In recent years human gut flora has been proven to effectively be associated with numerous psychological and physical problems like Depression and irritable bowel syndrome (IBS). Gut flora undergoes drastic fluctuations to maintain the balance between the trillions of microbes that inhabit the gut. While maintaining homeostasis the gut flora releases numerous molecules that function with gut cells and nerves, such as Brain-derived neurotrophic factor (BDNF) and gut-brain axis (GBA) which benefit the human body in a symbiotic way. There are, of course, some bacteria that impact the body in a negative way, producing molecules that are harmful to the mind and body. An imbalance in the gut flora can cause detrimental effects on the human body and mind, such as in patients with Major Depressive Disorder who have significantly higher populations of Actinobacteria and lower populations of Bacteroidetes. With traditional ways of treating infections, like antibiotics, the mechanism of action isn’t specific enough to only target pathogens. This study was designed to directly compare growth of Probiotic vs MDD associated bacteria in a simulated intestinal tube by inoculating tubes simultaneously with equal volume of each. Then letting them compete one vs the other in normal flora unintentionally. This was done to minimize microbial colonization known as the Restaurant Hypothesis. Statistically significant data was found when B. bifidum was used to inhibit both C. aerofaciens and A. israelii the MDD associated bacteria, when grown on BHI plates. C. aerofaciens was also shown to be inhibited with the PCR assay. After being added in equal concentrations L. acidophilus and B. bifidum consistently grew more and had a lower CT value. This means that their bacterial concentration was higher compared to C. aerofaciens.

**Introduction**

The human microbiome has a major impact on a person’s wellbeing, physically and mentally. Research has shown that the gut microbiome is especially influential, and that changes in the gut microbiome cause changes in receptors in the brain (Lach et al., 2018) and has been shown to have links specifically to depression (Sharan et al., 2016). This study will deal with how beneficial enteric flora, specifically Bifidobacterium bifidum and Lactobacillus acidophilus, affect the populations of bacteria that are present in Major Depressive Disorder, specifically species that belong to the two phyla: Actinobacteria and Bacteroidetes.

The Effects that Major Depressive Disorder (MDD) has on the host’s gut microbiome is explained best by analyses of the microbiomes of human subjects with MDD, and it was found that significantly higher Actinobacteria and lower Bacteroidetes species were present in these patients (Zheng et al., 2016). Fecal transplants from these patients were performed on mice. After the transplant, the mice began to show symptoms of MDD (Kelly et al., 2016). Normalizing the microbiome eradicated these symptoms, especially if done at an early age (Tetel et al., 2018). This suggests that there is a connection between these species of bacteria and symptoms of MDD. There was increased activity in the hypothalamic-pituitary-adrenal axis (HPA) stress response and anxiety and depression symptoms in those with the different microbiome.

Testing was conducted on the inhibitory effects of Lactobacillus acidophilus and Bifidobacterium bifidum against Collinsella aerofaciens, Actinomyces israelii, Alpistipes shahii and Bacteroides fragilis by inoculating the center of Brain Heart Infusion (BHI) plates with 25 microliters of a broth culture of BHI, incubating anaerobically, and then spraying with a sterile perfume diffuser a diluted (OD600 0.3 ± 0.05) broth of an MDD associated bacteria and quantitatively, and qualitatively, measuring inhibition. Additional experiments will include mixing the two groups together in a simulated gut tube and measuring differences using SBA plates and PCR to quantify the proportional inhibitory relationship between the mixtures.

**Methods**

BHI plates were inoculated in the center with probiotic species by pipetting a concentration of an OD_{600} 0.3 ± 0.05 in BHI broth to measure competitive inhibition when the MDD (Major Depressive Disorder) species were sprayed through a sterile perfume bottle onto the plates, after they were inoculated in BHI broth to the same dilution of the probiotics. L. acidophilus and B. bifidum were the probiotics, B. fragilis, C. aerofaciens, A. shahii, and A. israelii were the MDD species used. Inhibition was measured using a ruler, to the nearest 10⁻¹ millimeter, and the quality of the inhibition was also noted.

Inhibition between the probiotic vs. MDD bacteria was also done by inoculating gut simulation tubes with one probiotic and one MDD bacteria in equal concentrations. The gut simulation was treated with UV for 1hr to minimize flora contamination, prior to inoculation. The organisms were evaluated in two conditions – the probiotic plus the normal flora of the gut sim. tube vs the MDD bacteria and a single bacteria vs the normal flora of the gut sim. tube.

qPCR was used to measure concentrations of each bacteria. CT values were used to compare the different concentrations.

**Discussion**

The gut microbiome of an individual has potential to influence every system in the body, including the brain. After a disruption of the gut microbiome, deleterious effects can become apparent, and these effects can influence symptoms of mental health disorders, such as MDD. Overall results of decreased FCs for the MDD species may be due to space and resource limits of the bacteria grown together. A. israelii and A. shahii showed the most significant decreasing FCs, revealing that when grown with probiotics it will effectively inhibit them, giving mixed results since it decreased both phyta instead of just the Actinobacteria phylum. Additionally, C. aerofaciens showed a significant increase when grown with B. bifidum. Results from experimentation conducted in this study have shown beneficial microbial flora have potential to influence microbial species related to the gut microbiome of a person with MDD.

While more studies need to be done to expand the understanding of interactions between these bacteria, the results of this study are both encouraging and cautionary. Significant inhibition was seen, but not as initially intended. Probiotics could be a new supplemental treatment of MDD with less side effects than current treatments, however more research needs to be done to confirm their effectiveness. This experiment could be improved upon using an additional freeze-dried step on the gut mix to improve accuracy, and improved simulation of real-life situations.

**Limitations**

- This experiment was limited by restrictions due to the COVID pandemic, as well as time, and funds. This experiment could be improved upon using Tagman with the PCR, an additional freeze-dried step on the gut mix to improve accuracy, and improved simulation of real-life situations.
- Removal of normal flora would also provide a clearer picture of the effect of each probiotic.

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