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Flowers or Flora?: Understanding the Effects of Probiotics on Depression

Nicole Collopy
Dominican University of California

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Flowers or Flora?

Understanding the Effects of Probiotics on Depression

Nicole Collopy

Dominican University of California

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Abstract

The human gut is populated with hundreds of types of bacteria and fungi. In North America, both intestinal and mood disorders are prevalent, presenting health problems that challenge health care professionals and patients alike. A scholarly literature review was conducted to explore this important relationship.

The investigation of the literature revealed that there is an association between gut health disorders and mood disorders, mainly anxiety and depression. This is due to *bi-directionality* between the brain-gut pathways, meaning that gastrointestinal health may have an effect on mental health and vice versa.

This connection between gut and mood can have substantial implications for the treatment of both GI and mental health patients. Probiotics, bacteria that are beneficial to the body, have grown in popularity as a gut health supplement and may have an effect on the brain. Studies were conducted to test whether specific gut flora have effects on mood.

There were many differences identified between the gut flora of those with and without major depressive disorder. Specific bacterium, such as *Lactobacillus plantarum* P8, *Bifidobacterium longum* NCC300, and *Lactobacillus rhamnosus* HN001; have undergone randomized, double-blind, placebo-controlled trials and have been found to decrease feelings of depression as well as anxiety. Different probiotic strains have different effects on the mind, and these findings may be among the newest avenues of prevention and/or treatment for depression and anxiety. The next step will be additional research to validate these findings.

Acknowledgements

I would like to thank my professor, Dr. Patricia Harris, for all of her guidance and support throughout the process of writing this thesis. Without her, I would have been truly lost and overwhelmed. I would also like to thank my parents and younger sister for supporting me throughout my education and always pushing me to strive for excellence. I dedicate this research to my late sister Gracielle and all of those who suffer from mental health disturbances.

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Introduction

Have you or has someone you've known suffered from depression and anxiety? Chances are, we all know someone who has had their life affected by these disorders; and we have all experienced these feelings on some level. What if the key to unlocking these problems lied within every one of us -- inside of our gut? The research topic being explored within this paper is the relationship between gut health and mood disorders. The question of interest is: Does the use of probiotic supplementation have an effect on feelings of depression?

The connection between gut health and mental health should be of great significance as approximately 6.7% of adults in the United States suffer from major depressive disorder (ADAA, 2018). There has been an observed association between gut health disorders and mood disorders, mainly anxiety and depression, with a higher prevalence in subjects with inflammatory bowel diseases (IBD) compared to subjects without IBD. At the same time, those who demonstrate anxiety and depression have an increased likelihood of developing abnormal gastrointestinal (GI) symptoms. This raises the possibility of *bi-directionality* between the brain and the gut, meaning that GI health may have an effect on mental health and vice versa. Due to the prevalence of both of these issues in North America, this relationship is important to explore as it can have large implications for the treatment of both GI and mental health patients. For example, adding psychological therapies to the treatment regime of those with IBD may lower their risk of disease relapse and reduce need for pharmacological treatment. Probiotics, bacteria that are beneficial to the body, have grown in popularity as a gut health supplement which may have an effect on the brain. There is much reason to suspect and explore the relationship between probiotic supplements and depression.

Literature Review

The objective of this research was to find evidence that supports the existence of brain-gut bidirectionality, and evidence that supplementing probiotics has an effect on the brain and feelings of depression. This review of literature was compiled using multiple peer-reviewed journals found through the Dominican University of California library databases, including ScienceDirect and CINAHL. Eight articles were selected to give a holistic view of the brain-gut pathway and how probiotics affect the brain. The literature review will be divided into three categories. First, articles that discuss the pathway between the brain and gut. Second, articles that assess gut flora and how it affects mood. And third, articles in which probiotics are tested to determine their effects on mental function. Study designs and methods vary between the articles and will be described throughout the literature review. See the Literature Review Table in Appendix A for a summary of each article

The Brain-Gut Pathway

The articles chosen for this portion of the literature review contain data that support the existence of the brain-gut pathway. Gracie, Guthrie, Hamlin, and Ford (2018) are credited IBD researchers that wanted to answer the question: do mood disorders increase signs and symptoms of inflammatory bowel disease and vice versa? These researchers conducted a longitudinal study of 405 patients diagnosed with inflammatory bowel disease or ulcerative colitis. Data was collected at baseline and again after two years. This included IBD diagnosis, symptoms, somatization, fecal calprotectin levels (indicative of IBD flare ups), anxiety and depression data, and hospitalizations for IBD. The researchers used many standardized scales to compare their data to a control without IBD; and analysis revealed that the brain-gut pathway did, in fact, exist. Although the study contained a low prevalence of depression, results showed that anxiety was

associated with the later development of IBD flare-ups; and patients who were diagnosed with IBD with no anxiety at baseline showed abnormal anxiety later in the study. This was one of the first studies to prove that the brain and gut interact bidirectionally, and was the inspiration for this research paper.

The research team led by Kappelman, Long, Martin, Kinneer, and Chen (2013) used an incredibly large cohort of patients with inflammatory bowel disease to analyze and evaluate the association between patient-reported outcomes and disease activity. In 2014, this team recruited 10,634 individuals through the Crohn's and Colitis Foundation of America. They performed a cross-sectional, longitudinal analysis of disease activity indices, patient recorded outcomes, and disease-specific quality of life instruments. These researchers used the Patient Reported Outcome Measurement Information System (PROMIS) to compare the data from IBD patients with the general population. They used other standardized scales such as the Simple Clinical Colitis Activity Index and the Short IBD Questionnaire to track disease activity for each specific type of IBD. Compared to the general population, patients with IBD reported more depression, anxiety, sleep disturbance, and social interaction. All six of the PROMIS domains indicated that functioning became worse as disease activity increased, and became better as disease activity decreased. Although this study was specific to IBD patients, the use of such a large sample size greatly increases the credibility and reliability of the study, making the results more applicable to the general population.

Both of the articles in this portion of the literature review focus on IBD patients, but the findings can be taken and applied to the general population. Whether abnormal levels of anxiety and depression are a result of inflammatory bowel disease itself or simply a secondary byproduct of intestinal discomfort, it is clear that there is a connection between gut health and mood.

Gut Flora and Mood

Since it has been established that there is a connection between gut health and mood, it is natural to delve further into the gut and question what exactly is acting on the brain. Within the gastrointestinal system lives an abundance of bacterial flora that aid with digestion, communicate with the immune system, and produce vitamins. In this portion of the literature review, we will examine different strains of bacteria found in the gut, and their connection with mood.

In the beginning of 2019, a Taiwanese study was published with the aim of identifying microbiota targets for major depressive disorder and mood-related traits (Chung et al., 2019). The researchers recruited thirty-six patients with major depressive disorder (MDD) and thirty-seven subjects without MDD. Depression, anxiety, and stress symptoms were obtained in the initial interview using the Beck Inventories and the Perceived Stress Scale. Then, subjects were administered a food questionnaire that contained information about six basic food groups, sugar, and diet supplements. From this the researchers were able to analyze the amount of carbs, fats, proteins, and fatty acids being consumed by the subjects based on the frequencies in which they ate certain foods. Within two weeks of the initial interview, stool samples were collected and microbiota DNA was extracted using phenol-chloroform or the QIAamp DNA Stool Mini Kit. Although the sample size for this experiment was small, extensive "16S ribosomal RNA gene sequencing was conducted" (Chung et al., 2019, p. 76).

The results of this study reveal multiple targets for depression. This includes four phyla, seven families, and twelve genera (Please see Appendix B). At the phylum level, Actinobacteria was found in higher prevalence within MDD patients than with controls. Within the family level, Streptococcaceae and Bifidobacteriaceae were abundant in patients with MDD while

Prevotellaceae was more abundant in the healthy controls. Finally, at the genus level *Clostridium XI, Parabacteroides, Blautia,* and *Prevotella* were found to be associated with MDD. This was along with *Holdemania, Eggerthella, Streptococcus,* and *Sutterella,* which the authors note as a similar trend from their research.

The findings from this study are unique and groundbreaking for this topic. Although the data was collected from a small study of people in Taiwan who may have different lifestyles and dietary habits from people in other parts of the world, the findings are an excellent foundation for understanding which bacteria affect major depression.

In 2017, a large group of Italian researchers led by Guida performed clinical studies to examine the effects of imbalanced microflora on the gut and brains of mice. Their study was approved by the Animal Ethics Committee of the Università della Campania of Naples. The control group of mice drank water for fourteen days while the test group drank sterile water mixed with Ampicillin, Streptomycin, and Clindamycin (ASC). The mice underwent a multitude of tests to determine depressive-like behavior, motor coordination, muscle strength, recognition memory, working memory, nociceptive pain, and sociability. A Western blot analysis was done on brain tissue and samples were collected from each mouse for microbiota analysis and inflammatory mediator evaluation.

Analysis of the data shows that the administration of antibiotics to perturb natural gut microbiota leads to "depressive-like behaviour and impaired social activity associated with biochemical and functional changes in the hippocampus" (Guida et al., 2017, p. 240). The consumption of the ASC mixture created a floral imbalance with a loss of bacterial diversity. This increased the presence of Proteobacteria and Actinobacteria in the gut which the authors believe led to hippocampal rearrangement and depressive behavior. Although this study was

done on mice, this is the first step to understanding how bacteria can be affecting the human

brain and how this relates to depression.

In both of the studies mentioned, the findings indicate that the microbiota of subjects

experiencing depression differ from subjects without depression. Actinobacteria was

significantly more prevalent in humans with major depressive syndrome as well as mice with

depressive behaviour. Of course, more research can be conducted in this area, specifically

targeting Actinobacteria or some of the other bacteria correlated with depression. However, both

of these studies provide a clear understanding that the intestinal flora of depressed subjects are

dysbiotic compared to non-depressed subjects. From this information one can now ask: what if

there was a way to treat this?

Using Probiotics As Treatment

The following articles involve trials using probiotic supplements. The use of probiotics is

growing in popularity as a potential future medical treatment and as a daily health supplement.

There is still much to learn about different strains of bacteria and what effects each one has on

the brain. The following articles contain recent experiments attempting to learn more about them.

Lew and his team conducted randomized, double-blind, placebo-controlled trials using

Lactobacillus plantarum P8 (2018). The goal of the experiment was to investigate the effects of

probiotics on the alleviation of stress in stressed adults. Subjects were tested for psychological

distress using the aforementioned Perceived Stress Scale questionnaire, along with the

Depression, Anxiety, and Stress Scale (DASS-42), every four weeks. Memory and cognitive

functions were tested at baseline and at the end of the twelve week study. Blood samples were

also collected at these times to analyze cortisol, cytokines, and full blood count. 132 subjects

were originally recruited for the study but only 103 fully complied until the end. By the end of

the study, most of the effects of treatments (P8 and placebo) were insignificantly different from one another. However, even though both treatments reduced stress levels from "moderate" at baseline to "normal" at week 12; during week 4, 8, and 12 the P8 group had much lower stress than the control. Closer analysis of the DASS-42 over this time period attributes this to reduction of "touchiness", reduced irritation, increased calmness, and increased tolerance against interruptions. This goes to show that *L. plantarum* P8 may have a potential future as an anxiolytic supplement.

In *Stress matters: Randomized controlled trial on the effect of probiotics on neurocognition,* the team set out to investigate the effects of a multispecies probiotic on the neurocognitive measures of emotion (Papalini et al., 2019). They wanted to test whether probiotics could be used to buffer against the effects of stress on memory. This was a randomized, double blind, placebo-controlled trial that utilized fifty-eight healthy patients. Each participant was assessed before treatment and again four weeks later. During these assessments they performed a working memory test and received an MRI measurement. Some of the tests included the *emotional face-matching paradigm* which tested for emotional reactivity by choosing words to match pictures of faces, the *color-word stroop paradigm* where subjects were to choose the color of the word ink while ignoring what the word says, and the digit span test where subjects had to repeat numbers forwards or backwards. During the four week period subjects were instructed to take the probiotic every day.

The results of the questionnaires and MRIs did not appear to be different between the probiotic and placebo group. There was, however, a difference in stress-induced working memory between the groups as the probiotic groups had increased performance on their second

stress-induced backward digit span performance. These results indicated that the group taking probiotics had developed a larger buffer against the negative effects of stress on their memory.

Pinto Sanchez and her team conducted research on the probiotic *Bifidobacterium longum* NCC3001 to evaluate its effects on anxiety and depression for patients with irritable bowel syndrome (2017). This study was mentioned multiple times throughout other research and was used as a guiding framework for other probiotic studies. Data was collected from forty-four IBS patients at baseline, six weeks, and ten weeks. To qualify, the subjects needed a diagnosis of IBS, needed to be experiencing diarrhea or mixed-stool patterns, and had to have anxiety and/or depression. Anxiety and depression were analyzed by the Hospital Anxiety and Depression Scale. Blood, stool, and urine samples were collected to test for inflammatory markers and microbiota profiles. MRIs were used to assess brain activity.

After six weeks, the patients in the *B. longum* group had decreased depression scores compared to the placebo group. There was no changes found for patients with anxiety. Sensitivity analysis was used to understand the relationship between B. longum and depression and the theme was that lower depression was more likely to occur with those who felt adequate relief from IBS symptoms. MRIs showed that the probiotic group showed reduced engagement in the amygdala, frontal, and temporal cortices in response to fear stimuli. There was also heightened engagement of the occipital regions in response to fear. With these results, the authors drew the conclusion that *Bifidobacterium longum* NCC3001 decreased depression scores, but not anxiety scores. They go on to discuss how depression and anxiety are related to amygdala hyperactivity but both SSRIs and *B. longum* were able to benefit the patient by decreasing its activity. This goes to show how probiotics may be able to take a stand in the world of antidepressants.

The final article to be reviewed is *Effect of Lactobacillus rhamnosus HN001 in Pregnancy on Postpartum Symptoms of Depression and Anxiety: A Randomised Double-blind Placebo-controlled Trial* (Slykerman et al., 2017). These trials were conducted in order to evaluate the effect of the bacteria *Lactobacillus rhamnosus* HN001 when administered during pregnancy. The team specifically wanted to evaluate the effects of the bacteria on maternal anxiety and depression during the postpartum time period. This study received approval from the New Zealand Multi Regional Ethics Committee and was deemed safe for pregnancy. 423 women were recruited for this study and were randomly split into a HN001 group and a placebo group. The mothers were interviewed at 14-16 weeks gestation as their baseline. They received the probiotic or placebo from the initial interview all the way until six months postpartum if breastfeeding. The Edinburgh Postnatal Depression Scale and State Trait Anxiety Inventory (6 item) were used to help mothers describe their psychological wellbeing from when their child was one to two months old. This was done at six or twelve months postpartum.

At the trials' conclusion, 380 participants completed the study. Eleven of the mothers completed the questionnaires at the six month visit and 112 completed them at the twelve month visit. Mothers who received probiotic treatment reported significantly lower depression and anxiety scores than the placebo group. These scores tended to increase the longer that the mother took to complete the questionnaire. The researchers believe that this is because many mothers expect to be tired during their first year of motherhood and do not realize that they may be depressed. It may be hindsight that allows the mothers to realize how depressed and anxious they were feeling in the first two months postpartum. The number of women who reported clinically significant depression above the standard cut-off point did not differ between the HN001 group and the placebo group. However, women receiving the HN001 treatment were significantly less

likely to have anxiety above the cut-off point. This study is one of the first of its kind as it studies

the effects of probiotics on pregnant women and postpartum depression. This study could have

been more accurate if they had collected questionnaire data from mothers all at the same time, or

if they administered the questionnaires to each mother twice. This study has great implications

for treatment of postpartum depression and the future of antidepressants.

Literature Review Conclusion

Satisfactory evidence was found on the primary question of interest, "does the use of

probiotic supplementation have an effect on feelings of depression?" According to the research,

the use of different probiotics can have multiple different effects on feelings of depression

including relief of stress, increased calmness, reduced irritation, improved working memory

under stress, and reduced amygdala activity. In addition, certain strains of probiotics also have an

effect on anxiety and possibly IBD flare ups. *Lactobacillus plantarum* P8, *Bifidobacterium*

longum NCC300, and *Lactobacillus rhamnosus* HN001 are three specific probiotic strains that

have demonstrated effects on depression and anxiety.

The greatest limitation that I have found within the studies is the small sample size within

all except Kappelman and Lew's studies. Data is not as reliable within such small samples, but

because this research is so new it is an acceptable framework. All of the data collected from each

team was located within a specific geographic radius. This can be considered a limitation

because the dietary habits and lifestyle elements of different regions can have an effect on the

microbiota that form in their subject's studies. This is a consideration to keep in mind while

forming future experiments and studies if one is hoping to focus in on a certain group of people.

Although this research is paving a new route for medical treatments, I do not believe it is

worthy of guiding standard practice quite yet. With the introduction of new bacteria to people's

bodies can come new risks and adverse effects. While many practitioners may agree that

probiotics are a safe dietary supplement, more research needs to be done before it can be

recommended as an alternative to SSRIs. There is no proof that probiotics can cure depression or

that they work better than traditional antidepressants, but it may be worthy of mentioning for

patients newly diagnosed with depression. Or even to neurotypical people who may be feeling

some of "the blues." I believe the research is worthy of guiding future experiments on the topic.

A gap that I have identified within this research is more specified trials to determine

which probiotic strain would be the absolute best for decreasing depression. This gap is not

surprising considering how novel the topic is, and within the next decade there are bound to be

many more studies revealing which probiotics work the best.

Proposal for Further Study

The gap that I have identified within my research are a lack of studies specifically on the

probiotics that have been identified for having effects on depression. By doing further studies on

these probiotics, such as *Lactobacillus plantarum* P8, *Bifidobacterium longum* NCC300, and

Lactobacillus rhamnosus HN001, we can determine whether certain strains can be used as

antidepressant therapy. The research study that I would like to propose is a double-blind,

randomized, placebo-controlled clinical trial comparing the effects of specific probiotics on

feelings of depression. The primary research question is: Which probiotic decreases symptoms of

depression in the fastest and most significant way - *Bifidobacterium longum* NCC300,

Lactobacillus plantarum P8, or both?

As stated in the review of literature, *Bifidobacterium longum* NCC300 was correlated

with decreased depression. This may be linked to the fact that the probiotic decreases amygdala

activity, which controls the autonomic nervous system and feelings of fear. Selective serotonin

reuptake inhibitors (SSRIs) are similar to NCC300 in this way. This could imply that higher

doses of NCC300, or doses of NCC300 combined with other elements, could be a competitor

with SSRIs for the treatment of depression. In the study by Lew, *Lactobacillus plantarum* P8

was not being specifically tested for its effect on depression, but it was correlated with "the

alleviation of selected stress, anxiety, memory and cognitive symptoms in stressed adults"

(2018). All of these factors play a role in clinical depression, so it is not unreasonable to

hypothesize that P8 could have antidepressive effects. For this proposed study, research should

be conducted to determine the efficacy of specific, commonly-used SSRIs. Standardized scales

should be identified so the efficacy of NCC300 and P8 can be compared. By doing this, these

probiotics and SSRIs can be compared to see whether they have similar effects on the feelings of

depression.

The primary aim of this quantitative research is to investigate the effects of

Bifidobacterium longum NCC300, *Lactobacillus plantarum* P8, and the two combined on

feelings of depression. Another aim of this study is to compare data collected about the efficacy

of NCC300 and P8 as antidepressants with the efficacy of commonly used SSRIs such as Celexa,

Lexapro, Prozac, and Zoloft.

This study would absolutely be cleared by an institutional review board to ensure that

subjects are protected from harm. The Dominican University of California has an Institutional

Review Board that would be able to do this, but due to the nature of the experiment, extra

institutional reviews would be beneficial. With the use of human subjects there is always a risk

of bringing harm to the subject. Because this study involves the supplementation of bacteria,

extra precautions and screening must occur to ensure that no adverse reactions will come to the

patient, especially those with comorbidities. Before taking any part in the study, all subjects will

need to be educated on what the study will entail and sign a consent form to participate.

The population of this study will be patients age 18-65 who have been diagnosed with

depression. Inclusion criteria will be a diagnosis of depression without any current

pharmaceutical treatments for mental health and the ability to carry out the full experiment.

Exclusion criteria would include current SSRI treatment, current antibiotic therapy, recreational

drug use, body mass index that is severely out of the healthy range, severe medical illnesses, or

mental illness that interferes with the subject's ability to participate or understand the study. For

this particular study, purposive sampling can be used and subjects can be recruited through

psychologists, therapists, and psychiatrists offices. By doing this, we would be finding

participants who fit the specific criteria without having to sort through and screen every applicant

for their mental health history. From here, the applicants who have passed the screening can be

randomized. My proposed sample size would be at least 500 participants. This sample size was

chosen to account for people dropping out of the study with the intention of having at least 100

participants in the placebo-controlled group, 100 in the *Bifidobacterium longum* NCC300 group,

100 in the *Lactobacillus plantarum* P8 group, and 100 in the combination group. Subjects would

be randomized after the screening and assigned different identification numbers every time they

were assessed.

After screening is done and consent is obtained, the initial baseline interview should be

conducted. This should include the subjects' demographics, diagnoses, current medications, and

feelings of anxiety and depression based on the DASS-42 and BDI-II. The Depression, Anxiety

and Stress Scale (DASS-42) uses a 42 item questionnaire comprised of three sub-scales. Each

scale contains 14 items that assess symptoms such as dysphoria, hopelessness, self-deprecation,

loss of interests, autonomic arousal, subjective experiences of anxiety, relaxing difficulties, irritability, and impatience. This standardized scale is a useful tool because it is used in other health care settings and controls have been established. For the purposes of this experiment, it will be easier to compare subjects' results with the results from other studies. Although the focus of the study is depression, it is not uncommon to find that stress, depression, and anxiety go hand in hand and influence one another. Using the DASS-42 provides holistic information about the subjects' depression and mental state. The Beck Depression Inventory II is a standardized scale that measures severity of depressive by allowing subjects to rate their feelings on a 1-4 scale depending on severity (Please see Appendix C). This includes feelings of sadness, guilt, hopelessness, anhedonia, suicide, crying, agitation, loss of appetite, and more. By using this second questionnaire we can analyze more specified data regarding depression.

Once the initial interview has been conducted, subjects should receive random numbers assigning them to the control group, the *Bifidobacterium longum* NCC300 group, the *Lactobacillus plantarum* P8 group, or the combination group. Instructions should be given on how and when to ingest the probiotic. Subjects should be instructed to ingest the probiotic powder at approximately the same time every morning. The two questionnaires should be repeated after six weeks and again after twelve weeks. By doing this, there should be enough longitudinal data to be able to identify trends and a comparative analysis.

In order to analyze the data from the four groups; NCC300, P8, combination, and placebo; a one-way analysis of variance (ANOVA) should be performed. This form of analysis can be used to determine whether there are statistically significant differences between the four groups. Following this analysis, depression scores can be compared and contrasted with one another along with previous data collected about SSRIs.

Theoretical Framework

This framework for my proposed study comes from evidence-based physiology. Studies have demonstrated that there is a brain-gut pathway that is affected by the flora within the gastrointestinal system. Clinical trials concluded that a change in the microbiota of the gut could lead to alterations in mood such as "depressive-like behaviour and impaired social activity associated with biochemical and functional changes in the hippocampus" (Guida et al., 2017, p. 240). Studies conducted specifically about probiotic supplements have indicated a correlation between probiotics and decreased feelings of depression, anxiety, stress, and related symptoms. By understanding the quantitative data behind these studies we are able to delve deeper into these probiotics to find whether or not they can be used as antidepressant treatment. Without the research that preceded this proposal, there would not be a reason to suspect that probiotics would have anything to do with clinical depression.

Conclusion

Throughout the research, satisfactory evidence was found and the primary question of interest, "does the use of probiotic supplementation have an effect on feelings of depression?" was answered. Probiotics work on the human body when they are digested, releasing chemicals that are absorbed in the gastrointestinal tracts and work on the human brain. The effects of these bacteria can include relief of depressive symptoms along with relief from stress and anxiety. *Lactobacillus plantarum* P8, *Bifidobacterium longum* NCC300, and *Lactobacillus rhamnosus* HN001 are three specific probiotic strains that have demonstrated effects on depression and anxiety. Findings show that different probiotics have different effects on the mind, which is why much more research should be done on this topic.

Using this information, health care professionals can develop new ways of treating depression and anxiety. When a patient is newly diagnosed with depression, the use of therapy and probiotics could be the first plan of treatment before switching to pharmaceutical drugs like selective serotonin reuptake inhibitors. Or perhaps probiotics will be so well-studied that they become the more natural solution to mental health crises and replace pharmaceuticals as the standard of care altogether.

My proposed research should be one of the first stepping stones in learning whether probiotics can be used as antidepressants. Before probiotics can be considered as a formal treatment, much more research needs to be done to explore which bacteria have a negative effect on depression. Researchers need to narrow down on which strains are the most beneficial for treatment and do titration studies to determine the best dosage for patients. My study will help to narrow down and evaluate whether *Bifidobacterium longum* NCC300, *Lactobacillus plantarum* P8, or a combination of these would even be a consideration for further studies. From there, these will need to be evaluated for adverse effects, contraindications, and any drug interactions. With time, and more research, there is much hope for the future of probiotics and the treatment of patients with depression.

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Appendix A

Literature Review Table

Authors/Citation	Purpose/Objective of Study	Sample - Population of interest, sample size	Study Design	Study Methods	Major Findings	Strengths	Limitations
Exploration of microbiota targets for major depressive disorder and mood related traits Chung, Y.-C. E., Chen, H.-C., Chou, H.-C. L., Chen, I.-M., Lee, M.-S., Chuang, L.-C., … Kuo, P.-H. (2019). Exploration of microbiota targets for major depressive disorder and mood related traits. *Journal of Psychiatric Research*, *111*, 74–82. https://doi-org.dominican.idm.oclc.org/10.1016/j.jpsychires.2019.01.016	Purpose: This study aimed to identify microbiota targets for major depressive disorder and mood-related traits. Objective: Assess nutrient contents and mood related traits of subjects in order to analyze and identify microbiota targets for major depressive disorder	-Sample: 36 major depressive disorder patients and 37 healthy controls. -This study took place in Taiwan. -Purposive sampling used to find population of interest: patients with major depressive disorder	- Grounded theory	- data analysis	-Bifidobacterium(7%) and Blautia (8%) had high abundance among MDD patients -Prevotella (16%) had high abundance in controls.	- IRB approved. -extensive data was collected containing a large amount of bacterium -this study is unique and groundbreaking for this topic	-Small sample size was used. -Due to cultural dietary differences, results may be less applicable for the average person in the United States.

Bi-directionality of Brain–Gut Interactions in Patients With Inflammatory Bowel Disease. Gracie, D. J., Guthrie, E. A., Hamlin, P. J., & Ford, A. C. (2018). Bi-directionality of Brain–Gut Interactions in Patients With Inflammatory Bowel Disease. *Gastroenterology (00165085)*, *154*(6), 1635. Retrieved from https://dominican.idm.oclc.org/login?url=https://search.ebscohost.com/login.aspx?direct=true&db=edo&AN=129332010&site=eds-live	Purpose: To identify a correlation between mood disorders and IBS. Objective: Survey and analyze IBD patients to answer the question: to mood disorders increase signs and symptoms of inflammatory bowel disease (IBD) and vice versa?	-Sample: convenience purposive sample -Patients with Crohn's or ulcerative colitis	-descriptive correlational designn	-longitudinal survey and fecal analysis	-evidence of brain-gut interactions for patients with inflammatory bowel disease. -anxiety associated with development of flare ups	-Use of standardized scales/tools with identified controls -longitudinal study provides more holistic data about patient conditions	- researchers did not collect a fecal sample from all of their subjects -low prevalence of depression in the study

Antibiotic-induced microbiota perturbation causes gut endocannabinoidome changes, hippocampal neuroglial reorganization and depression in mice. Guida, F., Turco, F., Iannotta, M., De Gregorio, D., Palumbo, I., Sarnelli, G., … Maione, S. (2018). Full-length Article: Antibiotic-induced microbiota perturbation causes gut endocannabinoidome changes, hippocampal neuroglial reorganization and depression in mice. *Brain Behavior and Immunity*, 67, 230–245. https://doi-org.dominican.idm.oclc.org/10.1016/j.bbi.2017.09.001	Purpose: Examine the effects of imbalanced gut flora on the gut and brains of mice Objective: Examine behavioral, biochemical, and electrophysiological alterations, as well as gut microbiota, of mice.	-Sample: randomized sample of mice	-experimental design	-clinical studies and analyses	-changes in gut bacterial composition may cause altered responses in behavior. -hippocampal rearrangement from microbiota disruption is likely responsible for depressive behaviors in the mice	-First study to indicate that microbiota disruption can lead to hippocampal rearrangement -Materials and methods explained well -tried using different methods of administering antibiotics to rule out possibility of bias due to administration route -multiple different tests used to gain more holistic view of effects between brain and gut	-use of mice in clinical trials can be considered abusive -trials may not be completely applicable to humans due to the use of animal subjects

Evaluation of the Patient-Reported Outcomes Measurement Information System in a Large Cohort of Patients With Inflammatory Bowel Diseases Kappelman, M. D., Long, M. D., Martin, C., DeWalt, D. A., Kinneer, P. M., Chen, W., … Sandler, R. S. (2014). Original article: Evaluation of the Patient-Reported Outcomes Measurement Information System in a Large Cohort of Patients With Inflammatory Bowel Diseases. *Clinical Gastroenterology and Hepatology*, *12*, 1315–1323.e2. https://doi-org.dominican.idm.oclc.org/10.1016/j.cgh.2013.10.019	Use data to analyse and evaluate the association between Patient-reported outcomes and disease activity	-10,634 individuals participated in this study. -purposive sampling recruited through Crohn's and Colitis Foundation of America	-cross sectional, longitudinal analysis	-Analyzed patient recorded outcomes to compare with control over time -Short IBD Questionnaire used	-IBD patients reported more depression, anxiety, fatigue, sleep disturbance, pain interference, and had less social satisfaction than patients without IBD. -patients who have undergone prior colectomies have reported better outcomes than UC patients in highest quartile of disease activity	-very large sample size increases credibility and relability of information -purposive sampling from specific source provided rich, highly applicable data -used standardized questionnaires that have been used in other studies	-pain interference data was integrated later in the study, so data is only available for a portion of the population

Randomized Control Trials: Probiotic Lactobacillus plantarum P8 alleviated stress and anxiety while enhancing memory and cognition in stressed adults: A randomised, double-blind, placebo-controlled study. Lew, L.-C., Hor, Y.-Y., Yusoff, N. A. A., Choi, S.-B., Yusoff, M. S. B., Roslan, N. S., … Liong, M.-T. (2018). Randomized Control Trials: Probiotic Lactobacillus plantarum P8 alleviated stress and anxiety while enhancing memory and cognition in stressed adults: A randomised, double-blind, placebo-controlled study. Clinical Nutrition. https://doi-org.dominican.idm.oclc.org/10.1016/j.clnu.2018.09.010	Investigate the effects of probiotics in the alleviation of stress in stressed adults and identify specific strains to treat specific populations	-randomized -stressed adults -110 adult subjects were used (55 with independent variable, 55 with placebo)	-double-blind -placebo-controlled study -longitudinal	-questionnaires and blood analysis	-L. Plantarum P8 reduced some stress and anxiety symptoms via anti inflammatory properties followed by different memory and cognitive abilities. -P8 exterts different cognitive functions in men and women	-double blind and place control improve validity and decrease bias -Study analyzes specific aspects of subjects such as drug and alcohol use, gender, personality traits.	-sample size could be larger -subjects were from Malaysia. This could be a limitation since diets may be different from the US and conclusions may not be as applicable.

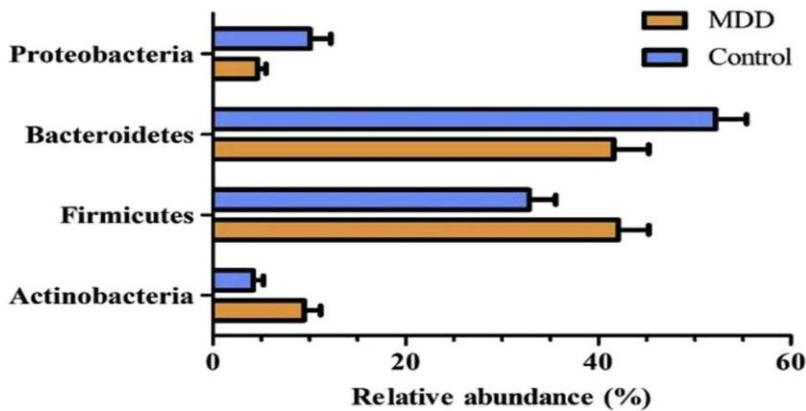|							
Stress matters: Randomized controlled trial on the effect of probiotics on neurocognition. Papalini, S., Michels, F., Kohn, N., Wegman, J., van Hemert, S., Roelofs, K., … Aarts, E. (2019). Stress matters: Randomized controlled trial on the effect of probiotics on neurocognition. Neurobiology of Stress, 10, 100141. https://doi-org.dominican.idm.oclc.org/10.1016/j.ynstr.2018.100141	Purpose: Investigate the effects of a multispecies probiotic on neurocognitive measures of emotion and test whether probiotics can buffer against the effects of stress on memory.	-Sample: 58 healthy participants -	-randomized controlled trial	-controlled trials -longitudinal -questionnaires, neuroimaging	-probiotics did not affect brain without stress-induction -with stress, probiotics show an increase in working memory performance -this is associated with intervention-related neural changes in frontal cortex	-used brain scan to collect further data on the effects of probiotic use. This is not something employed in all studies i have read -randomization and use of placebo and control group improves reliability and decreases bias	-This study excluded subjects with special diets, lactose intolerance, high alcohol use, endocrine/GI disorders, or have recently changed their diet. People with these ailments may be the people who could potentially benefit from probiotics, so including these people in the study may have been more beneficial for research purposes.

Original Research: Probiotic Bifidobacterium longum NCC3001 Reduces Depression Scores and Alters Brain Activity: A Pilot Study in Patients With Irritable Bowel Syndrome. Pinto-Sanchez, M. I., Hall, G. B., Ghajar, K., Nardelli, A., Bolino, C., Lau, J. T., … Bercik, P. (2017). Original Research: Probiotic Bifidobacterium longum NCC3001 Reduces Depression Scores and Alters Brain Activity: A Pilot Study in Patients With Irritable Bowel Syndrome. *Gastroenterology*, *153*, 448–459.e8. https://doi-org.dominican.idm.oclc.org/10.1053/j.gastro.2017.05.003	Evaluate the effects of *Bifodobacterium longum* on anxiety and depression in patients with IBS	-44 adults with IBS and diarrhea along with mild to moderate anxiety -randomized	-double-blind, placebo controlled study	-longitudinal -history taking, assessment	-probiotic BL reduces depression but not anxiety scores, increases quality of life for patients with IBS -indications that this probiotic reduces limbic reactivity	-using purposive sampling for this study was useful in studying a specific group of people and targeting specific variables -longitudinal study provides more holistic sets of data -rich data was collected on demographics and brain activation patterns	-small sample size was used

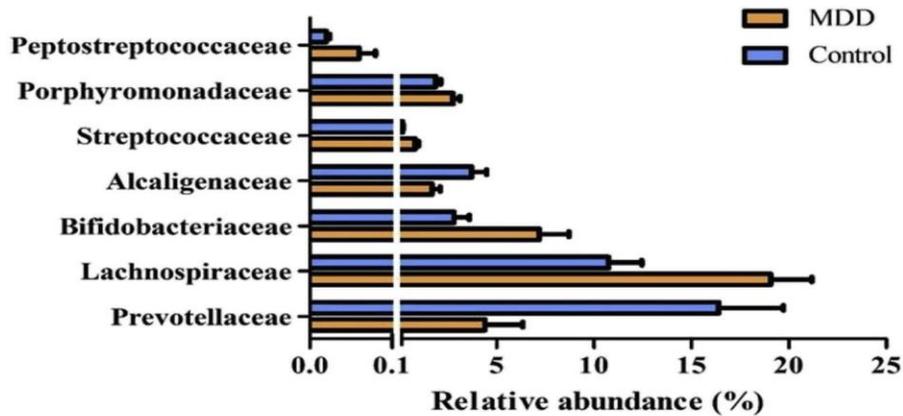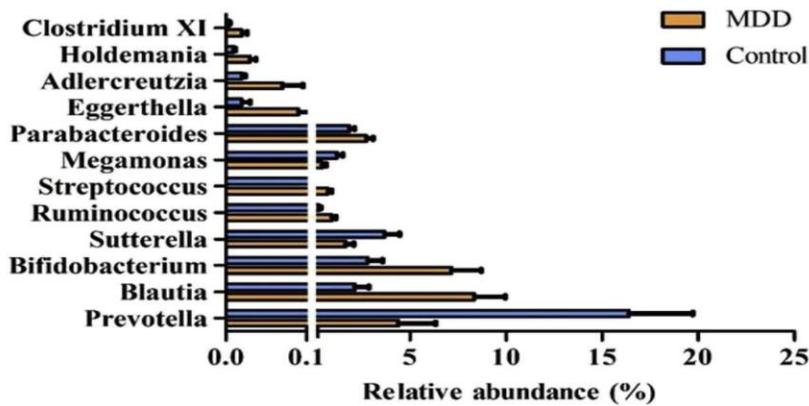|							
Effect of Lactobacillus rhamnosus HN001 in Pregnancy on Postpartum Symptoms of Depression and Anxiety: A Randomised Double-blind Placebo-controlled Trial Slykerman, R. F., Hood, F., Wickens, K., Thompson, J. M. D., Barthow, C., Murphy, R., … Mitchell, E. A. (2017). Research Paper: Effect of Lactobacillus rhamnosus HN001 in Pregnancy on Postpartum Symptoms of Depression and Anxiety: A Randomised Double-blind Placebo-controlled Trial. *EBioMedicine*, *24*, 159–165. https://doi-org.dominican.idm.oclc.org/10.1016/j.ebiom.2017.09.013	Evaluate the effect of the bacteria Lactobacillus rhamnosus HN001 when given in pregnancy. Also to evaluate its effects on maternal depression and anxiety postpartum.	-423 women at 14-16 weeks gestation -randomized	-double-blind placebo-controlled trials	-interviews at baseline -questionnaires when children were aged 6 and 12 months	-mothers in the probiotic group reported lower depression and anxiety scores.	-novel study because the subjects are pregnant women -large sample -randomized, double-blind, and placebo-controlled to boost reliability of information	-the screening tools used in this study were not made to be asked about the past. Since the mothers were reporting their feelings of anxiety and depression in retrospect, this may have caused bias

Appendix B

Phylum level

Family level

Genus level

Microbiota targets in patients with major depressive disorder and healthy controls. Adapted by Chung, et al. (2019, Section 3.2)

Appendix C

Beck's Depression Inventory

This depression inventory can be self-scored. The scoring scale is at the end of the questionnaire.

1.
- 0   I do not feel sad.
- 1   I feel sad
- 2   I am sad all the time and I can't snap out of it.
- 3   I am so sad and unhappy that I can't stand it.

2.
- 0   I am not particularly discouraged about the future.
- 1   I feel discouraged about the future.
- 2   I feel I have nothing to look forward to.
- 3   I feel the future is hopeless and that things cannot improve.

3.
- 0   I do not feel like a failure.
- 1   I feel I have failed more than the average person.
- 2   As I look back on my life, all I can see is a lot of failures.
- 3   I feel I am a complete failure as a person.

4.
- 0   I get as much satisfaction out of things as I used to.
- 1   I don't enjoy things the way I used to.
- 2   I don't get real satisfaction out of anything anymore.
- 3   I am dissatisfied or bored with everything.

5.
- 0   I don't feel particularly guilty
- 1   I feel guilty a good part of the time.
- 2   I feel quite guilty most of the time.
- 3   I feel guilty all of the time.

6.
- 0   I don't feel I am being punished.
- 1   I feel I may be punished.
- 2   I expect to be punished.
- 3   I feel I am being punished.

7.
- 0   I don't feel disappointed in myself.
- 1   I am disappointed in myself.
- 2   I am disgusted with myself.
- 3   I hate myself.

8.
- 0   I don't feel I am any worse than anybody else.
- 1   I am critical of myself for my weaknesses or mistakes.
- 2   I blame myself all the time for my faults.
- 3   I blame myself for everything bad that happens.

9.
- 0   I don't have any thoughts of killing myself.
- 1   I have thoughts of killing myself, but I would not carry them out.
- 2   I would like to kill myself.
- 3   I would kill myself if I had the chance.

10.
- 0   I don't cry any more than usual.
- 1   I cry more now than I used to.
- 2   I cry all the time now.
- 3   I used to be able to cry, but now I can't cry even though I want to.

11.
- 0   I am no more irritated by things than I ever was.
- 1   I am slightly more irritated now than usual.
- 2   I am quite annoyed or irritated a good deal of the time.
- 3   I feel irritated all the time.

12.
- 0   I have not lost interest in other people.
- 1   I am less interested in other people than I used to be.
- 2   I have lost most of my interest in other people.
- 3   I have lost all of my interest in other people.

13.
- 0   I make decisions about as well as I ever could.
- 1   I put off making decisions more than I used to.
- 2   I have greater difficulty in making decisions more than I used to.
- 3   I can't make decisions at all anymore.

14.
- 0   I don't feel that I look any worse than I used to.
- 1   I am worried that I am looking old or unattractive.
- 2   I feel there are permanent changes in my appearance that make me look unattractive
- 3   I believe that I look ugly.

15.
- 0   I can work about as well as before.
- 1   It takes an extra effort to get started at doing something.
- 2   I have to push myself very hard to do anything.
- 3   I can't do any work at all.

16.
- 0   I can sleep as well as usual.
- 1   I don't sleep as well as I used to.
- 2   I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
- 3   I wake up several hours earlier than I used to and cannot get back to sleep.

17.
- 0   I don't get more tired than usual.
- 1   I get tired more easily than I used to.
- 2   I get tired from doing almost anything.
- 3   I am too tired to do anything.

18.
- 0   My appetite is no worse than usual.
- 1   My appetite is not as good as it used to be.
- 2   My appetite is much worse now.
- 3   I have no appetite at all anymore.

19.
- 0   I haven't lost much weight, if any, lately.
- 1   I have lost more than five pounds.
- 2   I have lost more than ten pounds.
- 3   I have lost more than fifteen pounds.

20.
    0   I am no more worried about my health than usual.
    1   I am worried about physical problems like aches, pains, upset stomach, or constipation.
    2   I am very worried about physical problems and it's hard to think of much else.
    3   I am so worried about my physical problems that I cannot think of anything else.

21.
    0   I have not noticed any recent change in my interest in sex.
    1   I am less interested in sex than I used to be.
    2   I have almost no interest in sex.
    3   I have lost interest in sex completely.

INTERPRETING THE BECK DEPRESSION INVENTORY

Now that you have completed the questionnaire, add up the score for each of the twenty-one questions by counting the number to the right of each question you marked. The highest possible total for the whole test would be sixty-three. This would mean you circled number three on all twenty-one questions. Since the lowest possible score for each question is zero, the lowest possible score for the test would be zero. This would mean you circles zero on each question. You can evaluate your depression according to the Table below.

Total Score_____Levels of Depression

1-10_____These ups and downs are considered normal
11-16_____Mild mood disturbance
17-20_____Borderline clinical depression
21-30_____Moderate depression
31-40_____Severe depression
over 40_____Extreme depression

http://www.med.navy.mil/sites/NMCP2/PatientServices/
SleepClinicLab/Documents/Beck_Depression_Inventory.pdf