Humboldt Journal of Microbiology

Volume 23 Article 7

2023

Kom-Butcher of Pathogens

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2023, Volume 23

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ABSTRACT

The antimicrobial properties of kombucha are not well understood, but it is believed that they can be used against foodborne pathogens. In this study, its effectiveness was tested against two common food-borne and one yeast pathogens (*E. coli, S. aureus,* and *C. auris*). Three popular store-bought kombuchas (GT Synergy, Moss Beach, and Health-Aide Raw) and one home-brewed kombucha were used to study the antimicrobial properties of kombucha. Several nutrient broth tubes were individually inoculated with the microbes in question and were treated with kombucha (Figures 1-3). There were indications of bacteria settling at the bottom of all tubes except the three control samples (*E. coli, S. aureus,* and *C. auris*) that had no kombucha added. A white layer of pellicle (a byproduct of kombucha fermentation) was present on all three GT kombucha samples. The results indicate that homebrewed kombucha was more successful at inhibiting the growth of the chosen microorganisms when compared to the three store-bought versions. Related studies are important as kombucha is shelf stable on its own and has the potential to be used as a food preservative as well as an aid in the maintenance of our gut microbiome.

INTRODUCTION

Superfoods have always claimed to be full of properties that will have a positive impact on one's health and this has led these foods to be surrounded by controversy. The purpose of this study is to better understand the potential of raw, unpasteurized kombucha as an aid in the fight against food-borne pathogens and food spoilage [1]. Kombucha is a beverage made of glucose and tea (*Camellia sinensis*) fermented by a symbiotic culture of bacteria and yeast [2]. Kombucha converts sugar to alcohol and then to acetic acid during fermentation. It is widely believed that home-brewed kombucha has greater antimicrobial properties because the batches are fresher and contain live cultures, while store-bought kombucha have added ingredients and preservatives which affect its effectiveness and potency [11].

Researchers demonstrated four types of kombucha preparations with respect to their antimicrobial content [2]. These four kombucha preparations were fermented, neutralized, and heat-treated with respect to their antimicrobial activity against some pathogenic bacterial and fungal strains using agar well diffusion assay. The neutralization of kombucha pH is essential to prevent acidic conditions from killing the food-borne pathogens of interest before they have a chance to incubate. The raw kombucha exerted the strongest antimicrobial activities when compared with neutralized and heat-treated kombucha beverages. The organisms *Staphylococcus aureus* ATCC6538 (*S. aureus*) and *Escherichia coli* ATCC11229 (*E. coli*) were the most susceptible to the antimicrobial activity of kombucha beverage preparations [2]. The fermentation time has also been shown to play a role in the strength and potency of antimicrobial properties in kombucha. The antimicrobial molecules form after it has gone through the fermentation process, usually found after 14 days of fermentation [4,7].

Limited research has been conducted on kombucha, including the effectiveness of its antimicrobial and antifungal properties against food-borne pathogens [1,2]. The species *Candida auris* is a common fungus found in pasteurized kombucha and will be a key indicator of antifungal activity in our experiment [10].

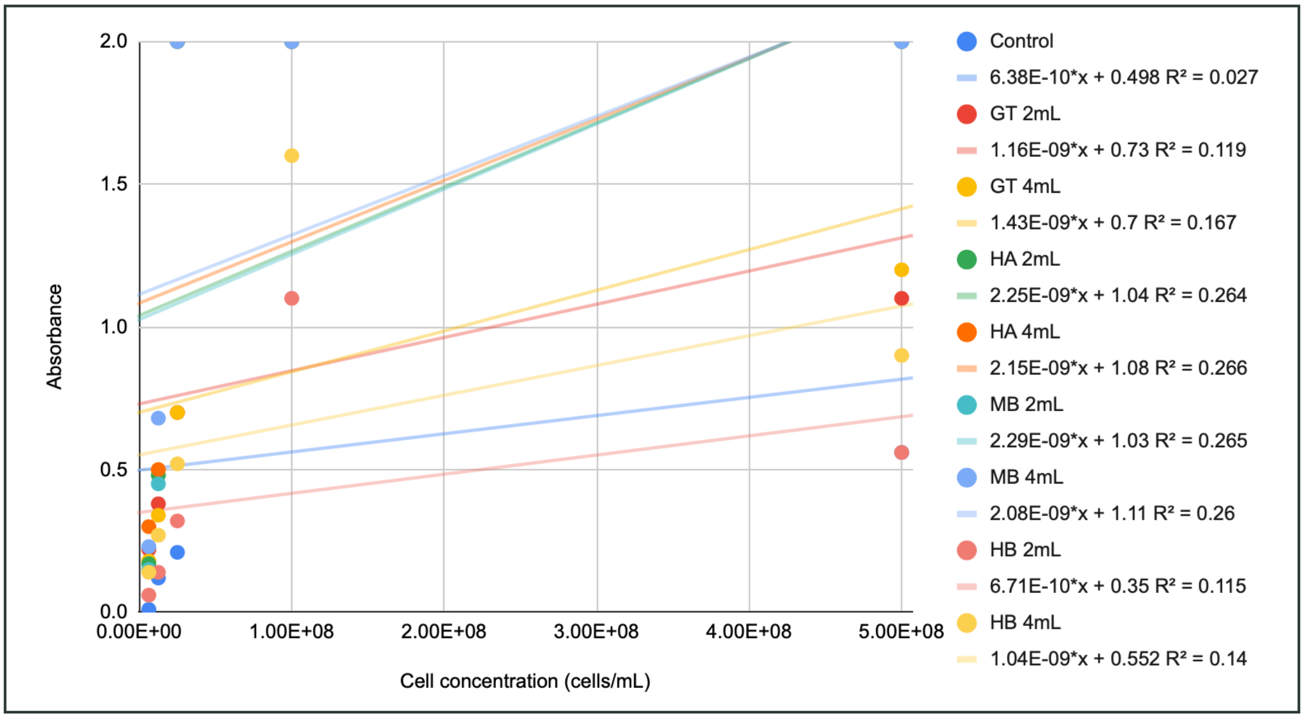
It is expected that there will be a higher amount of microbial growth in the trials with store-bought kombucha and little to no growth in the trials with homebrewed kombucha (HB) after an incubation period of 48 hours at 30°C. This temperature is the ideal environment needed for bacteria to grow in as well as for kombucha fermentation.

The objective of this study is to test whether or not there is a difference in the antimicrobial and antifungal properties in store-bought kombucha compared to home-brewed kombucha. The nutrient broth tubes were each treated with different volumes of kombucha in order to better understand the varying degree of antimicrobial activity in different kombucha [2].

MATERIALS AND METHODS

The store-bought kombucha used in this study were Health-Aide Raw (HA), GT Synergy Raw (GT), and Moss

Figure 1.
E. coli growth measured using a spectrophotometer. The HB 2mL treatment tube had the lowest amount of growth after a 48 hour incubation period. Both treatments had bacterial growth on the bottom of the tubes. The GT sample had a thin pellicle grow at the surface..



Beach (MB). These brands were chosen because they are popular and readily available in many grocery stores. It is impossible to know how long these samples were fermented or how long they had been sitting on the store shelves. The homebrewed (HB) sample was fermented for 2 weeks and then refrigerated to slow fermentation.

A sample of each kombucha type was plated on a nutrient agar plate and incubated at 30°C for 48 hours. There was very little growth on these plates and so it's believed that the live cultures found in kombucha should not grow in the nutrient broth and thus should not interfere with the results from the study.

A pH reading of the four kombucha was conducted and discovered to be extremely acidic so the pH was raised using sodium hydroxide (Table 1B). Twenty-seven nutrient broth tubes were inoculated with similar concentrations of *S. aureus, E. coli* (two common food-borne pathogens), or *C. auris* [1]. Each tube was then treated with either 2mL or 4mL of 7 pH (neutral) kombucha. The three control tubes were then inoculated only using their respective microorganisms (*E. coli, S. aureus,* and *C. auris*).

Each broth was incubated at 30°C for 48 hours [6]. After 48 hours the tubes were transferred to a 4°C fridge to slow down the rate of microbial growth until the lab was open after the weekend. A sample from each tube was taken and the absorbance was measured using a spectrophotometer to create an Absorbance vs Cell Concentration graph to compare the growth of the food-borne pathogens.

RESULTS

The absorbance readings from the spectrophotometer of each inoculated broth were then graphed. Results were then studied in order to observe the change in growth after the incubation period. Three absorbance graphs were made to measure the absorbance (y) and cell concentration (x) of *E. coli, S. aureus,* and *C. auris* after the 2mL and 4mL treatments. The results indicate that homebrewed kombucha was more successful at inhibiting the growth of the chosen microorganisms when compared to the three store-bought versions. The pH readings were confirmed before and after inoculating each broth. After the 48 hour incubation the MB treatment (pH 6.69) had less bacterial growth in the *E. coli* treatment (Figure 1). GT (pH 7.12) had an equal amount of growth in the *S. aureus* and *C. auris* nutrient broths (Figure 2-3). HA (pH 7.43) had a consistent increase in microbial growth for all three treatments (Figure 1-3). Simultaneously, HB nutrient (pH 7.32) had a constant decrease of bacterial

Figure 2.
S. aureus growth measured using a spectrophotometer. The HB 2mL and 4mL treatment tubes had the lowest amount of growth after a 48 hour incubation period. Both treatments had bacterial growth on the bottom of the tubes and had similar results. The GT sample had a thin pellicle grow at the surface.

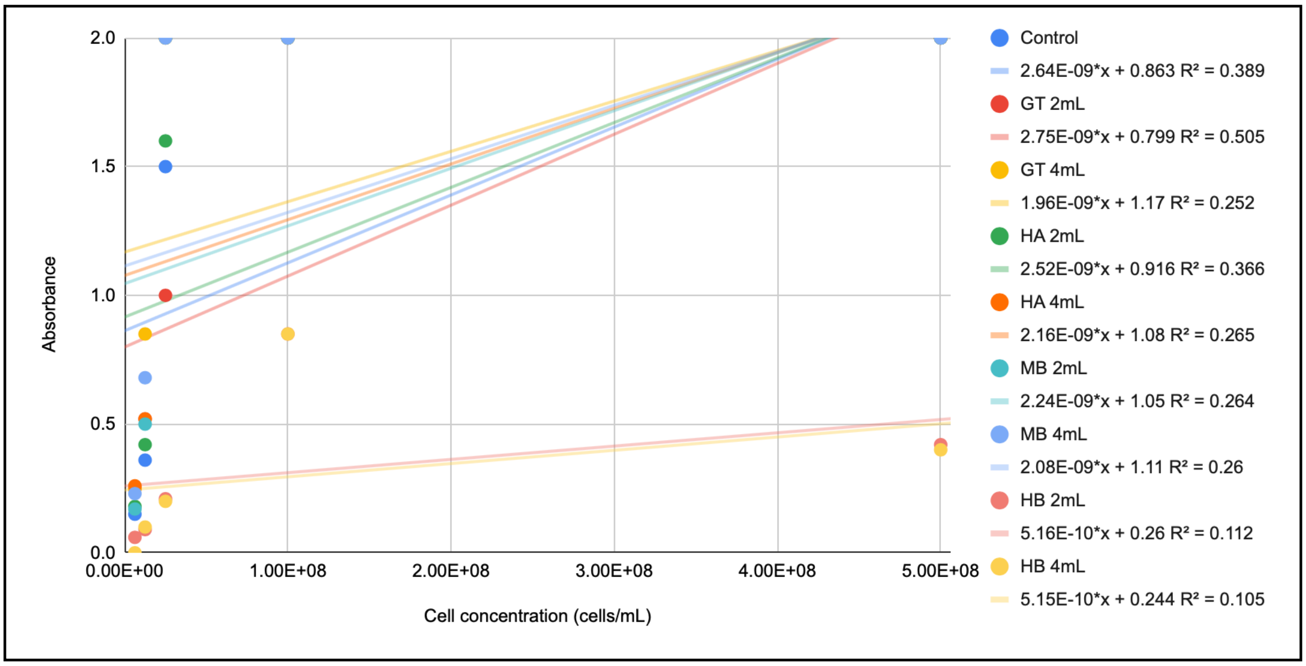


Figure 3.
C. auris growth measured using a spectrophotometer. The HB 2mL treatment tube had the lowest amount of growth after a 48 hour incubation period. Both treatments had bacterial growth on the bottom of the tubes. The GT sample had a thin pellicle grow at the surface..

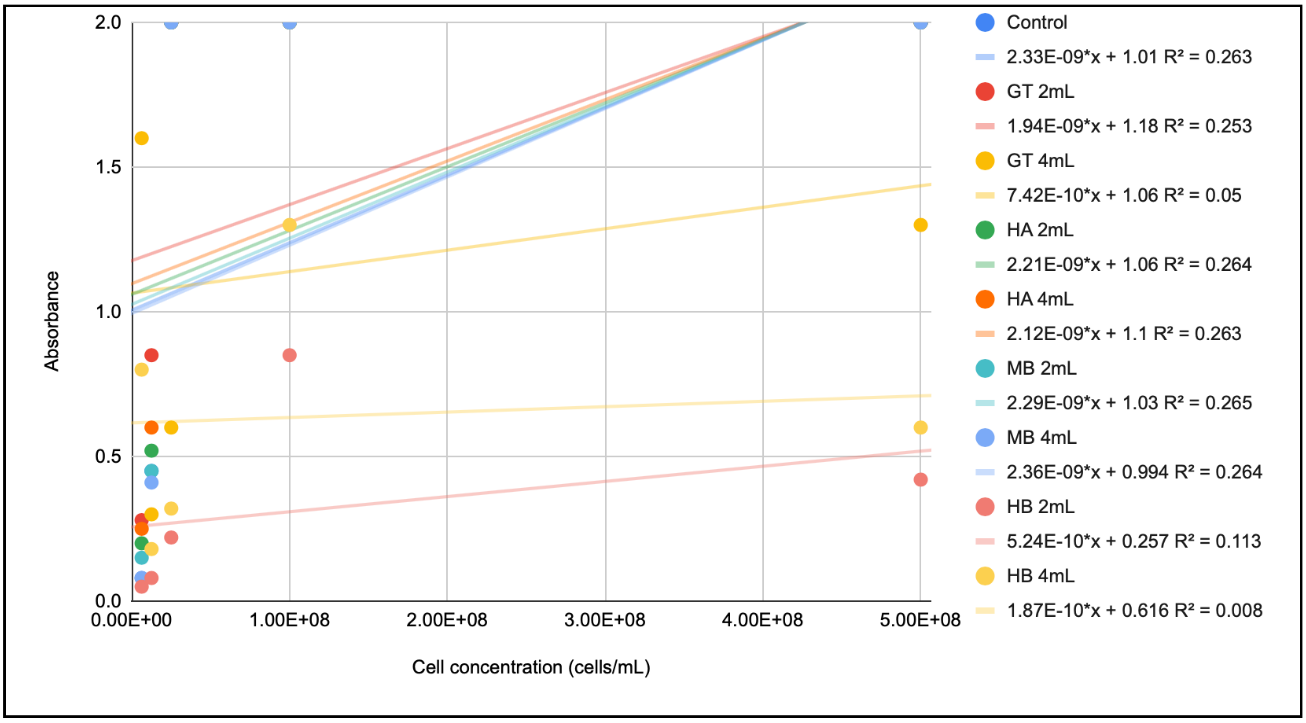


Table 1B.
pH readings before and after sodium hydroxide treatment to raise pH. Goal is to neutralize the kombucha pH (pH-7) to prevent acidic conditions from killing the pathogens before the incubation period. Our initial pH readings demonstrated very acidic conditions in all kombucha beverages which resulted from the fermentation process

Kombucha	Pasteurization	Initial pH	Final (neutralized) pH
Moss Beach	Pasteurized	2.93	6.69
GTS Synergy Raw	Pasteurized	2.72	7.12
Health-Aide	Pasteurized	2.78	7.43
Homebrew	Unpasteurized	2.86	7.32

growth on average for all three food spoiling pathogens (Figure 1-3).

DISCUSSION

In future experimentation, agar plating and serial dilutions should be the preferred method in order to accurately quantify the number of microbes present after different kombucha treatments. The different kombucha types should also have their live cultures looked at under a microscope before inoculating and after the 48 hour incubation period.

The formation of the pellicle in the GT tubes was not expected and should also be better understood. Based on the limited growth found on the nutrient agar plates and the pH neutralization of the kombucha, further fermentation should not have been possible. This may be due to the fact that GT has sugar added before or during the bottling process and so the kombucha microorganisms continue to ferment.

Comparative studies that examine antimicrobial and antifungal properties are limited. Based on these results, there is evidence that supports the claim that homebrewed kombucha contains stronger antimicrobial properties when compared to commercially available kombucha. These findings suggest that kombucha should be subject to more studies as it has the potential to be utilized against food spoilage.

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