The Synergy of Probiotics and Prebiotics: A Novel Approach on Depression

Grant Proposal

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Abstract (RQ) or Executive Summary (Eng)

As depression is a major disease that affects millions of people across the globe, there are many different methods to treat it. Of these methods, there are a few which are much more effective than others. However, not all of these have been fully researched, and one such area is probiotics and prebiotics. Probiotics and prebiotics have previously been used for other purposes, primarily for treating the gut. However, both have been shown to be effective at treating depression. A major factor in depression is the gut health of the individual. As a result, people with worsened gut health may be more likely to have depression. I am testing the effectiveness of probiotics and prebiotics on drosophila with varying gut conditions. This variance is to help to determine whether these two types of medications are better for usage in individuals with worse gut conditions or healthy gut conditions. Drosophila will be induced with depression using levodopa. Thereafter, their initial depression levels will be measured using a negative geotaxis assay. Thereafter, they will be split into three experimental groups which will be treated with bifidobacterium (the probiotic), inulin (the prebiotic), and both. After all treatment has been done, another negative geotaxis assay will be done to determine the depression levels after treatment. Finally, the feces of each group will go through DNA-sequencing to determine the gut health, which will then be used to develop a correlation between a treatment type and gut health type.

Keywords: Depression, Levodopa, Prebiotics, Probiotics, Gut health

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The Synergy of Probiotics and Prebiotics: A Novel Approach on Depression

Currently around 3.8% of the entire world's population suffers from a common yet often severe mental disorder, depression (Depressive disorder (depression), 2023). This 3.8% equates to around 304,000,000 people out of the 8 billion people. These individuals suffer from the mental disorder which can lead to obesity, physical illness, alcohol or drug misuse, anxiety, social isolation, suicide, alongside a plethora of other social, physical, and mental issues. (Depression (Major Depressive Disorder) - Symptoms and Causes, 2022). Depression is one of the many different mental disorders that are being understood at a deeper level as time progresses. As a result of this progression still taking place, many people die each day from such an devastating disease, due to a lack of understanding and effective treatments. Depression is part of the broader category of mental/psychiatric disorders (Mental disorders, 2022); these diseases tend to not be fully understood by the scientific community. The exact cause of depression, specifically, is yet to be identified, yet many different factors such as genetics, medications, and other medical conditions have contributed to the disorder in recent years (Depression: Causes, Symptoms, Types, and Treatment, 2023). Similarly, there are very few well researched treatments for depression including, brain stimulation therapy, antidepressants, and psychotherapy (Depression: Causes, Symptoms, Types, and Treatment, 2023). Even though these few methods have been researched deeply in recent years, other emerging methods show expansive potential. Two of these emerging methods are probiotics and prebiotics. Probiotics are living microorganisms that, unlike harmful microorganisms, benefit the human body. In fact, probiotics even help control potentially harmful microbes (Probiotics: What They Are, Benefits & Side Effects, 2023). In recent years, however, it has been shown that probiotics may be effective in treating depression, suggesting a link between the gut health and mental disorder of an individual (Bistas & Tabet, 2023). Prebiotics are a food source for microorganisms in the gut, including probiotics. When broken down, these prebiotics can become energy for colon cells, aid in mucus protection, or aid in reducing inflammation and immune reactions ("What Are Prebiotics and What Do They Do?", 2022). These are crucial roles that prebiotics play in the body and can therefore, be a large factor in depression treatment. As a result, probiotics and prebiotics being given as treatment together

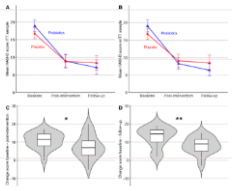

Figure 1: Change in Depression Level Based on Treatment. There is one treatment being given to the flies, probiotics, and it is being shown to be effective at reducing the depression score, which is on the vertical axis (Schaub et. al, 2022).

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have been shown to be effective in depression (Bistas & Tabet, 2023). Furthermore, recent studies have also shown that the gut-brain axis is a factor in depression (Chaiyasut et. al, 2023). The gut-brain axis is the network of neurons that send signals between the brain and gut ("What Is the Gut-Brain Connection?", 2023). However, past research in these different areas has yet to connect them together into one study. As a result, this study will focus on all three of these areas being tested together by doing trials on *Drosophila melanogaster*. Drosophilae match the conditions for this experiment as they can have depression and have a digestive system while also being relatively easy to maintain in a lab. The Drosophila will be depressed using levodopa, a dopamine precursor, and then will be tested using a placebo, probiotics, prebiotics, and both (Jiang et. al, 2017). Some of the Drosophila will have gut issues while other Drosophila will have normal guts, which will be determined at the end of the experiment using DNA-sequencing methods. For the test, there will be eight test groups consisting of placebo, prebiotics, probiotics, as well as probiotics and prebiotics: each of these groups will be for Drosophila with gut in proper condition and Drosophila with gut in a deteriorated condition. From this, the amount of Drosophila with a decline in symptoms of depression and/or a reduction depression levels would be counted and further analyzed using multiple statistical tests. These different statistical tests along with the data will provide a strong set of results. These results will then be used to show the effects of probiotics and prebiotics on drosophila individually, as well as the synergy between the two treatments. Alongside these implications, the results, through analysis, will show if the gut has a major effect on the state or existence of depression within the Drosophila. These results will also determine if probiotics or prebiotics are the better cure if the main cause of a case of depression is an unhealthy gut. Thereafter, the limitations of the study shall be discussed alongside a discussion about the impact of the study and its findings, as well as how they may impact future work in the field.

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Section II: Specific Aims

This proposal's objective is to determine whether prebiotics and probiotics are better at reducing depression levels in individuals with worse gut conditions or healthy gut conditions.

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Our long-term goal is to definitively determine whether prebiotics and/or probiotics are the better treatment to be administered to individuals with certain types of gut health for the purpose where the central hypothesis of this proposal is to fully understand if prebiotics and probiotics also have a synergetic effect which

could potentially make them a better treatment together. The rationale is that if this synergetic effect is present, the treatments would still be better for one gut condition or the other or may not show a statistically significant difference. The work we propose here will potentially change the way antidepressants are administered, and these two, specifically, could be used in certain cases where they may be more beneficial and effective for the patient.

Specific Aim 1: To identify the effectiveness of prebiotics and probiotics together in treating depression.

Specific Aim 2: To identify whether the effectiveness of using prebiotics and probiotics together to treat depression can differ based on the gut health of the organism.

Specific Aim 3: To identify whether prebiotics or probiotics are more effective in treating depression.

The expected outcome of this work is to find that prebiotics and probiotics are more effective at treating depression in individuals which have better gut health.

Section III: Project Goals and Methodology

Relevance/Significance

This study is particularly significant due to the prevalence as well as the severity of depression across the globe my those who have the neurological condition. Currently, around 304,000,000 people deal with depression ("Depressive disorder (depression)", 2023). One of the rather newly discovered factors in depression is the gut-brain axis, which relates the health of the gut to the health of the brain (mental health). This is also rather important as around 60 million people have digestive diseases in just the United States ("Digestive Diseases Statistics for the United States", 2014). Worldwide, digestive diseases, which deteriorate gut conditions are very common, with around 40% of the population experiencing them (Sperber et. al, 2021). As a result, a correlation between these two common sets of diseases has been shown through the gut-brain axis. Depression is also responsible for 850000 deaths around the world every year ("Global depression statistics", 2011).

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Innovation

This study gathers inspiration from past studies and combining different aspects regarding the medical science surrounding depression. Previously, studies had already used prebiotics as well as probiotics and tested their effectiveness at treating or reducing depression levels (Bistas et. al, 2023). There have also been studies

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testing whether the gut-brain axis exists and how significant it is. However, there has yet to be a study on whether prebiotics and probiotics are better at reducing depression levels in drosophila with bad gut conditions or healthy gut conditions. This study intends to test how effective the use of prebiotics and probiotics is on drosophila who have a worse gut condition and healthy gut condition.

Methodology

This study will start with the creation of four groups of drosophilae, one control and three experimental. The control group will be given regular drosophila food. The first experimental group will be given regular drosophila food induced with 10% bifidobacterium. The second experiment group will be given regular drosophila food induced with 10% inulin. The third experimental group will be given regular food induced with both 10% bifidobacterium and 10% inulin. Initially, all the groups of flies will be given levodopa as it will be in the food given to them. A negative geotaxis assay will be done on each group to determine baseline depression levels before treatments are given. Thereafter, the placebo or treatments will begin. After one breeding cycle, the drosophila will once again be taken out and each group will go through a negative geotaxis assay. Thereafter, the decrease in depression levels will be calculated for each group. For the final step of the experiment, the feces of each group will be gathered and put through DNA sequencing. DNA sequencing will determine the gut condition of the drosophilae.

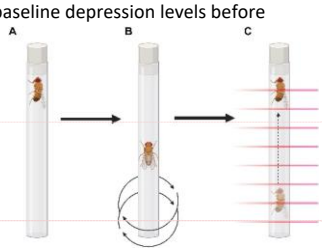

Figure 2: How a negative geotaxis assay works.

Specific Aim #1: Determine the effectiveness of prebiotics and probiotics together at treating depression. The objective is to determine if prebiotics and probiotics have a synergetic effect when used to treat depression. Our approach (methodology) is to test prebiotics and probiotics in three different groups of drosophilae against a control group of drosophilae. The first group will be of only prebiotic treatment and the second group will be of only probiotic treatment. The third group will be treated with both prebiotic treatment as well as probiotic treatment. The control group will just be given regular food. The prebiotic treatment will be 10% inulin within the regular drosophila food. The probiotic treatment will be of 10% bifidobacterium within regular drosophila food. A

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negative geotaxis assay will be done before and after treatment to measure the change in depression levels in the drosophila. After doing the initial geotaxis assay, the drosophila will be induced with depression using levodopa. Thereafter, the treatment will occur, and the final negative geotaxis assay will be conducted. Using the data for the negative geotaxis assays for all four groups we will be able to determine whether the prebiotics and probiotics have a synergetic effect. Our rationale for this approach is that it will allow for a proper control group and will also easily allow us to measure the different treatments as drosophila are an easy to work with model organism.

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Justification and Feasibility. The methods for this specific aim allow for the accurate testing of the effectiveness of probiotics and prebiotics together and determining if they have a synergetic effect as we test the two treatments independently as well as in combination. The treatments are also tested against a control. Although in this experiment I am using drosophila due to limitations, in another study, humans were used in a similar manner and its results showed that probiotics and prebiotics had a synergetic affect (-19.95 decrease on Beck's score versus -17.75 for placebo) (Bistas & Tabet, 2023). As a result, my study which uses drosophila, and a smaller variety of bacteria should have the same results.

Summary of Preliminary Data. The preliminary data for this specific aim involves testing the two assays used in this experiment on wild type flies (one of the control groups). The data gathered from the two assays is shown in the graphs. As both graphs show, there is a very high R^2 value, which shows a very high correlation between the number of flies being result of these high R^2 values, it can be assured that these assays accurately measure depression levels. In a similar manner the slopes of the linear regression graphs are measures for comparative depression levels. As a result, such slopes of experimental groups can be analyzed to determine if there is a synergetic effect.

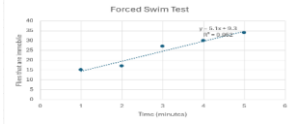

Figure 3: The results of the Forced Swim Test performed on wild type flies. The vertical axis measures the number of flies that are immobile at a timepoint. The horizontal axis measures time. The data points have been put through linear regression and the trendline is shown along with its equation and R^2 value.

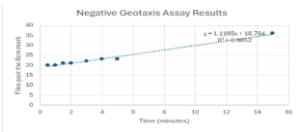

Figure 4: The results of the Negative Geotaxis Assay performed on the wild type flies. The vertical axis measures the number of flies past the 8cm mark. The horizontal axis measures time. The data points have been put through linear regression and the trendline is shown along with its equation and R^2 value.

Expected Outcomes. The overall outcome of this aim is to determine whether prebiotics and probiotics have a symbiotic effect. This knowledge will be used for part of the results as it is part of what is being measured.

Potential Pitfalls and Alternative Strategies. I expect that the use of drosophila instead of humans may result in slightly different results. Another pitfall would be the drosophila not being depressed due to not having a strong enough levodopa concentration. A third pitfall could also be the flies not being significantly affected by the treatment methods. Alternative strategies for this would be to increase the concentration of levodopa as well as that of the treatment which seems to be ineffective.

Specific Aim #2: Determine the effectiveness of prebiotics and probiotics together at treating depression in drosophila with healthy gut condition and worse gut condition. The objective is to determine if prebiotics and/or probiotics are better at treating depression in drosophila with healthy gut conditions or worsened gut conditions. Our approach (methodology) is to test prebiotics and probiotics in three different groups of drosophilae against a control group of drosophilae, as aforementioned. The negative geotaxis assays will also be conducted just as aforementioned. However, for this specific aim, after the treatment has been conducted DNA-sequencing will also be done on the feces of the drosophila. The DNA-sequencing will be done to determine which flies had worse gut condition and which flies had better conditions. DNA-sequencing will be able to accomplish this by determining the populations of bacteria in the gut.

Justification and Feasibility. The methods for this specific aim allow for the accurate testing of the effectiveness of probiotics and prebiotics together on different gut condition levels. As each treatment is separated and the gut condition of the flies can also easily be tested as a whole batch rather than on an individual level. This method of testing has also been proven to work. In another study the feces of humans were used to find presence of intestinal diseases using DNA sequencing (Jiang et al, 2020).

Expected Outcomes. The overall outcome of this aim is to determine whether probiotics and prebiotics are better for treating patients with depression with worse or healthy gut condition. This knowledge will be used for part of the results as it is part of what is being measured.

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Potential Pitfalls and Alternative Strategies. I expect that the use of drosophila instead of humans may result in slightly different results. Another pitfall would be the drosophila not being depressed due to not having a strong enough levodopa concentration. A third pitfall could also be the flies not being significantly affected by the treatment methods. Alternative strategies for this would be to increase the concentration of levodopa as well as that of the treatment which seems to be ineffective.

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Specific Aim 3: Determine whether prebiotics or probiotics are better at treating depression. The objective is to determine if prebiotics and/or probiotics are better at treating depression in drosophila with healthy gut condition or worsened gut condition. Our approach (methodology) is to test prebiotics and probiotics in three different groups of drosophilae against a control group of drosophilae as aforementioned. The negative geotaxis assays will also be conducted just as aforementioned. By testing the two treatments individually it will be apparent which one is better suited to treat depression.

Justification and Feasibility. This methodology will work for this specific aim as it can easily test the different treatments independently and then can compare them to the placebo. A similar study was also conducted in another research article, and it worked, and it showed that prebiotics were more effective (Bistas & Tablet, 2023).

Summary of Preliminary Data. The preliminary data for this specific aim involves testing the two assays used in this experiment on wild type flies (one of the control groups). The data gathered from the two assays is shown in the graphs. As both graphs show, there is a very high R^2 value, which shows a very high correlation between the number of flies being measured and time (minutes). As a result of these high R^2 values, it can be assured that these assays accurately measure

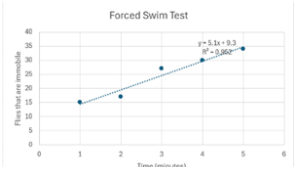

Figure 5: The results of the Forced Swim Test performed on wild type flies. The vertical axis measures the number of flies that are immobile at a timepoint. The horizontal axis measures time. The data points have been put through linear regression and the trendline is shown along with its equation and R^2 value.

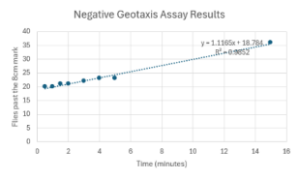

Figure 6: The results of the Negative Geotaxis Assay performed on the wild type flies. The vertical axis measures the number of flies past the 8cm mark. The horizontal axis measures time. The data points have been put through linear regression and the trendline is shown along with its equation and R^2 value.

depression levels. In a similar manner the slopes of the linear regression graphs are measures for comparative depression levels. As a result, such slopes of experimental groups can be analyzed to see if prebiotics or probiotics are more effective at treating depression.

Expected Outcomes. The overall outcome of this specific aim is to determine whether prebiotics or probiotics are better to treat depression.

Potential Pitfalls and Alternative Strategies. I expect that the use of drosophila instead of humans may result in slightly different results. Another pitfall would be the drosophila not being depressed due to not having a strong enough levodopa concentration. A third pitfall could also be the flies not being significantly affected by the treatment methods. Alternative strategies to this would be to increase the concentration of levodopa as well as that of the treatment which seems to be ineffective.

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Section IV: Resources/Equipment

The materials that I will be using are Bifidobacterium, Levodopa, Inulin, Drosophila, DNA-sequencing machine, pipettes, and drosophila food.

Section V: Ethical Considerations

The only ethical considerations for this study are the safety of drosophilae and that they do not die.

Section VI: Timeline

December 2: Grant Proposal Draft Completed

December 5: Preliminary Data collected and Analyzed

January 10: Grant Proposal Due

January 15: End of data collection

January 30: End of data analysis

February 10: Done preparing for February Fair

Section VII: Appendix

Section VIII: References

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