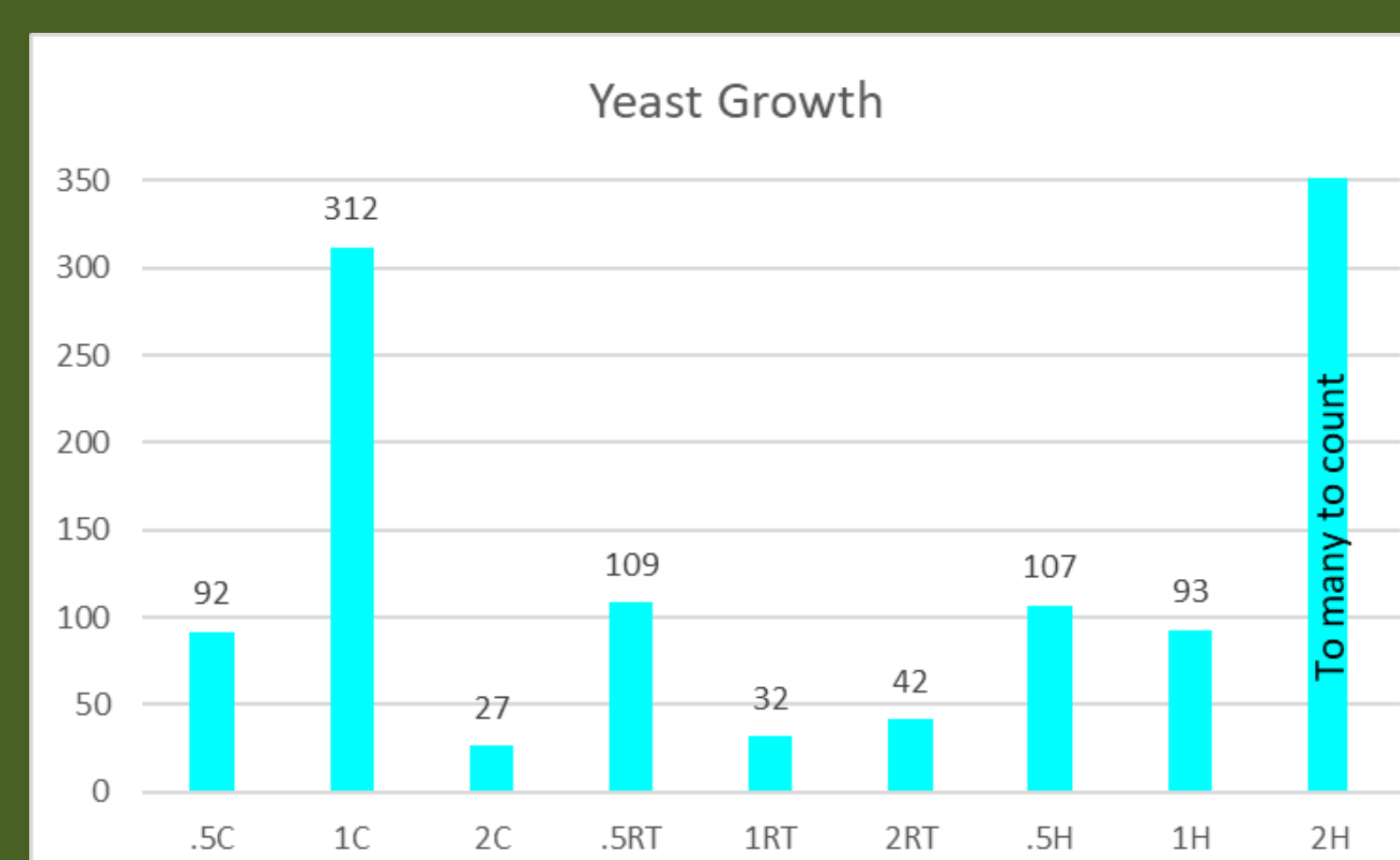


Figure 2: Colony growth of yeast on YGC agar plates. The 2H concentration had too many colonies to count

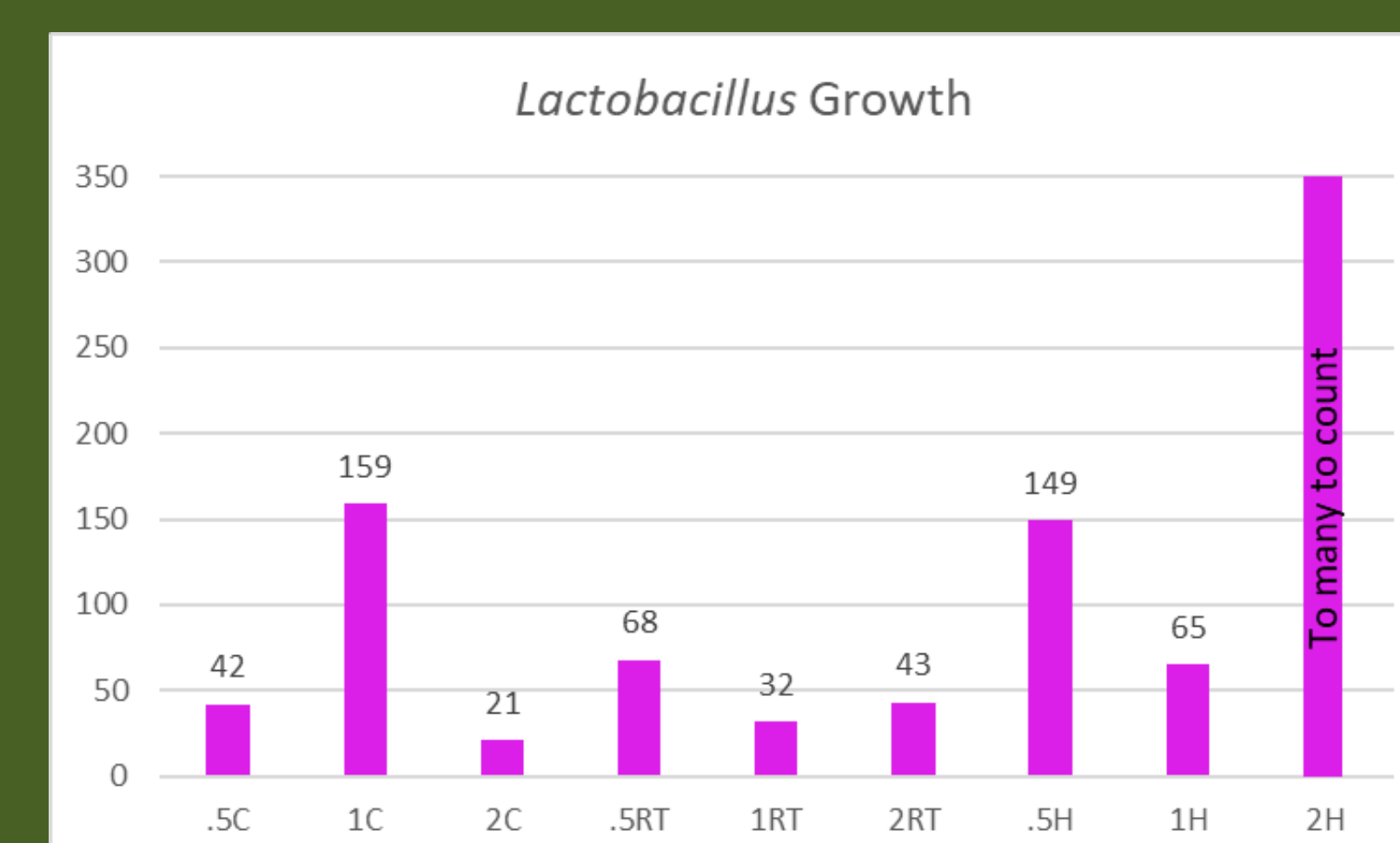


Figure 3: Colony growth of *Lactobacillus* on MRS agar plates. The 2H concentration had too many colonies to count