The Pursuit of Happiness: Determining the Ideal Treatment for Enriching Kombucha With Psychobiotics

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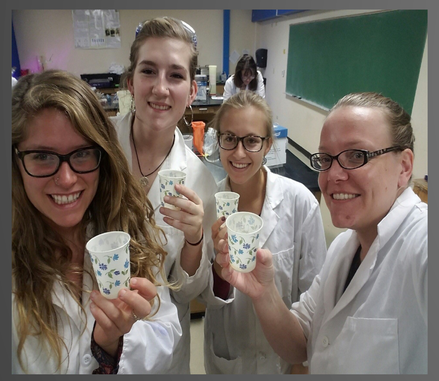
Introduction

In 2014, the United States Census Bureau estimated that approximately 24 percent of Albany County residents in Laramie, Wyoming lived in poverty[1]. The Downtown Clinic (DTC) of Laramie is a clinic developed strictly for the population of low income, uninsured citizens of Albany County, offering medical care. Since all the patients at the DTC live below the poverty line, many suffer from low food security and are unable to access or afford fruits, vegetables and high quality proteins. As a result, their diets are high in carbohydrates, refined sugars, and fats. Previous studies have shown a correlation between poor nutrition and various metabolic and neurological conditions[2]. Specifically, anxiety and depression are amongst the most common of these conditions seen at the DTC[3].

Zhou and Foster have shown that the microbiome has led to connections between the gut microbiome and proper neurotransmitter functioning in the brain connected to neurological disorders like anxiety and depression[4]. In fact, bacteria produce tryptophan, a precursor of serotonin, tyrosine, a precursor of dopamine, and other amino acids like glycine that help with neurotransmission in mammals[5]. However, not all microbiota produce these beneficial effects for improved mood. *Lactobacillus rhamn*osus, *Lactobacillus helveticus*, and *Bifidobacterium longum*[4], are gut microbiota, termed psychobiotics, that have been associated with reducing anxiety and depression-like symptoms. Consequently, there is a probiotic supplement on the market today to improve mood called Essential Probiotics. However, an easy to make and affordable probiotic food product that includes these psychobiotics does not exist. Therefore a novel probiotic kombucha will be augmented with these psychobiotics in order to ultimately provide the DTC patients with a recipe which will reduce anxiety and depression-like symptoms in their daily lives.

Objectives

- To enrich the SCOBY (Symbiotic Culture of Bacteria and Yeast) of kombucha with probiotics specifically associated with anxiety and depression so that we can provide the recipe to DTC patients.
- To determine if psychobiotcs, *L. rhamnosus*, *L. helveticus*, and *B. longum*, can be successfully inoculated into a kombucha drink.



Hypotheses

1). If an affordable, easy to make probiotic drink, such as kombucha, can be supplemented with probiotics *L. rhamnosus, L. helveticus*, and *B. longum* in the laboratory, then the microbes will grow to a titer reported in the literature to be efficacious in anxiety and depression symptom relief.
2). If the SCOBY from the psychobiotic kombuchas is re-dropped into fresh tea, then it will have taken up the previous psychobioitcs from the pill and thus psychobiotics will be observed in the the new kombucha.
3). If we inoculate kombucha with psychobiotics, then the kombucha will still successfully brew to completion and taste good.

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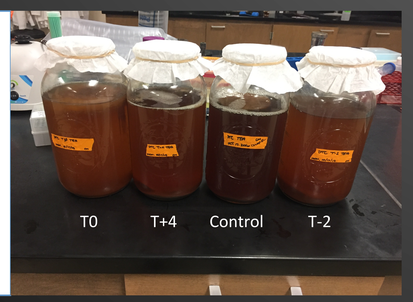

Results

All of our kombuchas throughout our experiments successfully fermented to completion and retained an ideal kombucha taste. The fermentation period ranged from 8-12 days and the average end pH was 2.8. When compared to our control, the addition of the psychobiotics had little to no effect on the fermentation period, end pH, and taste. Using our midpoint samples, we were able to calculate a titer for *Zygosaccharomyces* for all of our original kombuchas except the T0 batch. The remainder of the organisms did not grow on our enumeration plates. At the end of our first fermentation, *Gluconacetobacter* was isolated from all timepoints. *Zygosaccharomyces* was isolated from all the timepoints except T-2. T0 showed the highest percentage of *L. rhamnosis* and *L. helveticus*. We were unable to isolate *B. longum* from any of our kombucha samples (see Figure 1). Figure 1 also shows the highest titer achieved and it's respective batch. The presence or absence of each organism was tested for after our second batch of kombucha had completed. The results are shown in Table 1.

Presumed psychobiotic bacteria formed puffy white and medium red colonies that tested positive for sucrose and maltose and negative for sucrose and maltose respectively.

Organism	T0	T+4	T-2	Control
Glucanoacetobacter	+	+	N/A	+
Zygosaccharomyces	+	+	N/A	+
B. longum	_	_	N/A	-
L. rhamnosus	+	+	N/A	+
L. helveticus	+	_	N/A	+

Table 1: Presence/ absence of organisms in re-drop kombucha. *The T-2 did not finish fermenting before the last day of lab. ** There were two colonies growing on the control MRSCR plate, we did not identify these colonies.

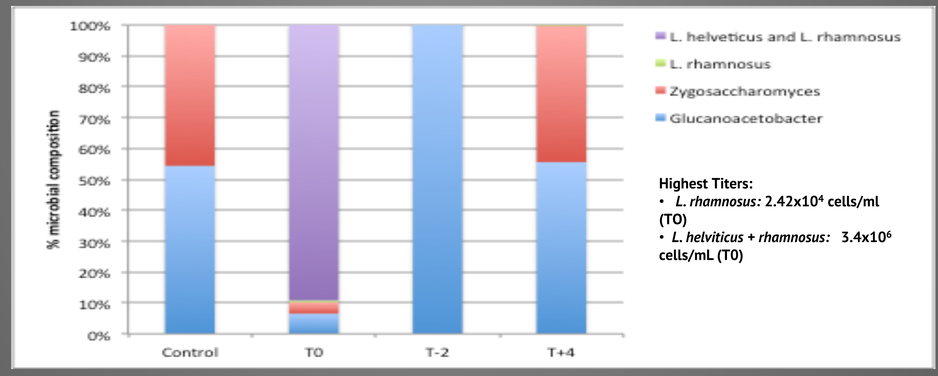

Figure 1: End percentage of microbial composition of each kombucha treatments.
Percent microbial composition of each kombucha treatment. * *L. rhamnosus* was present in the T+4, but in a very small percentage that cannot be seen in the graph (0.22%)

Discussion

We reject hypothesis one. Messaoudi et al determined the efficacious dose of psychobiotics to be 1x10^9 cells/mL [13] . As seen in figure one, our titers did not reach these levels. We believe that these titer values were not reached due to prolonged refrigeration of our samples. In regards to *B. longum,* in traditional kombucha, it is present at the beginning of fermentation, but is eventually killed off due to low pH. We believe that the pH of our kombucha was not suited to its growth [14]. We partially accept hypothesis 2. As seen in Table 1, both the T0 and T+4 contained the *Lactobacilli* species. However, the T0 contains both *Lactobacilli* while the T+4 does not. The T0 batch was most effective at uptaking the psychobiotics. In our control, colonies grew on our MRSCR plate. We know that *Lactobacilli* are common SCOBY bacteria [14]. We also did not perform biochemical tests on these colonies, and can therefore not confidently identify them. We accept hypothesis number 3. Our original kombuchas as well as our re-drops successfully fermented to completion. The experimental kombuchas had a taste that resembled our initial control batch, being sour and carbonated, similar to sparkling apple cider. The pH continuously dropped, signifying successful fermentation.

Biochemical tests were used to differentiate between our two *Lactobacilli species*[12]. Our results allowed us to suspect that the white puffy colony was *L. rhamnosus*, as it successfully fermented sucrose and maltose; while the medium red colony was *L. helveticus*, as indicated by a lack of fermentation. We believe that we successfully identified two of our psychobiotics after they were inoculated into our kombucha. However, due to time contraints, we were unable to enumerate *L. helveticus* on its own.

As indicated in Figure 1 and Table 1, the T0 kombucha was shown to have the highest titer count of psychobiotics, while also retaining the traditional kombucha taste. Also, in both of the experimental trials the T0 kombucha fermented to completion the fastest and had the most carbonation. Even so, kombucha still contains valuable probiotics, while remaining cost effective. Therefore, we feel confident that we can provide the DTC an alternative form of probiotic supplementation.

Methods

We prepared a starter tea that was composed of black tea and a sugar concentration of 0.14 g/mL[6]. This allowed us to develop a taste metric, pH standard, and timeline for the completion of the kombucha. Next, the novel experimental kombuchas were brewed. The SCOBYs were all added at day 0 to the sweet black tea, while the Essential Probiotic was added: two days before (T-2), four days after (T+4), or the same day (T0) as the SCOBY. Another control batch was also brewed and was started on day 0. During the process of fermentation, the pH and taste were monitored. Later, all of the kombuchas were enumerated on day five and at the end of the fermentation process. Five media types were used to enumerate all organisms in each kombucha:

- Hestrin- Schramm – Glucanoacetobacter[7]
- Potato Dextrose Agar + tetracycline (50µg/mL) – *Zygosaccharomyces*[8]
- *Bifidobacterium* (A novel medium containing propionic acid, methylene blue and bromophenol blue) - Bifidobacterium longum[9-10]
- de Man, Rogosa and Sharpe + Congo Red (MRSCRV) + vancomycin (3µg/mL) - Lactobacillus rhamnosus[11]
- MRSCR- *L. rhamnosus* and *L. helveticus*[11]

To differentiate between the two Lactobacilli colonies found on the MRSCR agar plates, sucrose and maltose sugar fermentation tests were used[12]. A puffy white colony and a medium red colony were pulled from the T0 -5 MRSCR plate and used for the previously mentioned biochemical tests. In the latter half of our experiment, we reused the SCOBYs from the previous experiment, and re-dropped them into a new batch of tea. We then looked for the presence or absence of the psychobiotics in each batch using the above media.

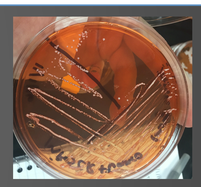

Figure 2: Suspected *L. rhamnosus growth on selective agar*

Conclusion

- The T0 kombucha had the best viability of augmented psychobiotics, while retaining a delicious kombucha taste; therefore, we recommend this timepoint for further uses.
- For a tasty kombucha, the following recipe is recommended for the patients of the Laramie Downtown Clinic:

✧ For 0.5 gallons of water, steep 4 black tea Lipton Tea bags for 4 minutes to make the tea.
Add 255 g of sugar to the tea.
Add 1 pill of Essential Probioitics, and 1 SCOBY(obtained from Amazon) on the same day to the sweetened tea.
Ferment the kombucha for 11 days.

- In the future, we would like to do enumerations of the re-drop kombuchas. We also would like to replicate our experiments and see if similar results are obtained.

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