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Pilot Trial of Probiotics in Major Depressive Disorder: A Randomized, Double-Blind, Placebo-Controlled Approach

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Abstract: Depression affects approximately 280 million people globally and is projected to become the leading cause of disease burden by 2030. Emerging evidence suggests that probiotics may play a role in managing depression by modulating the gut-brain axis. Therefore, as a pioneering research initiative, we conducted a pilot study in a Malaysian setting, presenting it as a preliminary report on the potential effects of probiotics on depressive symptoms in patients with mild major depressive disorder (MDD) and the associated stool-derived gut microbiome changes. The primary aim was to provide initial insights into the potential effects of probiotics as a stand-alone treatment for mild MDD while

also assessing the feasibility of the study. Malaysian adults aged 18–65 with clinically diagnosed mild MDD were randomized in a 3:1 ratio to receive either multi-strain probiotics (*Lactobacillus helveticus* R0052, *Lactobacillus rhamnosus* R0011, *Bifidobacterium longum* R0175, and *Saccharomyces cerevisiae boulardii*) or a starch-only placebo for six weeks. The primary outcome was overall depressive symptomatology assessed using the Beck Depression Inventory, with additional clinical measures including the Patient Global Impression and Clinical Global Impression scales. Gut microbiome profiling was conducted using 16S rRNA gene sequencing of stool samples. Of 81 initial respondents, 15 met the inclusion criteria and were randomised (probiotic: n=12, placebo: n=3). Key exclusions included current or recent (past month) use of antidepressants or psychiatric treatment and the presence of psychiatric comorbidities. Three probiotic participants dropped out, resulting in a per-protocol analysis of probiotic (n=9) and placebo (n=3) groups for clinical assessments, and probiotic (n=7) and placebo (n=3) for microbiome analysis. Probiotic supplementation led to statistically significant improvements in depressive symptoms, severity, and overall improvement (p<0.05), with no significant changes observed in the placebo group. Effect sizes post-intervention (d_{post}=0.3) and pre-post ($d_{pre\text{-}post}$=1.282) suggest that probiotics outperformed placebo, with a modest effect size comparable to clinical antidepressant trials. Gut microbiome analysis showed no significant differences in diversity measures but revealed distinct shifts in microbial composition, with increases in beneficial taxa associated with greater clinical improvement. Despite recruitment challenges, the intervention was well-tolerated, and compliance was high. While findings are promising, the small sample size limits generalisability. Larger studies are needed to confirm these results and explore the long-term effects of probiotics in depression management.

Keywords: mild major depressive disorder; probiotics; randomised controlled trial; gut microbiome; SDG 3 Good health and well-being

1. Introduction

Major depressive disorder (MDD) or depression is a prevalent, debilitating and potentially fatal neuropsychiatric disorder involving a plethora of heterogeneous phenotypes[1]. It affects approximately 280 million people worldwide and is projected to become the leading cause of disease burden by 2030[2–4]. The exact etiopathophysiology of depression remains unelucidated to date. The general understanding is that depression develops as a result of multifactorial etiology involving a complex interaction between the biological, genetic, environmental and psychological components[5–7].

Major depressive disorder (MDD) remains a clinical diagnosis without definitive laboratory assessments other than to exclude non-idiopathic causes of depression including hypothyroidism, iron deficiency anemia, Cushing's syndrome, and infections[8–11]. Consequently, a clinician's expertise is critical in establishing the diagnosis of MDD through

a detailed assessment including history, physical and mental status examinations, with adherence to the criteria outlined in established diagnostic manuals, while also determining appropriate management strategies[12–14]. The clinical diagnosis of MDD follows a categorical classification model, stratifying patients by symptom severity into mild, moderate, or severe cases, with treatment approaches tailored accordingly[15–18]. According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V), one of the primary diagnostic manuals used in clinical settings, the diagnosis of MDD requires the persistent presence of at least five symptoms outlined in the manual in a continuous period of two weeks that markedly affect an individual's functionality. One of the symptoms must be either persistent sad mood or anhedonia, or both, along with changes in weight, sleep patterns, psychomotor activities, fatigue, feelings of worthlessness or inappropriate guilt, diminished concentration, and/or suicidal thoughts[19,20]. The DSM-V classifies MDD severity as mild when there are minimal symptoms with manageable impairment, moderate when there are more symptoms with noticeable functional difficulty, and severe when most or all symptoms cause significant distress and major functional impairment, potentially with psychotic features[21].

The current management of depression entails the use of psychotherapy, pharmacological therapy, lifestyle intervention, and brain stimulation[7]. Clinically depressed patients are primarily managed in the outpatient setting. Hospitalisation is typically warranted for patients with severe depressive symptoms and suicidality. Mild MDD is typically managed with lifestyle interventions and psychotherapies including, but not limited to, cognitive behavioural therapy (CBT), behavioural activation therapy, and psychodynamic therapy that target negative thought processes and deep-rooted awareness to facilitate positive changes[7,22]. While CBT is the preferred initial treatment for mild MDD, the clinical guidelines committee suggests that pharmacologic treatment using antidepressants (AD) may be considered when CBT is inaccessible, costly, or if the patient has a history of moderate to severe MDD or prefers medication[18]. Pharmacology therapy, in combination with lifestyle intervention and psychotherapy is typically used to treat moderate and severe MDD. Brain stimulation method such as electroconvulsive therapy (ECT) is typically reserved for severe or treatment-resistant MDD[12,23,24].

Antidepressants (AD) targeting the monoaminergic system are routinely prescribed as a first-line pharmacological treatment for depression[25]. However, antidepressants have often been associated with low efficacy and effectiveness, therapeutic latency, detrimental side effects and withdrawal symptoms, and social stigma[7,26–28]. About 20–30% of patients do not respond well to the existing pharmacotherapies, and the remission rate for

monotherapy with the best antidepressants is merely 50.78%[29–31]. Non-adherence, reportedly as high as 50% among depressed patients is another major concern that poses severe consequences on the overall clinical outcome and healthcare economics[32]. The clinical management of depression has also been particularly challenging due to its heterogeneous presentation and multifactorial model of aetiology[33,34]. Therefore, it has become a common pursuit to ensure continuous and progressive efforts are in place to combat this disabling condition with severity likened to paraplegia or blindness[35,36].

Echoing the dire situation, a microbial-based therapeutic strategy based on probiotic administration emits a refreshing ray of hope within the clinical landscape of depression management[37–40]. The first publication appraising the possible beneficial role of probiotics in depression appeared in 2005[41]. Probiotics, consisting of non-pathogenic live microorganisms, have been hypothesised to ameliorate depression through anti-inflammatory effect, attenuation of the hypothalamic–pituitary–adrenal axis, modulation of neurotransmitters, and epigenetic mechanisms by modulating the gut microbiota[42–44]. The therapeutic potential of probiotics in depression was founded on unanimous findings of gut dysbiosis in depressed patients. Generally, it was established that depressed patients have poorer microbial diversity and inversely proportional abundance of pathobionts to beneficial microbiota as opposed to healthy controls[43,45–47]. Although there has not been a standardised pattern of gut dysbiosis, some commonly reported findings in patients with MDD include significant alterations within the main four phyla, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinomycetota*[48,49]. More specifically, at the genus level, there is a notable decrease in *Bifidobacterium*, *Lactobacillus*, *Faecalibacterium*, and *Ruminococcus* and an increase in *Prevotella, Clostridium, Klebsiella, Streptococcus and Oscillibacter* were observed in MDD patients[48,50,51]. Nonetheless, whether gut dysbiosis has a causative or consequential role in depressive disorder remains unanswered. Therefore, more studies are emerging to substantiate the causative role of gut microbes in depression[52,53].

The existing reviews have postulated the anti-depressive effects of probiotics based on the intertwining microbiota-gut-brain axis (MGBA) mechanisms in the pathophysiological occurrence of depressive disorder[43,54–58]. Furthermore, the beneficial effects of probiotics on depression have been substantiated in numerous systematic reviews and meta-analyses of clinical studies[37,38,55,59–64]. *Lactobacillus* spp. and *Bifidobacterium* spp. are the most widely studied probiotics in depression[56,65]. The efficacy of probiotics in mitigating depressive symptoms has been comparable to the conventionally used AD[66, 67,68]. In contrast to AD, probiotics have favourable side-effect profiles and no associated stigma barriers[69–71]. Besides their promising therapeutic potential, the widespread awareness and

consumption of commercial probiotics confer some advantages in terms of promoting acceptance and improving adherence to probiotics among patient cohorts[72,73]. These critical attributes of probiotics are garnering increasing attention from the scientific community to expand research directives towards exploring the potential of probiotics as a therapeutic alternative to combat crippling depressive disorder.

Although there have been numerous clinical studies of probiotics in depression, most studies include participants with depressive symptoms based on self-administered depressive scales without a formal diagnosis of MDD and depression as a comorbid. In terms of intervention, probiotics are often utilised as an adjunct treatment or supplementation in depressed patients[55]. Furthermore, probiotics may be used as a stand-alone intervention only in mild MDD, whereas for moderate to severe MDD, they are considered an adjunct to antidepressant therapy, in alignment with the clinical guidelines committee's recommendation that warrants pharmacological treatment for more severe cases[7,12,18,22]. The most recent systematic review and meta-analysis published in 2021, focusing on randomised controlled clinical trials of probiotics in depressed patients concluded by supporting the clinical use of probiotics as an adjunct to AD in the treatment of depression[55]. The first and most recent probiotic trial in MDD patients not on antidepressants was an open-label pilot study with eight participants, where probiotics were used as a stand-alone treatment for 8 weeks, showing significant improvements in affective symptoms by week 4, which were sustained at week 8[71]. Therefore, intervention research of probiotics specific to depressive disorder, particularly as a stand-alone intervention, is evidently scanty to date. It is necessary to develop clinical trials of probiotics with methodological approaches that allow the bridging of pre-clinical evidence and practical application of a new therapeutic recommendation within the clinical canon of depression management, aligning with established clinical guidelines.

Narrowing our focus to the Malaysian setting, there have yet to be any clinical trials of probiotics for depression done so far. Similar to the global trend, the prevalence of depression has been reportedly increasing in Malaysia with prevalence ranging as high as 14.3% to 81.7%[74]. Based on an epidemiological review, it was reported that around 2.3 million people from various ethnic backgrounds experience depression at least once in their lifetime in Malaysia[75]. According to the National National Health and Morbidity Survey (NHMS), the prevalence of depression has nearly doubled between the years 2011 and 2020[76,77]. Contrary to the common conception that the prevalence of depression is lower in developing countries compared to developed countries, the Malaysian data reveals lifetime prevalence, taking into account depressive symptoms, ranging from 3.9% to 46%[7,75]. The most recent cross-sectional study involving 10, 300 Malaysian participants aged between 35

and 70 years old has reported 3.7% of this population experiencing depressive symptoms[78]. Moreover, the work-productivity loss associated with mental health conditions, including depression in Malaysia, has been estimated to incur an economic cost of RM14.46 billion in 2018[79,80].

Within the clinical context of depression, various barriers to treatment adherence have been identified among Malaysian populations. Patient-specific (83%) and medication-specific (63%) barriers, including negative attitude, misconception, presence of comorbidities, detrimental side-effects, treatment cost and duration, contribute to the most significant portions of identified barriers to AD adherence among Malaysian patients[81,82]. Despite the alarming state of depression in Malaysia, one of the unanimously emphasized concerns has been the lack of depression-related research conglomeration in the Malaysian setting. There have been persistent calls for more united and prompt action to combat this debilitating disorder, considering the significant depression-associated individual and national burden of disease[74,75,78,80,83,84].

Considering the lack of intervention research on probiotics in clinical depression within the Malaysian setting and the need for carefully developed research approaches that are tailored specifically to patients with MDD, a pilot research initiative appears more relevant, timely and consistent with the global trends and needs. Therefore, as a pioneering research initiative, we conducted a pilot study in a Malaysian setting with a primary aim to provide initial insights into the potential effects of probiotics as a stand-alone treatment for mild major depressive disorder (MDD) and explore the feasibility of the study. We specifically focused on mild MDD for the stand-alone intervention of probiotics in alignment with clinical guidelines that do not mandate the use of antidepressants (AD). To our best knowledge, this is also the first study in Malaysia and worldwide to utilise probiotics as a stand-alone therapeutic intervention solely in outpatients with formally established clinical diagnosis of mild MDD and without any psychiatric comorbidity.

2. Materials and Methods

2.1. Ethics Statement

2.1.1. Approval

Ethics approval was obtained from the Monash University Human Research Ethics Committee (MUHREC) (Project ID: 28104). The study complied with the ethical principles and values outlined in the National Statement on Ethical Conduct in Human Research, the Australian Code for the Responsible Conduct of Research[85] and the Malaysian Good Clinical Practice Guidelines[86].

2.1.2. Consent

Subjects were informed of the study during their first scheduled clinic visits, where the patient information sheet was provided and explained to them. The consent forms would be signed and dated if they were willing to participate. All the patients' written informed consent was obtained before enrolment.

2.1.3. Withdrawal criteria and safety measures

Subjects may choose to withdraw at any time and may be withdrawn if the investigator deems that it is detrimental or risky for the subject to continue. Withdrawn subjects will not be replaced. The total score of the Beck Depression Inventory was closely monitored through regular visits and online follow-ups on a weekly basis. The suicide score on the BDI was also monitored closely for any deterioration to scores 2 or 3 that would require immediate withdrawal from the study. Participants who showed severe depressive symptoms and exhibited suicidal ideation during screening were immediately referred for further follow-up at the hospital and excluded from the trial. Using the pharmacovigilance form, the clinician on board assessed and reported any adverse events during the intervention phase.

2.1.4. Data management

Subjects' names were kept on a password-protected database and linked only with a study identification number for this research. The identification number instead of patient identifiers was used on the subject data sheets. All data were entered into a computer that was password protected. On completion of the study, data on the computer were copied to CDs while the data on the computer was erased. CDs and any hardcopy data will be stored in a locked investigator's office and maintained for a minimum of five years after completion of the study. The CDs and data will be destroyed after that period of storage. Subjects will not be allowed to view their personal study data, as the data will be consolidated into a database. Subjects may write to the investigators to request access to study findings.

2.2. Participants and Setting

Participants with mild major depressive disorder (MDD) were recruited from the Malaysian community, specifically individuals aged 18 to 65 years residing in Peninsular Malaysia. Recruitment was conducted through research advertisements primarily on social media platforms including Facebook (support groups for depression, re-shared posts by close acquaintances) and mobile messaging via WhatsApp. Advertisements were posted at regular three- to four-month intervals to maximise outreach. Eligibility screening included individuals with a prior MDD diagnosis who had defaulted on clinical follow-ups for over a month, as well as those experiencing newly developed depressive symptoms. Participants were excluded if they had used antidepressants or other psychopharmacological treatments for depression within the past month, as these could influence depressive outcomes.

Additionally, individuals with existing psychiatric comorbidities were not eligible. A complete list of inclusion and exclusion criteria is provided in Table 1. The trial was conducted at three study sites across the central and southern regions of Peninsular Malaysia. It began in December 2021 at the primary site in Larkin, Johor Bahru, Johor, and later expanded to the Klang Valley in May 2022 to enhance participant recruitment. The Klang Valley sites were located in Mid Valley, Kuala Lumpur, and Petaling Jaya, Selangor. Participants' study visits were conducted at three private clinic settings throughout the trial.

Table 1. Inclusion and exclusion criteria for participants in the study.

Inclusion criteria
• 18–65 years old • Malaysian citizen • Patients clinically diagnosable with mild MDD at the time of enrolment • No use of antidepressants, psychiatric medications, psychotherapy, or complementary therapy within the past one month before enrolment • No use of antibiotics, dietary supplements, probiotic supplements, or NSAIDs within the past one month before enrolment • No existing psychiatric comorbidities • No possible organic causes of depression (e.g., thyroid disorders, anemia, brain diseases) • No severe medical comorbidities or gastrointestinal disorders requiring treatment or causing altered bowel habits within the past one month before enrolment • No immunocompromised conditions (e.g., AIDS, malignancy, long-term corticosteroid treatment) • Not pregnant or breastfeeding • No known allergies to milk, yeast, or soy • Understand and comply with the study requirements • Provision of written informed consent
Exclusion criteria
• < 18 and > 65 years old • Non-Malaysian • Use of antidepressants, psychiatric medications, psychotherapy, or complementary therapy within the past one month before enrolment • Use of antibiotics, dietary supplements, probiotic supplements, or NSAIDs within the past one month before enrolment • Presence of existing psychiatric comorbidities • Presence of possible organic causes of depression (e.g., thyroid disorders, anemia, brain diseases) • Presence of severe medical comorbidities or gastrointestinal disorders requiring treatment or causing altered bowel habits within the past one month before enrolment • Presence of immunocompromised conditions (e.g., AIDS, malignancy, long-term corticosteroid treatment) • Pregnant or breastfeeding • Known allergies to milk, yeast, or soy • Unable to understand and comply with the study requirements • Unwilling to provide written informed consent

2.3. Diagnosis Establishment

Participants included in the trial were clinically diagnosed with mild major depressive disorder (MDD) by a consultant psychiatrist and had normal blood investigation results at the time of enrolment, during their first face-to-face baseline screening visit. Four consultant psychiatrists were assigned for clinical assessments throughout the trial. The diagnosis of mild MDD was established through a structured clinical interview conducted by a consultant psychiatrist, following the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria. The severity of depressive symptoms was determined using the 'Severity for Depressive Disorders' criteria outlined in DSM-5, which considers symptom intensity, functional impairment, and distress[19]. Patients meeting the criteria for mild MDD, characterised by the presence of depressive symptoms with minimal impairment in social and occupational functioning, were eligible for the trial. Following clinical interview, eligible participants underwent baseline blood investigations including thyroid function tests to measure levels of thyroxine (T4), triiodothyronine (T3), and thyroid-stimulating hormone (TSH). Abnormal levels of these thyroid hormones can indicate thyroid dysfunction, thus, participants displaying these abnormalities will be excluded. To ensure consistency in assessments, inter-rater reliability measures were implemented. Though the raters were familiar with the validated rating scales used in the study, they underwent re-training on the Beck Depression Inventory[87], Patient Health Questionnaire-9 (PHQ-9), Clinical Global Impression of Severity (CGI-S), and Clinical Global Impression of Improvement (CGI-I) before the study commenced. Additionally, each diagnosis and severity classification was reviewed and discussed between two psychiatrists before confirmation, minimising variability and enhancing diagnostic reliability.

2.4. Study Design and Procedure

This study was a pilot double-blind, randomised, placebo-controlled trial of probiotics in patients with clinically diagnosed mild MDD. The study design planned seven weeks for each patient, including baseline screening assessments during the first week, followed by an uninterrupted intervention phase over the remaining six weeks (42 days). Throughout the 6-week intervention phase, patients' overall depressive symptoms were closely monitored through a structured approach. Refer to Supplementary Table S1. This monitoring occurred through a combination of clinical interviews, rating scales, and phone text conversations. Clinical interviews and rating scales were conducted at least once every week as per the scheduled plan. Additionally, phone text conversations were held two days after each consultation to ensure continuous monitoring and timely follow-up. Beck Depression Inventory, Patient Health Questionnaire-9 (PHQ-9), Patient Global Impression of Severity (PGI-S), Patient Global Impression of Improvement (PGI-I) were patient rating scales whereas Clinical Global Impression of Severity (CGI-S) and Clinical Global Impression of Improvement (CGI-I) were clinician rating scales administered to obtain the clinical data on the overall depression symptomatology, severity of illness and improvement

from the initiation of intervention. Patients' stool samples were collected at Week 1, pre-intervention and the end of Week 6, post-intervention, for the gut microbiome analysis. The entire study procedure is presented in Supplementary Table S1.

2.4.1. Preliminary (phone screening) and baseline screenings (face-to-face visit at Week 0)

A non-clinician research member performed a brief preliminary screening on all the interested participants over the phone to assess the basic eligibility to participate in the trial. Age, citizenship, accessibility to the study site and a brief, relevant psychiatric history was checked with the participants before scheduling their first face-to-face appointment with the consultant psychiatrist for the baseline screening visit.

Baseline screening assessments were performed at Week 0, during patients' first face-to-face visit to the study site. The study and procedures were explained to all the participants, and written informed consent was obtained before the enrolment assessment. Basic demographic questionnaires and validated depression self-rating scales, including PHQ-9, BDI and PGI-S, were administered after obtaining participants' consent.

A clinical interview by a consultant psychiatrist was followed to determine if the patient was suitable for the trial. The diagnosis was established by the consultant psychiatrist based on an integrated clinical judgement including Mental State Examination and with reference to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). The clinician completed the CGI-S rating after the consultation. Baseline blood investigations, including complete blood counts and thyroid function tests, were performed on all the participants who were otherwise deemed eligible for the study upon clinical assessment by the interviewing clinician. Participants underwent thyroid function tests to measure levels of thyroxine (T4), triiodothyronine (T3), and thyroid-stimulating hormone (TSH). Abnormal levels of these thyroid hormones can indicate thyroid dysfunction, thus, participants displaying these abnormalities will be excluded. Patients with normal blood investigation results were informed by phone and scheduled for the second face-to-face visit within the following week, at Week 1, where the intervention phase commenced.

2.4.2. Intervention phase (Week 1 till Week 6) and clinical data collection

The intervention phase lasted six weeks, counted to 42 days, for each patient. Each patient was required to have a minimum of four face-to-face clinical interview sessions with the consultant psychiatrist. Alternatively, face-to-face meetings through Zoom were arranged for those who could not attend in person to the study site during the mid-intervention phase, usually at Week 2 or Week 4, but only for those who were not scheduled for replenishment of investigation product (IP).

Patient rating scales, including Beck Depression Inventory, Patient Global Impression of Severity (PGI-S), and Patient Global Impression of Improvement (PGI-I) were administered on a weekly basis. Clinical interviews involved Mental State Examination and

side effects assessment followed by administration of clinician rating scales on Weeks 1, 2, 4 and 6. Clinical Global Impression of Severity (CGI-S) and Clinical Global Impression of Improvement (CGI-I) scales were rated by the clinician for every patient following each clinical review. Of these, PGI-I and CGI-I scales were administered on alternate weeks from Week 2 onwards, one week after the initiation of intervention. Every clinical interview and rating following the start of intervention at Week 1 were done at the end of the subsequent scheduled week within a maximum extension period of two days. The assessments on dietary and physical activity, compliance and adverse events were performed during clinical interviews, and data were obtained based on patient information.

2.5. Randomisation and Blinding

Patients who met all the study criteria and enrolled on the trial were randomly allocated into either an active group receiving probiotic supplements or a control group receiving a placebo compound for six weeks in a ratio of 3:1. Randomization and dispensing of the medication, either probiotic or placebo, was handled by an independent research assistant, who was otherwise not involved in the study. The website randomizer.org. was utilised to generate a simple randomisation table. All the participants, investigators and clinicians performing assessments were blinded by using a unique patient identification code rather than any identifying information. The blinding protocol was carefully maintained until the trial's conclusion.

2.6. Investigational Product

A probiotic formulation consisting of vegetable capsules with a novel combination of 4 different probiotic strains consisting of three bacterial strains and one yeast was used in this study. This product was supplied by Medispec (M) Sdn Bhd upon purchase, a company based in Malaysia that specialises in the supply and distribution of medical and healthcare products. Participants in the probiotic group were instructed to take one probiotic capsule orally twice daily, either with food or 30 minutes before food. They were instructed to take one capsule in the morning and another at night, approximately 12 hours apart. Each capsule contained freeze-dried live *Lactobacillus helveticus* R0052 (19.3mg), *Lactobacillus rhamnosus* R0011 (19.9mg), *Bifidobacterium longum* R0175 (25.5mg) and *Saccharomyces cerevisiae boulardii (125mg)* strains. Each bacterial strain was at a dosage of $\geq 5 \times 10^9$ colony-forming units (CFU), while yeast was at a dosage of $\geq 2.5 \times 10^9$ CFU, forming a total dosage of $\geq 17 \times 10^9$ CFU per capsule. Participants in the placebo group were instructed similarly to those in the probiotic group to take one placebo capsule orally twice daily, in the morning and at night, either with food or 30 minutes before food. This product was supplied by Herbal Science Sdn. Bhd., a pharmaceutical company based in Malaysia. Each placebo capsule contained only starch (400mg) in a powdered form and was indistinguishable from the probiotic capsule in terms of colour, packaging, smell, and taste. The dispensing of the medication, including both probiotics and placebo, was handled by an independent research

assistant, who was not involved in any other aspect of the study to maintain blinding. This individual was responsible for ensuring that the investigational products were properly supplied, stored, and dispensed in compliance with the study protocol. Additionally, the independent research assistant monitored and verified the return of unused medication at the end of the study.

2.7. Compliance, Diet and Physical Activity Monitoring

Compliance was monitored by asking patients to return the container containing probiotic or placebo capsules at each visit and through phone calls. Regular reminders to consume probiotics were sent via text messages via phone to ensure compliance. Participants were asked to maintain their regular diet and physical activity during the study. Participants were asked to discontinue any probiotic supplements and foods containing added probiotics or prebiotics. Dietary and physical activity records for any two weekdays and one weekend for each week throughout the trial period were obtained from participants at each face-to-face follow-up and assessed for any changes to their routine diets and physical activities.

2.8. Outcome Measures and Data Analysis

2.8.1. Clinical measures and analysis

The primary outcome measure was the patient's total Beck Depression Inventory scores, which indicate the severity of depressive symptoms. Total scores at baseline (Week 0), mid-intervention (Week 4), and at the end of the trial (Week 7) were used for the analytical assessment. The Beck Depression Inventory, a patient-rating scale, is among the commonest and primary tool used in research settings to report depression outcome following probiotic intervention[88–90]. A reduction in total BDI scores is synonymous with a positive improvement in depression outcome and the scorings follow categorical classification of depression severity with total scorings of l0–13 indicating minimal depression, 14–19 mild, 20–28 moderate and 29–63 severe depression[91]. Additionally, the 7-point scales Patient Global Impression of Severity (PGI-S) and Clinical Global Impression of Severity (CGI-S) were used to rate the severity of illness at baseline (Week 0) and at the end of the intervention (Week 6). Meanwhile, Patient Global Impression of Improvement (PGI-I) and Clinical Global Impression of Improvement (CGI-I) were used to rate the improvement of illness from the initiation of the intervention (Week 2) to the end of the intervention (Week 6). The assessment using CGI and PGI scales provide a global view on the patients' and clinicians' impression on the disease severity and improvement over a stipulated period of time. These are single-item, seven-point scales with lower ratings indicating positive changes[92,93]. These are supplementary assessments typically used in clinical and research settings that provide useful information that divulge the consistency associated with the intended intervention[94].

All statistical analyses were done using Statistical Package for Social Science version 27 (SPSS Inc., Chicago, IL, USA), Prism 9.0 and GPT-4o. Analysis of the clinical scores for

BDI, CGI and PGI was completed on a per-protocol basis. Independent samples t-test was done to compare means between study groups[95], and paired-samples t-test was used to compare means within study groups[96]. Data were expressed as means and standard deviations with 95% confidence intervals for unequal variances. Two-tailed p-values less than 0.05 were considered statistically significant in all the comparisons. The effect size was calculated with Hedge's g corrections to Cohen's d for post-intervention scores between study groups, while the overall effect size between study groups pre-and-post-intervention was derived based on Morris (2008)[94,97]. The determined effect size provides an overall impression of the probiotic's anti-depressive effect compared to the placebo. The effect size was calculated based on mean differences of BDI scores between probiotic and placebo groups for post-intervention (d_{post}) and pre-and-post-intervention ($d_{pre\text{-}post}$). Unlike p-value, which informs the occurrence of an effect and is dependent on sample size, effect size determines the magnitude of an effect independent of sample size. Determining the effect size provides a meaningful evaluation of the practical significance of an intervention. According to Cohen's classification, an effect size, d=0.2 is small, d=0.5 is medium, and $d \geq 0.8$ is large[98–100]. The inter-rater agreement reliability using Cohen's Kappa (κ) and percentage distribution were assessed for the probiotic group for both severity (PGI-S and CGI-S) and improvement (PGI-I and CGI-I)[94].

2.8.2. Stool sample processing and gut microbiome analysis

Stool samples from all the patients enrolled in the trial were collected before (Week 1/ Day 0) and at the end of the intervention (Week 6/ Day 42). Patients were instructed to store their sealed stool samples immediately in the freezer compartment before their scheduled visitation. All the stool samples were collected from the patients and stored in a freezer at -80◦C until DNA extraction.

Analysis was performed on only stool samples with a minimum weight requirement of 2 grams. The genomic DNA was extracted based on the manufacturer's protocol with slight modifications using the SPINeasy DNA Kit for Fecal/Soil (MP Biomedicals). The extracted total DNA was measured using a nanophotometer. Polymerase chain reaction was used to amplify the V3–V4 variable region of the prokaryotic 16S rRNA gene. The sequencing libraries were generated using NEB Next® Ultra™ II FS DNA PCR- free Library Prep Kit (New England Biolabs, USA, Catalog # : E7430L) following the manufacturer's recommendations. The library was checked with Qubit and real-time PCR for quantification and bioanalyser for size distribution detection. Quantified libraries were pooled and sequenced on Illumina (Novogene). The demultiplexed paired-end reads from 16S rRNA gene amplicon sequencing were imported into Quantitative Insights Into Microbial Ecology (QIIME2 version 2022.2) and installed in a conda environment for data analysis[101]. The reads were quality-filtered using the QIIME2 plugin DADA2 to remove low-quality sequences and primer sequences, and chimeras were removed using DADA2 default parameters before clustering them into amplicon sequence variants (ASVs)[102]. Multiple

sequence alignment of the ASVs was performed using MAFFT[103] and the highly gapped regions were masked before the construction of phylogeny using the FastTree2[104]. The number of reads per sample was rarefied according to the sampling depths. Rarefaction analysis was performed at the ASV levels to determine if the samples were sequenced to a sufficient depth.

Alpha diversity indices were computed in QIIME2 to assess the community diversity (Shannon and Simpson indices) and richness (Chao1). The beta diversity Bray-Curtis, weighted and unweighted UniFrac[105] indices were calculated using the *R* package phyloseq[106] to estimate the dissimilarities between the samples. Principal coordinate analysis (PCoA) on the stool-derived gut microbiome was conducted using phyloseq with the Bray–Curtis, unweighted and weighted UniFrac distances to visualise the microbiome differences between probiotic and placebo groups, and significant differences between the groups were determined using the adonis2 function of vegan 2.6-4 implemented in *R*[107]. Taxonomic assignment of the ASVs was carried out using a q2-feature-classifier plugin[108] with reference to the EzBioCloud database[109]. The visualisations, such as the boxplots and PCoA, were done using the ggplot package in *R*.

3. Results

3.1. Participants Recruitment, Baseline Demographics and Clinical Measures of Study Groups

A total of 15 (12 completed the trial, three drop-outs) out of 81 initial respondents were included in the trial. The participant recruitment was conducted between December 2021 and December 2022 with study locations at three different regions within Peninsular Malaysia through online research advertisements. Patient recruitment for the Johor site commenced in December 2021 and garnered 53 respondents up to December 2022. The study location was then expanded to the Klang Valley region in May 2022 to improve the recruitment rate. There were 28 respondents for the Klang Valley sites between May 2022 and December 2022. Participants recruitment between December 2021 and December 2022 is presented in Supplementary Figure S1.

57 out of 81 initial respondents interested in participating in the trial were immediately excluded after the preliminary screening over the phone, commonly due to lack of accessibility to the study locations and existing use of antidepressants (AD). The remaining 25 participants were scheduled for their first face-to-face appointments for further screenings. Out of 25 participants, 9 participants were excluded for not showing up (n=1), following primary screenings by consultant psychiatrists (n=5) and blood investigations (n=3). The remaining 16 participants who fulfilled all the inclusion and exclusion criteria were included in the trial. Based on a predetermined 3:1 randomisation ratio, a total of 12 participants were assigned to the probiotic group, while a total of 3 were assigned to the placebo group. In the

probiotic group, there were three drop-outs; two were lost to follow-ups at Week 1 and Week 3 into the intervention phase, respectively, and one was withdrawn from the study at Week 2 due to adverse events. Per protocol analysis was performed for both the study groups on all the participants who completed the clinical assessments (probiotic *n*=9, placebo *n*=3) and participants whose stool samples were adequate for gut microbiome analysis (probiotic *n*=7, placebo *n*=3). The lack of stool samples for the microbiome analysis is mainly due to the participants providing inadequate samples due to irregular bowel habits. The patient recruitment flow is presented in Supplementary Figure S2, with reasons specified for exclusions at every stage.

Patients' baseline demographics and clinical scores are presented in Supplementary Table S2. Mean age was 32.33 (6.59) with a range of 23–42 years old in the probiotic group and 28.66 (44.72) with a range of 25–34 years old. Both study groups included female and male participants, however, female participants greatly outnumbered the male participants in both groups at a percentage of 88.9% and 66.7% in probiotics and placebo groups respectively. The study patients were amongst three different main ethnicities in Malaysia namely Malay, Chinese and Indian, corresponding to the largest percentage of Malay patients at 55.6% (*n*=5 out of a total of 12 patients across both groups) in the probiotics group, followed by Chinese patients at 22.2% (*n*=2) in probiotics group and 100% (*n*=3) in placebo group and the remaining 22.2% (*n*=2) of Indian patients in probiotics group. Most of the study patients had a Bachelor's degree (44.4% in the probiotics group and 100% in the placebo group) in terms of education level and full employment (66.7% in the probiotics group and 33.3% in the placebo group) in terms of employment status. In terms of non-psychiatric comorbidities, there were patients on oral medication for hypertension (*n*=2) and endometriosis (*n*=1) in the probiotics group. Psychiatric history revealed that there was a total of five study patients with a previous clinical diagnosis of MDD and/or Generalized Anxiety Disorder (GAD) who mostly defaulted clinical follow-up for reasons including treatment side effects and lack of perceived improvement. All these patients were not on any clinical follow-up or any form of psychotherapy and/or pharmacotherapy for at least a minimum of 6 months to a maximum of two years at the time of their first study visits. The baseline information on patients' regular diet and exercise patterns was obtained and assessed for any major change over the trial period through clinical interviews with reference to their respective weekly dietary and physical activity records. All patients maintained their regular diet and physical activity throughout the intervention phase. Baseline scores for total PHQ-9 and BDI scores were referred for diagnosis establishment during a clinical interview by a consultant psychiatrist. The mean for PHQ-9 scores for the probiotic group was 13.44 (6.26), and for the placebo group was 7.66 (2.08), whereas for total BDI for the probiotic group was 26.11 (11.83) and for the placebo group was 13.66 (3.78).

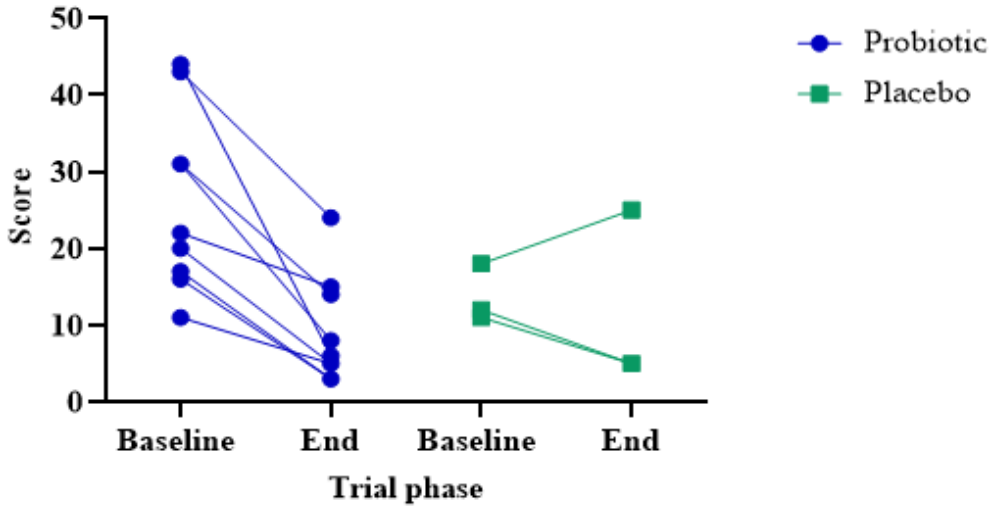
3.2. Clinical Outcomes of Depression

The primary clinical outcome measure for overall depressive symptomatology was measured using the Beck Depression Inventory, whereas secondary clinical outcomes included the assessment of depression severity using the Patient Global Impression of Severity (PGI-S) and Clinical Global Impression of Severity (CGI-S), as well as the improvement of depression following the initiation of the intervention, measured using the Patient Global Impression of Improvement (PGI-I) and Clinical Global Impression of Improvement (CGI-I).

3.2.1. BDI

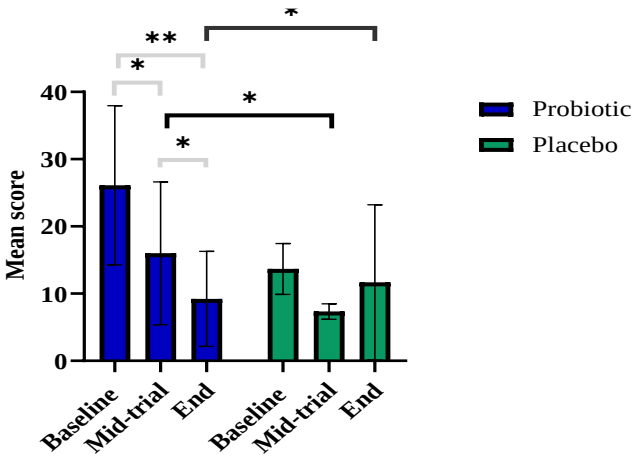
A significant reduction of total BDI scores was observed within the probiotic group pre-and-post intervention with a mean of 26.11 (11.8) to 9.22 (7.06) with $p<0.001$. No significant change was observed in the placebo group pre-and-post intervention from 13.66 (3.78) to 11.67 (11.54) with $p=0.701$. A significant reduction of scores was observed in the probiotic group from baseline to mid-trial and mid-trial to post-intervention with a mean of 16 (10.61) at mid-trial with p-values of .018 and .01, respectively. However, no significant reduction of scores was observed in the placebo group, with a mean of 7.33 (1.15) with *p*-values of .076 and .566, respectively. The individual patient's BDI scores within study groups and changes in mean BDI scores within and between study groups are shown in Figures 1 and 2, respectively. As shown in Figure 1, all participants in the probiotic group showed a reduction in their BDI scores, with the largest improvements seen in Participants 005 (from 44 to 6), 003 (from 31 to 8), and 002 (from 43 to 24). The placebo group showed mixed results, with Participant 006 experiencing a worsening of symptoms (from 18 to 25). Participants 007 and 010 showed modest improvements, but these changes were much less substantial compared to those in the probiotic group.

The post-intervention mean score was lower in the probiotic group but did not significantly differ between probiotic and placebo groups, with a difference of -2.44 ($p=0.756$). However, the post-intervention mean scores yielded a modest effect size of $d_{post}=0.3$ (62%) with a 95% CI: -1.012, -1.612. The absolute effect size considering the pre-and-post-intervention differences, $d_{pre\text{-}post}$ was 1.282.



	Probiotic									Placebo		
	001	**002**	**003**	**004**	**005**	**008**	**011**	**012**	**013**	**006**	**007**	**010**
Baseline	17	43	31	22	44	31	11	20	16	18	11	12
End of trial	3	24	8	15	6	14	5	5	3	25	5	5

Figure 1. Individual BDI scores of participants in the probiotic (n=9) and placebo (n=3) groups at baseline and end of trial.



* p < .05
** p < .001

Figure 2. Changes in mean BDI scores of participants within and between probiotic (n=9) and placebo (n=3) groups at baseline, mid-trial and end of trial.

3.2.2. PGI-S and CGI-S

The severity of depression pre-and-post-intervention rated by the patient and clinician were assessed using PGI-S and CGI-S, respectively. The mean of PGI-S in the probiotic group significantly reduced from 2.67 (0.866) to 1.89 (1.269) with p=0.023 pre-and-post intervention whereas no significant change was seen in the placebo group from 1.67 (0.577) to 1.33 (0.577) with p=0.423. No significant difference in the scoring was observed between the probiotic and placebo groups post-intervention, with a mean difference of 0.556 (p=0.535).

The mean of CGI-S in the probiotic group significantly reduced from 2.89 (0.601) to 1.89 (0.782) with p=0.003 pre-and-post intervention, whereas no significant change was seen in the placebo group from 2.67 (0.577) to 2.00 (1.00) with p=0.423. No significant difference in the scorings was observed between the probiotic and placebo groups post-intervention, with a mean difference of -0.111 (p=0.872).

The Cohen's kappa, κ measuring the inter-rate agreement reliability between PGI-S and CGI-S within the probiotic group post-intervention was -0.019 (p=0.929), indicating no agreement between the raters, although no statistical significance was observed. The cross-tabulation of percentage distribution for the rated items revealed 44.4% on PGI-S and 33.3% on CGI-S for "normal, not at all ill", 44.4% and 44.4% for "borderline mentally ill", 0% and 22.2% for "mildly ill" and 11.1% and 0% for "markedly ill" suggesting discrepancies between patients' and clinicians' perspectives on the severity of illness.

Based on the severity assessment, patients' and clinicians' ratings in the probiotic group mainly were "normal, not at all ill" and "borderline mentally ill" (patient-rating of 88.8% and clinician-rating of 77.7%) compared to "mildly ill" (clinician-rating of 22.2%) and "markedly ill" (patient-rating of 11.1%). Therefore, these discrepancies in percent agreement correspond to "no agreement" between both raters (κ = -0.019).

3.2.3. PGI-I and CGI-I

The improvement of depression pre-and-post-intervention rated by patient and clinician were assessed using Patient Global Impression of Improvement (PGI-I) and Clinical Global Impression of Improvement (CGI-I) from the initiation of treatment, respectively. The mean of PGI-I in the probiotic group significantly reduced from 3.67 (0.866) to 2.33 (1.118) with p=0.023, whereas no significant change was seen in the placebo group from 1.67 (0.577) to 1.33 (0.577) with p=0.423 following the initiation of intervention. No significant difference in the scoring was observed between the probiotic and placebo groups post-intervention, with a mean difference of 0.556 (p=0.535). The mean of CGI-I in both study groups significantly reduced from 2.89 (0.601) to 1.89 (0.782)) with p=0.016 pre-and-post intervention in the probiotic group and from 4.33 (1.528) to 2.67 (1.528) with p=0.038 in the placebo group. However, no significant difference in the improvement scorings was observed

between the probiotic and placebo group post-intervention, with a mean difference of -0.333 (p=0.602).

The Cohen's kappa, κ measuring the inter-rate agreement reliability between PGI-I and CGI-I within the probiotic group post-intervention was 0.368 (p=0.07), suggesting a fair agreement between both the raters, although no statistical significance was observed. The cross-tabulation of percentage distribution for the rated items revealed 33.3% on PGI-I and 33.3% on CGI-I for "very much improved", 11.1% and 22.2% for much improved, 44.4% and 33.3% for minimally improved and 11.1% and 11.1% for no change suggesting some discrepancies between patients' and clinicians' perspectives on the improvement illness. Based on the improvement assessment, the ratings mainly were "improved" (patient-and-clinician-ratings of 88.8%) compared to "no change" (patient-and-clinician-ratings of 11.1%). Therefore, these percent agreement correspond to a "fair agreement" (κ = 0.368) between both raters.

3.3. Clinical Assessments on Patients' Diet and Physical Activity, Compliance and Adverse Events

Patients' diet and physical activity were clinically assessed based on info provided by patients with reference to their respective weekly dietary and physical activity records. All the trial patients maintained their routine diets and physical activities throughout the study. Patients were considered compliant if no more than one-day doses (twice per day) were missed over a week based on patient-provided information and returned capsules at every clinical visit. Most patients were compliant and did not miss the scheduled consumption of the investigation product (IP) throughout the intervention phase. Only one patient in the probiotic group missed three scheduled doses consecutively over two days after two weeks into the intervention phase. The patient had an onset of diarrhea and fever and was treated by the patient's regular General Practitioner (GP) as "food poisoning". The patient resumed the scheduled intake of probiotics immediately after symptomatic improvement and successfully completed the trial. In the probiotic group, there were also two drop-outs that were lost to follow-ups at Week 1 and Week 3 into the intervention phase, respectively. Based on individual checking, both the patients stated transportation issues and high travel-related costs as reasons for discontinuation.

One patient in the probiotic group complained of the onset of gastrointestinal symptoms (mild abdominal discomfort and bloating, frequent watery stools) one day after the consumption of IP. The patient continued taking IP for the next two days until the regular follow-up call over the phone, where the patient expressed concerns that the symptoms were due to the IP intake. The patient sought treatment from the patient's regular GP on day three from the onset of symptoms and was treated as "acute gastroenteritis". The patient's symptoms were considered possible gastrointestinal side effects from the IP and immediately

withdrawn from the trial upon the clinician's assessment on day four from the onset of the symptoms.

3.4. Stool-Derived Gut Microbiome Changes

3.4.1. Taxa-specific relative abundance of study groups

The microbiome data for the probiotic group (*n*=7) consisted of seven phyla, 13 classes, 17 orders, 35 families and 89 genera. In contrast, the placebo group (*n*=3) consisted of four phyla, nine classes, 10 orders, 19 families and 57 genera. The relative abundances for high abundance taxa at phylum and family levels were obtained for three phyla (*Actinomycetota*, *Firmicutes*, *Proteobacteria*) and seven families (*Bifidobacteriaceae*, *Lactobacillaceae*, *Coriobacteriaceae*, *Enterobacteriaceae*, *Enterococcaceae*, *Peptostreptococcaceae*, *Bacillaceae*) for probiotic and placebo groups. The graphical presentations are provided in Figures 3 and 4. No statistically significant differences were seen within and between the study groups before and after intervention at phylum and family levels. However, at the phylum level, the average relative abundances of *Actinomycetota* after intervention increased in the probiotic group but decreased in the placebo group. However, the opposite was seen for the *Proteobacteria*, where the average relative abundance decreased in the probiotic group while slightly increasing in the placebo group after intervention. *Firmicutes* increased in both groups, but the increase was greater in the placebo group. At family levels, the average relative abundances of *Bifidobacteriaceae*, *Enterococcaceae* and *Bacillaceae* increased in the probiotic group but decreased in the placebo group after intervention. On the other hand, *Lactobacillacaceae*, *Enterobacteriaceae*, and *Peptostreptococcaceae* decreased in the probiotic group while increasing in the placebo group after intervention. *Coriobacteriaceae* decreased for both study groups after the intervention, with a greater decrease in the placebo group. At the genus level, the average relative abundances for *Bifidobacterium* increased for the probiotic group but decreased for the placebo group, while *Lactobacillus* increased for both groups with a greater increase in the probiotic group. The graphical presentation is provided in Figure 5.

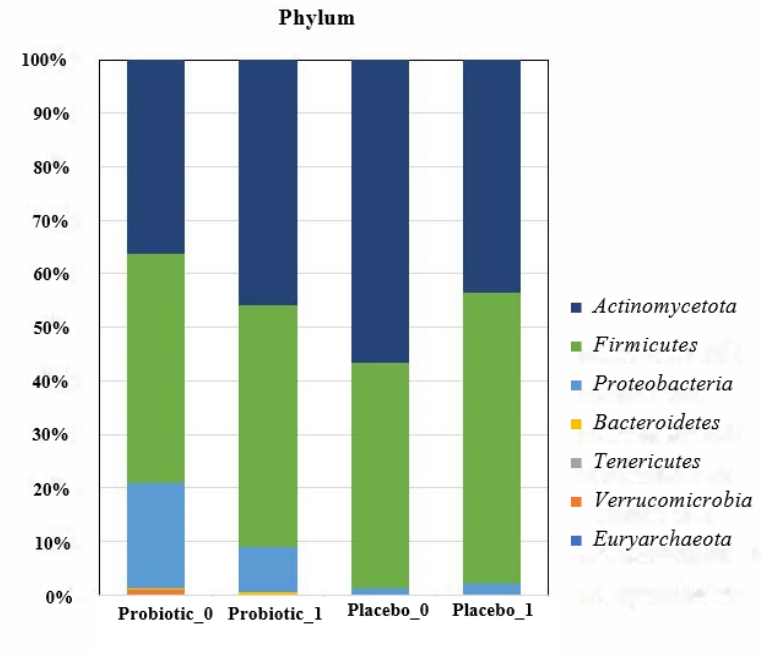


Figure 3. Taxonomic bar plot of average relative abundance at the phylum level before (_0) and after (_1) intervention for probiotics (*n*=7) and placebo (*n*=3) groups.

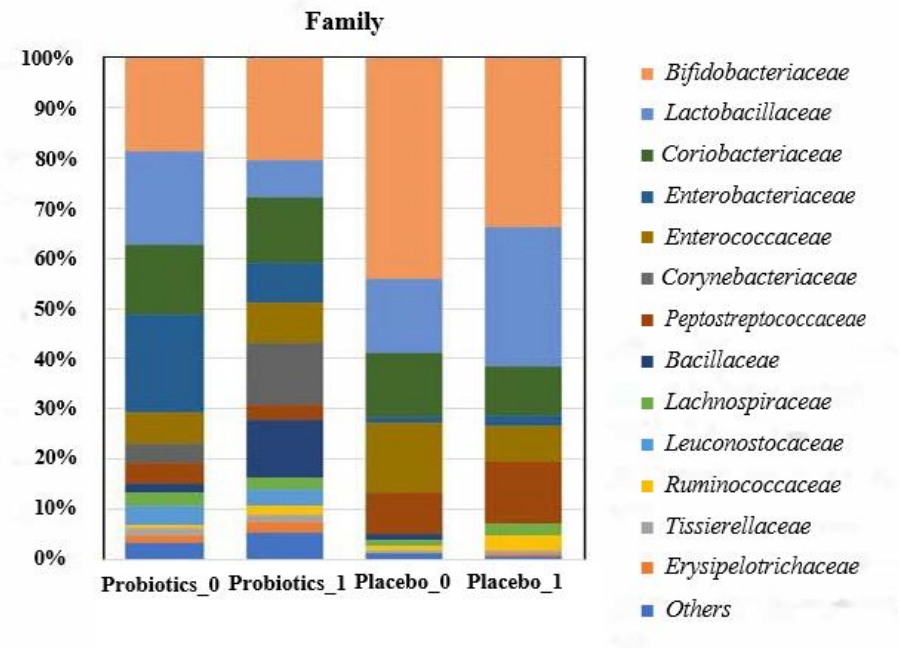


Figure 4. Taxonomic bar plot of average relative abundance at the family level before (_0) and after (_1) intervention for probiotics (*n*=7) and placebo (*n*=3) groups.

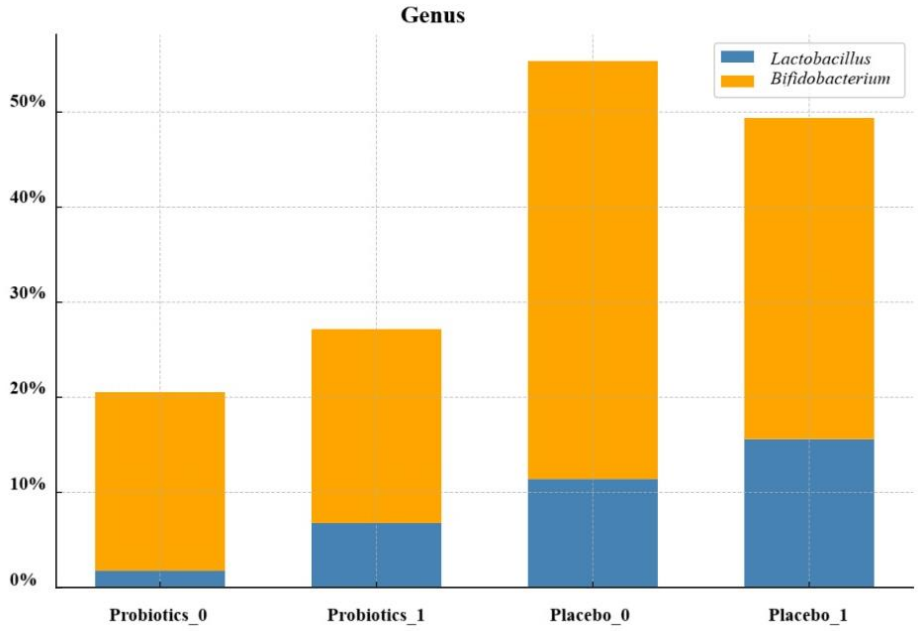


Figure 5. Taxonomic bar plot of average relative abundance at the genus level before (_0) and after (_1) intervention for probiotics (*n*=7) and placebo (*n*=3) groups.

3.4.2. Taxa-specific relative abundance of individual participants

The relative abundances for high abundance taxa at family levels (*Bifidobacteriaceae, Lactobacillaceae Coriobacteriaceae, Enterobacteriaceae, Enterococcaceae, Peptostreptococcaceae*) were obtained for individual participants in probiotic (*n*=7) and placebo (*n*=3) groups before and after intervention as shown in Figure 6. In the probiotic group, distinct patterns in bacterial relative abundance were observed among participants. *Bifidobacteriaceae* and *Coriobacteriaceae* exhibited notable reductions in Participants 001, 004, and 013, while *Enterobacteriaceae* increased in 001 and 013 but was nearly undetectable in 004. Participants 005 and 012 demonstrated substantial increases in *Bifidobacteriaceae* and *Lactobacillaceae*. Variability was evident in Participants 008 and 011, where *Lactobacillaceae* and *Peptostreptococcaceae* increased, while *Enterobacteriaceae* and *Coriobacteriaceae* decreased. Participant 013 displayed a mixed response, characterised by reductions in *Bifidobacteriaceae*, *Lactobacillaceae*, and *Coriobacteriaceae*, alongside sharp increases in *Enterobacteriaceae* and *Enterococcaceae*. Overall, most participants, including 005, 011, and 012, exhibited increases in *Bifidobacteriaceae* and *Lactobacillaceae*, whereas *Enterobacteriaceae* displayed variable trends. *Peptostreptococcaceae* consistently declined, reaching undetectable levels in 001 and 004. *Lactobacillaceae* showed mixed trends, with notable increases in 011 but moderate decreases in 001 and 004. In the placebo group, a more consistent pattern of change emerged. Most individuals, including 006, 007, and 010, showed either decreases or no significant changes in *Bifidobacteriaceae.* Participant 006 exhibits decreases across all bacterial families, except for *Lactobacillaceae*, which shows a notable increase. In contrast, Participant 007 and Participant 010 display mixed changes, with *Enterobacteriaceae*,

Enterococcaceae, and *Peptostreptococcaceae* increasing in abundance, while *Bifidobacteriaceae* and *Coriobacteriaceae* show reductions.

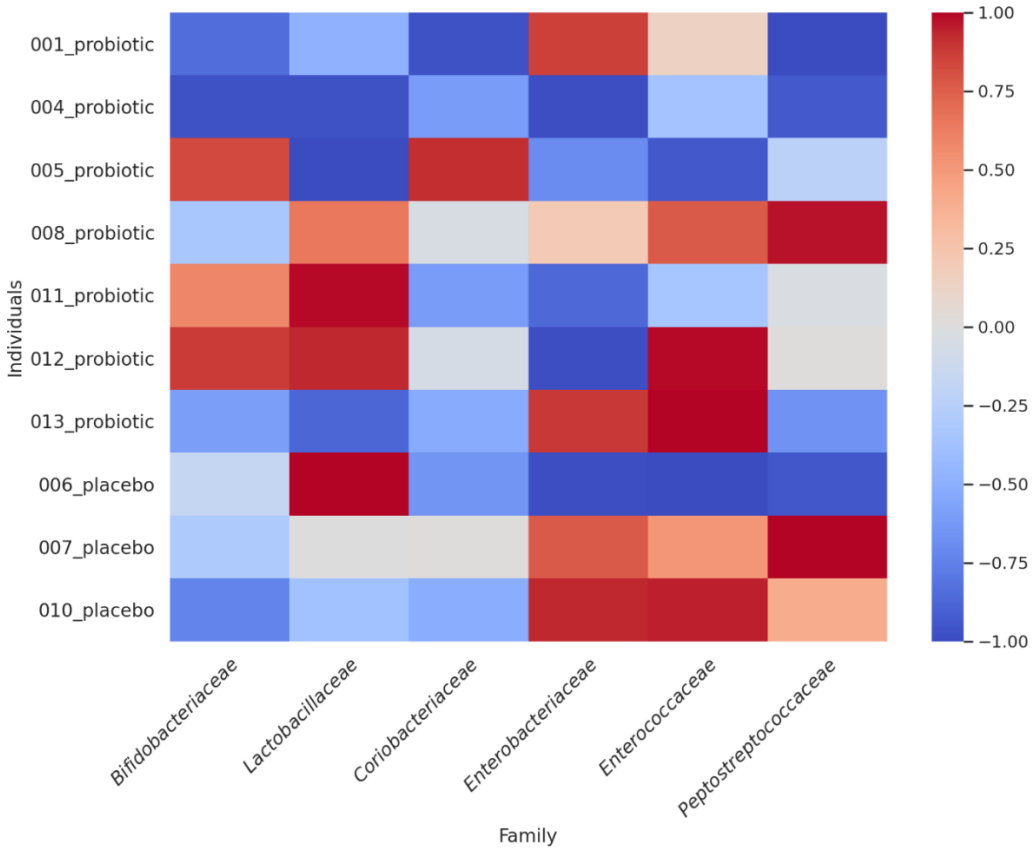


Figure 6. Relative abundance changes of bacterial families among individuals before and after intervention for probiotics (*n*=7) and placebo (*n*=3) groups, normalized between -1 and 1.

3.4.3. Alpha diversity

The alpha diversity indices before and after probiotic treatment showed no significant differences for the Chao-1-diversity index ($F_{1,12}$ = 0.014, p = 0.909), Shannon index ($F_{1,12}$ = 0.014, p = 0.909), Simpson index ($F_{1,12}$ = 0.118, p = 0.737), and a number of observed features ($F_{1,12}$ = 0.014, p = 0.909).

No significant differences were seen before and after placebo treatment with the Chao-1-diversity index ($F_{1,12}$ = 0.2, p = 0.678), Shannon index ($F_{1,12}$ = 0.112, p = 0.754), Simpson index ($F_{1,12}$ = 0.134, p = 0.733), and number of observed features ($F_{1,12}$ = 0.2, p = 0.678). Similarly, no significant differences were seen between probiotic and placebo groups before and after intervention.

No significant differences were seen between probiotic and placebo groups after intervention. The graphical presentations for all the alpha diversity indices are provided in Supplementary Figure S3.

3.4.4. Beta diversity

The beta diversity metrics before and after intervention with probiotics showed no significant differences for the Bray-Curtis dissimilarity index (*R2*=0.04789, *p*=0.875), unweighted UniFrac distance (*R2*=0.02366, *p*=0.984) and weighted UniFrac distance (*R2*=0.01855, *p*=0.972).

Similarly, no significant differences were seen before and after placebo treatment with the Bray-Curtis dissimilarity index (*R2*=0.03013, *p*=1.00), unweighted UniFrac distance (*R2*=0.08081, *p*=0.8) and weighted UniFrac distance (*R2*=0.03926, *p*=0.900).

No significant differences were seen between probiotic and placebo groups after intervention. Supplementary Figure S4 provides the graphical presentations for all the beta diversity metrics. In addition to beta diversity indices, a Venn diagram is included as Supplementary Figure S5 to illustrate the number of common and unique ASV within and between treatment groups, comparing ASV presence before (_0) and after (_1) intervention in probiotics (*n*=7) and placebo (*n*=3) groups.

4. Discussion

4.1. Correlation of Clinical Outcomes of Depression and Gut Microbial Alteration

Our study provides insights into the impact of a six-week probiotic intervention on depressive symptoms and gut microbiome, contributing to the growing body of evidence supporting the gut-brain axis as a target for therapeutic interventions in depressive disorder[43,45–47]. The relationship between clinical outcomes and gut microbiome findings in this study highlights intriguing connections that suggest the potential role of probiotics in alleviating depression through the gut-brain axis. Our study found that the probiotic group demonstrated consistent and significant improvements across all measures, including the Beck Depression Inventory, Clinical Global Impression of Severity (CGI-S), Patient Global Impression of Severity (PGI-S), Clinical Global Impression of Improvement (CGI-I), and Patient Global Impression of Improvement (PGI-I). In contrast, the placebo group showed mixed results, with some modest improvements but also worsening symptoms in one participant, and no significant changes in BDI, CGI-S, PGI-S, or PGI-I, except for a significant reduction in CGI-I, indicating clinician-rated improvement. No significant differences in clinical outcomes were found between these study groups. Similarly, no statistically significant differences in relative abundances or diversity measures were obtained. Although the observed changes in taxa-specific relative abundances and microbial diversity indices did not reach statistical significance, distinct patterns between the probiotic and placebo groups were evident. These patterns suggest that probiotics may modulate the gut microbiome in ways that correlate with improvements in depressive symptoms. The

correlation between gut microbiome changes and clinical outcomes in both the probiotic and placebo groups reveals distinct differences in microbial composition and their corresponding effects on depressive symptoms. The clinical data suggest that probiotics have a significant yet modest effect on depression, and the gut microbiome analysis offers insights into the potential gut-microbiome modulating impact of probiotics underlying these effects. The therapeutic potential of probiotics in depression is rooted in consistent findings of gut dysbiosis in depressed patients, characterized by reduced microbial diversity and an inverse relationship between pathobionts and beneficial microbiota compared to healthy controls[43,45-47]. Our focus is on the relative abundances of predominant taxa at the phylum, family, and genus levels, examining their correlation with depressive outcomes, aligning with the reporting approach commonly adopted in clinical trials of probiotics[110–115]. In our study, we report notable changes observed at the phylum, family, and genus levels in the probiotic and placebo group post-intervention, which aligned with the depressive outcomes, although no statistical significance was obtained for the compositional changes and diversity measures. Several systematic reviews indicated that no significant alteration was observed in gut microbial composition following probiotic supplementation over a period ranging from four to eight weeks although significant depressive outcomes were reported as early as four weeks[62,116,117]. On the other hand, a handful of clinical studies of probiotics in depressed patients demonstrated significant changes and differences between probiotic and placebo groups in terms of either alpha or beta diversity measures as early as four weeks post-intervention[111,114,115].

In the probiotic group, one of the key observations includes increases in *Actinomycetota*, particularly *Bifidobacterium* and *Firmicutes*, particularly *Lactobacillus*, which positively correspond to the hallmark combined probiotic strains of *Lactobacillus* and *Bifidobacterium* used in this study for their known significant anti-depressive potential[55,56,112,113,117,118]. Additionally, there was a decrease in *Proteobacteria*, a phylum often linked to dysbiosis and inflammatory states, both of which are implicated in depression[119,120]. Therefore, the reduction in *Proteobacteria* likely contributed to the observed improvements in clinical outcomes. At the family level, the increase in *Bifidobacteriaceae* is linked to enhanced gut health and mental health outcomes as members of this family play a crucial role in modulating inflammation, producing short-chain fatty acids (SCFAs), and supporting gut barrier function[121,122]. Furthermore, the increase in *Bacillaceae*, which is known to strengthen gut barrier integrity, and the production of neuroactive compounds such as gamma-aminobutyric acid (GABA), further supports the observed improvements in depressive outcomes[123,124]. A clinical trial that examined the impact of probiotics as an adjunct to antidepressants in MDD patients reported an elevated level of *Bacillaceae* following probiotic consumption over eight weeks and correlated this finding to its beneficial depression-related outcome[125]. Conversely, an increase in *Enterococcaceae* is often linked to worsened depressive symptoms, though certain studies suggest some strains within this family may have anti-depressive potential[122,126–129]. In

contrast, the decrease in *Enterobacteriaceae*, and *Peptostreptococcaceae* in the probiotic group suggests a reduction in potentially harmful bacteria that could exacerbate gut dysbiosis and inflammation, as elevated levels of these taxa have been commonly reported in depressed patients and were positively associated with depressive outcomes[118,122,130–135]. Moreover. Nonetheless, the study that examined the pathological role of gut microbiota in inflammatory bowel disease (IBD) and depression concluded that the interaction between *Enterobacteriaceae, Enterococcaceae, Lactobacillaceae,* and *Bifidobacteriaceae; Bifidobacterium longum* exerts a positive effect in controlling IBD, neuroinflammation and depression by regulating the potent gut microbial by-products associated with occurrence of depression in IBD[122]. At the genus level, there was an increase in *Bifidobacterium* and *Lactobacillus*, two hallmark strains commonly linked to gut health, neurotransmitter regulation, and mood improvement. Known for producing GABA and SCFAs, these genera play a vital role in supporting the gut-brain axis and depressive outcomes through modulation of gut inflammation and neurotransmitter synthesis, thereby influencing mood regulation and potentially alleviating depressive symptoms[67,136–139]. Furthermore, earlier findings of gut microbiome studies that reported lower levels of these genera in patients with depression[140,141]. Moreover, the combination of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 are commonly researched probiotics, known to demonstrate significant anti-depressive potential in animal and human models[142–148]. Hence, the observed changes in relative abundances and discussed potential mechanisms may explain the substantial reductions in depressive symptoms as well as improvements in both patient-rated and clinician-rated outcomes, particularly among Participants 005, 003, and 002, who showed the most significant clinical improvements.

In contrast, the placebo group showed more uniform changes in bacterial abundances alongside minimal to no improvement in depression symptoms, as indicated by stable or fluctuating BDI scores and no significant change in severity assessments. Key observation includes a greater increase in *Firmicutes* but with a corresponding increase in microbial taxa often positively associated with depression, such as *Enterococcaceae* and *Peptostreptococcaceae* and a decrease in negatively associated taxa, including *Actinomycetota*, particularly *Bifidobacterium*, and *Bacillaceae*[119,120,122–124,126–129,136–138]. Furthermore, there was a slight increase in *Proteobacteria* in the placebo group, which is often associated with dysbiosis and gut inflammation[119,120]. Additionally, the decreases in potentially beneficial families *Bifidobacteriaceae* and *Bacillaceae* and increases in *Peptostreptococcaceae* and *Enterobacteriaceae*, families associated with dysbiosis and inflammation, further suggest a potential imbalance in the gut microbiota, contributing to dysbiosis that may influence the depressive outcome via the gut-brain axis[45,118,133–135,149,150]. Moreover, the decrease in *Bifidobacterium* in the placebo group further contributes to the lack of beneficial taxa that support gut integrity and neurotransmitter synthesis, thus impacting the regulation of mood and stress responses[136–138,151,152]. While *Lactobacillus* also increased in the placebo group, the magnitude of this increase was smaller compared to the

probiotic group. Additionally, in commonality, *Coriobacteriaceae* decreased in both groups, with a greater decrease in the placebo group, where an increase in *Coriobacteriaceae* has been positively linked to gut dysbiosis and detrimental effects on metabolism and inflammation[121]. *Coriobacteriaceae* was also found to be significantly higher in stress-induced mice models[153]. The observed patterns suggest the natural progression of an individual gut microbiome in the absence of targeted intervention in the placebo group, possibly explaining the more modest or fluctuating clinical progress and worsening of depressive symptoms in some individuals, particularly Participant 006[154–159].

Overall, the probiotic group showed mostly favourable microbial changes, particularly the increases in potentially beneficial taxa, including *Bacillaceae* and *Bifidobacteriaceae* families and *Bifidobacterium* and *Lactobacillus* genera, with reductions in potentially pathogenic taxa including *Proteobacteria*, *Peptostreptococcaceae* and *Enterobacteriaceae* which correlated with substantial overall improvements in depressive outcomes. We also speculate that the probiotic intervention may have led to targeted changes in beneficial microbes, rather than broad changes in microbial diversity, which may resonate with the overall favourable and significant depressive outcome in this group. In contrast, the placebo group demonstrated microbial shifts linked to gut dysbiosis with increases in potentially pathogenic taxa, including *Proteobacteria*, *Peptostreptococcaceae* and *Enterobacteriaceae* and depletion in potentially beneficial taxa, including *Bacillaceae* and *Bifidobacteriaceae* families and *Bifidobacterium* genus, which were may reflect the mixed or worsening clinical outcomes in this group. Generally, the comparison between the probiotic and placebo groups underscores the potential role that specific gut microbial shifts play in influencing clinical outcomes. The contradictory effects observed could also be attributed to the strain-specific differences, where certain strains produce beneficial effects on depression outcomes, while others may trigger inflammation or behave opportunistically, leading to varied outcomes across studies[42,160]. While individual variations in microbial responses were evident owing to host-specific and environmental factors, the overall findings highlight the potential of probiotics to positively influence depression through the modulation of gut microbiota and the gut-brain axis[43,154–159,161–163]. This variability underscores the relevance of and the growing emphasis on precision medicine, where individualised microbiome profiling could inform targeted probiotic interventions[42,164,165]. By identifying microbial patterns associated with treatment response, precision approaches may enhance therapeutic efficacy, offering a more personalised strategy for managing depression[42,166–168].

4.2. Probiotics Selection

Patients in our study were given multi-strain probiotics containing *Lactobacillus helveticus* R0052, *Lactobacillus rhamnosus* R0011, *Bifidobacterium longum* R0175, and *Saccharomyces cerevisiae boulardii*. The selection of probiotics is from the pool of probiotics with GRAS status[169,170]. They are selected for their antidepressive potential based on pre-clinical research consisting of mostly bacterial strains of *Lactobacillus* and

Bifidobacterium. These bacterial species are the typical indigenous gut microbiota of human origin. Therefore, they confer properties ideal for therapeutic use as probiotics in the human population[171,172]. Yeast strains, such as *Saccharomyces boulardii,* are also probiotics that are usually used in combination with bacterial probiotics[168,173]. *Saccharomyces boulardii* confers both therapeutic and preventive roles in diarrhoeal diseases caused by bacteria, and promotes gastrointestinal health[174]. *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 strains are among the most commonly studied combination probiotics that are known to demonstrate significant anti-depressive potential in animal and human models[116 142–148,175,176]. In mice models subjected to chronic stress, *L. helveticus* R0052 and *B. longum* R0175 combination probiotics significantly improved depressive-like behaviors, reduced corticosterone levels, prevented stress-induced reduction of hippocampal neurogenesis of noradrenaline, and restored gut barrier. The restoration of elevated cortisol levels is one of the main parameters of normalization of HPA-axis. The improved synaptic plasticity within the hippocampus and hypothalamic regions has been linked to the augmented adult hippocampal neurogenesis. This genetic basis is correlated to the increased expression of several hypothalamic genes involved in neurotransmission and synaptic plasticity, thus enhancing the neuronal network within the hypothalamus to exert anti-depressive effects[143].

Consistently, in clinical trials of combination probiotics *L. helveticus* and *B. longum*, clinical depression and mood outcomes were significantly improved in MDD patients and healthy human volunteers respectively with reduced urinary cortisol levels at the end of probiotic trials[177]. *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 have also been found to exert significant effect on the augmentation of BDNF levels in patients with depression[178–180]. In 66 healthy human volunteers, probiotics supplementation consisting of *L. helveticus* R0052 and *B. longum* R075 over 30 days significantly improved their mood and overall psychological well-being that were reported through a few self-reported measures, including Hospital Anxiety and Depression Scale (HADS). The reduced free urinary cortisol levels were linked to the possible attenuation of the HPA axis[142]. The administration of probiotic *L. rhamnosus* mitigated depressive behaviors in mice models by reducing the stress-induced plasma corticosterone levels[181]. *Lactobacillus rhamnosus* R0011 is another commercially available probiotic that have been commonly marketed as a supplement in combination with prebiotics for improved mood outcome in human subjects[182]. *Lactobacillus rhamnosus* has been found to modify the expression of central GABA receptors and elevate GABA levels. This mechanism was also linked to its possible influence on the downregulation of HPA-axis via a neural route (i.e., vagus nerve) to exert anti-depressive effect[183,184]. In a comprehensive post-market review based on animal and human studies, the combination probiotics *L. helveticus* R0052 and *L. rhamnosus* R0011 have been found to protect the gut barrier integrity and elicit inflammatory response by suppressing inflammatory cytokines IL-1β, IL-8 and TNF-α that have been implicated in the pathomechanism of depression[185,186]. *L. rhamnosus* R0011 also has been found to improve diarrhoeal-associated outcomes in adult subjects[187]. In a RCT inovlning 32 adults, the

supplementation of combination probiotics of *L. helveticus* R0052, *B. longum* R0175 and *L. rhamnosus* R0011 with added prebiotics for 30 days was shown to reduce depression symptoms by 55% and improve overall mood by 25% based on assessment using Profile of Mood Stress (POMS) questionnaire[188]. Therefore, the findings in our study are consistent with the demonstrated anti-depressive potential of *Lactobacillus* and *Bifidobacterium* strains that have plausible roles in attenuating depressive outcome.

4.3. Study Feasibility

To the best of our knowledge, our study is the first pilot study evaluating the preliminary effects of probiotics in patients with clinical depression in Malaysia. Our preliminary report provides a solid base to expand further into full-fledged randomised controlled trials (RCT) of probiotics that involve Malaysian patients with MDD. Although this remains a small-scale project, we gather various methodological and clinical insights to drive more research initiatives in this area in its entirety.

The key factors that provide insight into the feasibility of this study include participant recruitment, tolerability to the intervention, and compliance with the study protocol. Participants were recruited from the Malaysian community in Klang Valley and Johor Bahru. The rate of respondents varied between none and 31 in a month from December 2021 to December 2022, with the recruitment rate between none and three in a month (Please refer to Supplementary Figure S1). Participant recruitment was the most significant obstacle, with low response rates likely influenced by the pandemic, which restricted access to hospital settings. Furthermore, the imposed strict study inclusion criteria and limited research advertisement platforms further contributed to the low recruitment rate.

The tolerability of the intervention in this study was generally favourable, with most participants able to complete the trial without reporting significant adverse effects. However, Two patients in the probiotic group experienced gastrointestinal-related issues during the study. One patient missed three consecutive doses due to diarrhea and fever and was diagnosed as having “food poisoning” by a general practitioner, but resumed the probiotics after recovery and completed the trial. This event highlights the possibility of gastrointestinal side effects, although they were mild and likely transient. Another patient developed gastrointestinal symptoms (abdominal discomfort, bloating, and frequent watery stools) one day after taking the probiotics, sought medical treatment for acute gastroenteritis, and was withdrawn from the study after a clinician’s assessment, with the symptoms attributed to potential side effects of the probiotics. The patient’s withdrawal was in line with the study protocol to ensure participant safety, and the occurrence of such side effects is consistent with what has been reported in other studies involving probiotics. Gastrointestinal side effects associated with probiotics, although uncommon, have been reported in patients with underlying inflammatory bowel diseases and compromised health, thus becoming subject to individual receptivity and health status[147,189–191]. In our study, the adverse event was

isolated, and most participants did not experience any significant tolerability issues, suggesting that probiotics were well-tolerated by the majority of the study population.

In terms of compliance assessment, patients were considered compliant if no more than one-day doses (twice per day) were missed over a week based on patient-provided info and returned capsules at every clinical visit. Most patients were compliant and did not miss the scheduled consumption of the investigation product (IP) throughout the intervention phase. Only one patient in the probiotic group missed three scheduled doses consecutively due to the onset of "food poisoning" but resumed the scheduled intake of probiotics immediately after symptomatic improvement and successfully completed the trial. Therefore, we present an overall satisfactory compliance from our study patients that is consistent with findings from other clinical studies of probiotics in depressed cohorts that generally report a high compliance rate[115,147, 92]. We also reported two drop-outs that were lost to follow-ups at Week 1 and Week 3 into the intervention phase, respectively, due to transportation issues and high travel-related costs. Other studies have reported a drop-out rate of 30% in probiotics and 13% in placebo groups and predicted a 25% drop-out rate in the probiotic group[114,115].

Our study demonstrated modest yet positive feasibility despite encountering some challenges. Participant recruitment was affected by the pandemic and strict eligibility criteria, resulting in fluctuating recruitment rates. The probiotic intervention was generally well-tolerated, with minimal gastrointestinal side effects reported, and only one participant withdrew due to these effects. Compliance with the study protocol was high, although a small number of participants dropped out due to logistical issues, such as transportation and travel costs. Another significant hurdle faced was in obtaining stool samples for microbiome analysis, as some participants provided inadequate samples often due to irregular bowel habits. Despite these challenges, the study proved feasible and provided important insights into the potential of probiotics for treating depression, informing the design of future, larger-scale trials.

5. Conclusions

As a pioneering study in Malaysia, our preliminary report provides evidence that multi-strain probiotics containing *Lactobacillus helveticus* R0052, *Lactobacillus rhamnosus* R0011 and *Bifidobacterium longum* R0175 may exert significant anti-depressive effects as a stand-alone treatment for mild major depressive disorder (MDD), with the probiotic group showing significant improvements in depressive symptoms ($p<0.05$) compared to no changes in the placebo group. The effect sizes ($d_{post}=0.3$; $d_{pre\text{-}post}=1.282$) indicate that probiotics outperformed placebo, with a post-intervention effect comparable to clinical antidepressant trials. While these findings are promising, it is important to note that they are derived from a limited sample size and based on straightforward statistical approaches. While gut microbiome diversity measures remained unchanged, differences in microbial composition suggest potential modulation, with increases in beneficial taxa linked to greater clinical

improvements. The study was feasible, with high compliance and minimal adverse effects, though challenges in recruitment and sample collection were noted. While our findings are promising and the approach is feasible, the small sample size and exploratory nature of the study limit generalizability. Small sample sizes, while not ideal for drawing firm conclusions on efficacy, are often necessary in early-stage research, particularly in pioneering studies where ethical considerations are paramount, especially in depressive disorders. Our study establishes a critical groundwork for future research, with larger sample sizes being a primary focus for strengthening subsequent investigations. Future research is needed to validate our findings and examine the long-term impact of probiotic interventions in larger, more diverse populations to elucidate the clinical significance of probiotics in treating depression. Nonetheless, we conclude by supporting the progressive expansion of probiotics as a potential stand-alone therapeutic within the clinical management of patients with mild MDD and as a preventive intervention for individuals at risk of developing MDD, while also advocating for their role as an adjunct treatment in more severe cases.

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References

1. Barrio C, Arias-Sánchez S, and Martín-Monzón I. The gut microbiota-brain axis, psychobiotics and its influence on brain and behaviour: A systematic review. Psychoneuroendocrinology 2021: 105640.
2. WHO. Depressive disorder (depression). 2023 [Accessed Jan 10]; Available from: https://www.who.int/news-room/fact-sheets/detail/depression.
3. WHO. *The global burden of disease: 2004 update*. 2008: World Health Organization.
4. Lee L-H. Probiotics in Depression Management: Efficacy, Mechanisms and Future Directions. Prog Microbes Mol Biol 2025; 8(1).
5. Saveanu RV and Nemeroff CB. Etiology of depression: genetic and environmental factors. Psychiatric Clinics 2012; 35(1): 51–71.
6. Hu Y, Yiu V, and Clark R. Etiology of Depression: Biological and Environmental Factors in the Development of Depression. J Stud Res 2021; 10(4).
7. Marx W, Penninx BW, Solmi M, *et al*. Major depressive disorder. Nat Rev Dis Primers 2023; 9(1): 44.
8. Benrimoh D, Pomerleau V, Demoustier A, *et al*. *Why We Still Use "Organic Causes": Results From a Survey of Psychiatrists and Residents. JNP 31, 57–64*. 2018.

9. Shafi M, Taufiq F, Mehmood H, *et al*. Relation between depressive disorder and iron deficiency anemia among adults reporting to a secondary healthcare facility: a hospital-based case control study. J Coll Physicians Surg Pak 2018; 28(6): 456–559.
10. Sonino N and Fava GA. Psychiatric disorders associated with Cushing's syndrome: epidemiology, pathophysiology and treatment. CNS Drugs 2001; 15: 361–373.
11. Doyle C, Swain WA, Ewald HAS, *et al*. Inflammation, infection and depression: an evolutionary perspective. Evol Hum Sci 2019; 1: e14.
12. Gautam S, Jain A, Gautam M, *et al*. Clinical practice guidelines for the management of depression. Indian J Psychiatry 2017; 59(Suppl 1): S34.
13. Sheehan DV, Lecrubier Y, Sheehan KH, *et al*. The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 1998; 59(20): 22–33.
14. Norris DR, Clark MS, and Shipley S. The mental status examination. Am Fam Physician 2016; 94(8): 635–641.
15. Cameron IM, Cardy A, Crawford JR, *et al*. Measuring depression severity in general practice: discriminatory performance of the PHQ-9, HADS-D, and BDI-II. Br J Gen Pract 2011; 61(588): e419–e426.
16. Soleimani L, Lapidus KA, and Iosifescu DV. Diagnosis and treatment of major depressive disorder. Neurol Clin 2011; 29(1): 177–193.
17. Zimmerman M, Martinez JH, Young D, *et al*. Severity classification on the Hamilton depression rating scale. J Affect Disord 2013; 150(2): 384–388.
18. Qaseem A, Owens DK, Etxeandia-Ikobaltzeta I, *et al*. Nonpharmacologic and pharmacologic treatments of adults in the acute phase of major depressive disorder: a living clinical guideline from the American College of Physicians. Ann Intern Med 2023; 176(2): 239–252.
19. APA. *Diagnostic and statistical manual of mental disorders: DSM-5*. Vol. 5. 2013: American psychiatric association Washington, DC.
20. Uher R, Payne JL, Pavlova B, *et al*. Major depressive disorder in DSM-5: Implications for clinical practice and research of changes from DSM-IV. Depress Anxiety 2014; 31(6): 459–471.
21. APA. *Diagnostic and statistical manual of mental disorders: DSM-IV*. Vol. 4. 1994: American Psychiatric Association Washington, DC.
22. Cuijpers P, Quero S, Noma H, *et al*. Psychotherapies for depression: a network meta-analysis covering efficacy, acceptability and long-term outcomes of all main treatment types. World Psychiatry 2021; 20(2): 283–293.
23. Otte C, Gold SM, Penninx BW, *et al*. Major depressive disorder. Nat Rev Dis Primers 2016; 2(1): 1–20.
24. Kupfer DJ, Frank E, and Phillips ML. Major depressive disorder: new clinical, neurobiological, and treatment perspectives. Lancet 2012; 379(9820): 1045–1055.
25. Czéh B, Fuchs E, Wiborg O, *et al*. Animal models of major depression and their clinical implications. Prog Neuropsychopharmacol Biol Psychiatry 2016; 64: 293–310.
26. Guy A, Brown M, Lewis S, *et al*. The 'patient voice': patients who experience antidepressant withdrawal symptoms are often dismissed, or misdiagnosed with relapse, or a new medical condition. Ther Adv Psychopharmacol 2020; 10: 2045125320967183.
27. Dell'Osso B, Albert U, Carrà G, *et al*. How to improve adherence to antidepressant treatments in patients with major depression: a psychoeducational consensus checklist. Ann Gen Psychiatry 2020; 19: 1–8.
28. Tang EK, Loo KY, Thye AYK, *et al*. The Impact of Antidepressants on Gut Microbiome and Depression Management. Prog Microbes Mol Biol 2024; 7(1): a0000446.

29. Phillips C. Brain-derived neurotrophic factor, depression, and physical activity: making the neuroplastic connection. Neural Plast 2017.
30. Alemi F, Min H, Yousefi M, *et al*. Effectiveness of common antidepressants: a post market release study. EClinicalMedicine 2021; 41: 101171.
31. Rush AJ, Trivedi MH, Wisniewski SR, *et al*. Bupropion-SR, sertraline, or venlafaxine-XR after failure of SSRIs for depression. N Engl J Med 2006; 354(12): 1231–1242.
32. Ho SC, Chong HY, Chaiyakunapruk N, *et al*. Clinical and economic impact of non-adherence to antidepressants in major depressive disorder: a systematic review. J Affect Disord 2016; 193: 1–10.
33. Lynall M-E and McIntosh AM. *The heterogeneity of depression*. 2023, Am Psychiatric Assoc. p. 703–704.
34. Cuijpers P, Stringaris A, and Wolpert M. Treatment outcomes for depression: challenges and opportunities. Lancet Psychiatry 2020; 7(11): 925–927.
35. Herrman H, Patel V, Kieling C, *et al*. Time for united action on depression: a Lancet–World Psychiatric Association Commission. Lancet 2022; 399(10328): 957–1022.
36. Horwitz AV and Wakefield JC. Two Sides of Depression: Medical & Social. Daedalus 2023; 152(4): 212–227.
37. Musazadeh V, Zarezadeh M, Faghfouri AH, *et al*. Probiotics as an effective therapeutic approach in alleviating depression symptoms: an umbrella meta-analysis. Crit Rev Food Sci Nutr 2023; 63(26): 8292–8300.
38. Zhao S, Liang S, Tao J, *et al*. Probiotics for adults with major depressive disorder compared with antidepressants: a systematic review and network meta-analysis. Nutr Rev 2024: nuad171.
39. Loo K-Y, Thong JYH, Tan LT-H, *et al*. A Current Overview of Next-Generation Probiotics and Their Prospects in Health and Disease Management. Prog Microbes Mol Biol 2024; 7(1).
40. Kong GY-E, Letchumanan V, Tan LT-H, *et al*. Gut microbiome in obsessive compulsive disorder: potential of probiotics as an adjuvant therapy. Prog Microbes Mol Biol 2022; 5(1): a0000272.
41. Logan AC and Katzman M. Major depressive disorder: probiotics may be an adjuvant therapy. Med Hypotheses 2005; 64(3): 533–538.
42. Johnson D, Letchumanan V, Thum CC, *et al*. A Microbial-Based Approach to Mental Health: The Potential of Probiotics in the Treatment of Depression. Nutrients 2023; 15(6): 1382.
43. Johnson D, Thurairajasingam S, Letchumanan V, *et al*. Exploring the role and potential of probiotics in the field of mental health: Major depressive disorder. Nutrients 2021; 13(5): 1728.
44. Thye AY-K, Tan LT-H, Law JW-F, *et al*. Long COVID-19: Psychological symptoms in COVID-19 and probiotics as an adjunct therapy. Prog Microbes Mol Biol 2022; 5(1).
45. Barandouzi ZA, Starkweather AR, Henderson WA, *et al*. Altered composition of gut microbiota in depression: a systematic review. Front Psychiatry 2020; 11: 541.
46. Zhu F, Tu H, and Chen T. The Microbiota–Gut–Brain Axis in Depression: The Potential Pathophysiological Mechanisms and Microbiota Combined Antidepression Effect. Nutrients 2022; 14(10): 2081.
47. O'Callaghan AA and Corr SC. Establishing boundaries: the relationship that exists between intestinal epithelial cells and gut-dwelling bacteria. Microorganisms 2019; 7(12): 663.
48. Rong H, Xie X-h, Zhao J, *et al*. Similarly in depression, nuances of gut microbiota: Evidences from a shotgun metagenomics sequencing study on major depressive disorder versus bipolar disorder with current major depressive episode patients. J Psychiatr Res 2019; 113: 90–99.
49. Liu Y, Zhang L, Wang X, *et al*. Similar fecal microbiota signatures in patients with diarrhea-predominant irritable bowel syndrome and patients with depression. Clin Gastroenterol Hepatol 2016; 14(11): 1602-1611. e5.

50. Lin P, Ding B, Feng C, *et al*. Prevotella and Klebsiella proportions in fecal microbial communities are potential characteristic parameters for patients with major depressive disorder. J Affect Disord 2017; 207: 300–304.
51. Aizawa E, Tsuji H, Asahara T, *et al*. Possible association of *Bifidobacterium* and *Lactobacillus* in the gut microbiota of patients with major depressive disorder. J Affect Disord 2016; 202: 254–257.
52. Zhang Z-W, Gao C-S, Zhang H, *et al*. Morinda officinalis oligosaccharides increase serotonin in the brain and ameliorate depression via promoting 5-hydroxytryptophan production in the gut microbiota. Acta Pharmaceutica Sinica B 2022.
53. Chevalier G, Siopi E, Guenin-Macé L, *et al*. Effect of gut microbiota on depressive-like behaviors in mice is mediated by the endocannabinoid system. Nat Commun 2020; 11(1): 1–15.
54. Nadeem I, Rahman MZ, Ad-Dab'bagh Y, *et al*. Effect of probiotic interventions on depressive symptoms: A narrative review evaluating systematic reviews. Psychiatry Clin Neurosci 2019; 73(4): 154–162.
55. Nikolova VL, Cleare AJ, Young AH, *et al*. Updated review and meta-analysis of probiotics for the treatment of clinical depression: adjunctive vs. stand-alone treatment. J Clin Med 2021; 10(4): 647.
56. Yong SJ, Tong T, Chew J, *et al*. Antidepressive mechanisms of probiotics and their therapeutic potential. Front Neurosci 2020: 1361.
57. Poluektova E and Danilenko V. Probiotic Bacteria in the Correction of Depression Symptoms, Their Active Genes and Proteins. Russ J Genet 2021; 57(9): 1017–1025.
58. Poluektova E, Yunes R, and Danilenko V. The putative antidepressant mechanisms of probiotic bacteria: relevant genes and proteins. Nutrients 2021; 13(5): 1591.
59. El Dib R, Periyasamy AG, de Barros JL, *et al*. Probiotics for the treatment of depression and anxiety: A systematic review and meta-analysis of randomized controlled trials. Clinical Nutrition ESPEN 2021; 45: 75–90.
60. Chudzik A, Orzyłowska A, Rola R, *et al*. Probiotics, prebiotics and postbiotics on mitigation of depression symptoms: modulation of the brain–gut–microbiome axis. Biomolecules 2021; 11(7): 1000.
61. Purton T, Staskova L, Lane MM, *et al*. Prebiotic and probiotic supplementation and the tryptophan-kynurenine pathway: A systematic review and meta analysis. Neurosci Biobehav Rev 2021; 123: 1–13.
62. Alli SR, Gorbovskaya I, Liu JC, *et al*. The gut microbiome in depression and potential benefit of prebiotics, probiotics and synbiotics: A systematic review of clinical trials and observational studies. Int J Mol Sci 2022; 23(9): 4494.
63. Zhu, Tian P, Zhao J, *et al*. A psychobiotic approach to the treatment of depression: A systematic review and meta-analysis. J Funct Foods 2022; 91: 104999.
64. Musazadeh V, Zarezadeh M, Faghfouri AH, *et al*. Probiotics as an effective therapeutic approach in alleviating depression symptoms: an umbrella meta-analysis. Crit Rev Food Sci Nutr 2022: 1–9.
65. Xiao Y, Zhai Q, Zhang H, *et al*. Gut colonization mechanisms of *Lactobacillus* and *Bifidobacterium*: an argument for personalized designs. Annu Rev Food Sci Technol 2021; 12: 213–233.
66. Wei C-L, Wang S, Yen J-T, *et al*. Antidepressant-like activities of live and heat-killed Lactobacillus paracasei PS23 in chronic corticosterone-treated mice and possible mechanisms. Brain Res 2019; 1711: 202–213.
67. Yunes R, Poluektova E, Vasileva E, *et al*. A multi-strain potential probiotic formulation of GABA-producing *Lactobacillus plantarum* 90sk and *Bifidobacterium adolescentis* 150 with antidepressant effects. Probiotics Antimicrob Proteins 2020; 12(3): 973–979.
68. Ko CY, Lin H-TV, and Tsai GJ. Gamma-aminobutyric acid production in black soybean milk by Lactobacillus brevis FPA 3709 and the antidepressant effect of the fermented product on a forced swimming rat model. Process Biochem 2013; 48(4): 559–568.

69. Samtiya M, Dhewa T, and Puniya AK. *Probiotic Mechanism to Modulate the Gut-Brain Axis (GBA)*, in *Microbiome-Gut-Brain Axis*. 2022, Springer. p. 237–259.
70. Cabana MD, Salminen S, and Sanders ME. Probiotic Safety—Reasonable Certainty of No Harm. JAMA Intern Med 2019; 179(2): 276–276.
71. Wallace CJ and Milev RV. The efficacy, safety, and tolerability of probiotics on depression: Clinical results from an open-label pilot study. Front Psychiatry 2021; 12: 132.
72. Liang D, Wu F, Zhou D, *et al*. Commercial probiotic products in public health: Current status and potential limitations. Crit Rev Food Sci Nutr 2023: 1–22.
73. Baral KC, Bajracharya R, Lee SH, *et al*. Advancements in the pharmaceutical applications of probiotics: Dosage forms and formulation technology. Int J Nanomedicine 2021: 7535–7556.
74. Mud Shukri MI, Minhat HS, Ahmad N, *et al*. Prevalence and factors associated with depression, anxiety and stress in Malaysia during COVID-19 pandemic: A systematic review. PLoS One 2023; 18(7): e0288618.
75. Mukhtar F and Oei TP. A Review on the Prevalence of Depression in Malaysia. Curr Psychiatry Rev 2011; 7: 000-000.
76. Health IfP. *National Health and Morbidity Survey (NHMS) 2019: Vol. I: NCDs–Non-Communicable Diseases: Risk Factors and Other Health Problems*. 2020, Ministry of Health Malaysia Putrajaya, Malaysia.
77. Health IfP. National Health and Morbidity Survey 2015 (NHMS 2015). Vol. II: Non-Communicable Diseases, Risk Factors & Other Health Problems. Minist. Health Malays. 2015; 2: 185–186.
78. Tamil AM, Ismail NH, Jaafar MH, *et al*. Depressive symptoms among adults: Baseline findings of PURE Malaysia cohort study. Heliyon 2024; 10(1).
79. Mat N, Samsudin S, and Applanaidu S. A review on the association of mental disorders and workers' productivity in Malaysia. International Journal of Public Health and Clinical Sciences 2021; 8(3): 1–15.
80. Chua SN. The economic cost of mental disorders in Malaysia. Lancet Psychiatry 2020; 7(4): e23.
81. Ho SC, Jacob SA, and Tangiisuran B. Barriers and facilitators of adherence to antidepressants among outpatients with major depressive disorder: a qualitative study. PLoS One 2017; 12(6): e0179290.
82. Jacob SA, Ab Rahman AF, and Hassali MAA. Attitudes and beliefs of patients with chronic depression toward antidepressants and depression. Neuropsychiatr Dis Treat 2015: 1339–1347.
83. Ng C. A review of depression research in malaysia. Med J Malaysia 2014; 69: 42–45.
84. bin Hassan MF, Hassan NM, Kassim ES, *et al*. Issues and challenges of mental health in Malaysia. International Journal of Academic Research in Business and Social Sciences 2018; 8(12): 1685–1696.
85. National Health and Medical Research Council ARC, & Universities Australia. *Australian code for the responsible conduct of research*. 2018: National Health and Medical Research Council.
86. NPRA. 2021 [Accessed Available from: https://www.npra.gov.my/index.php/en/guideline-for-the-submission-of-product-samples-for-laboratory-testing/clinical-trial/clinical-trial-guidelines.html.
87. Wlodarska M, Luo C, Kolde R, *et al*. Indoleacrylic acid produced by commensal peptostreptococcus species suppresses inflammation. Cell Host Microbe 2017; 22(1): 25-37. e6.
88. von Glischinski M, von Brachel R, and Hirschfeld G. How depressed is “depressed”? A systematic review and diagnostic meta-analysis of optimal cut points for the Beck Depression Inventory revised (BDI-II). Qual Life Res 2019; 28: 1111–1118.
89. Goh KK, Liu Y-W, Kuo P-H, *et al*. Effect of probiotics on depressive symptoms: A meta-analysis of human studies. Psychiatry Res 2019; 282: 112568.

90. Ng QX, Peters C, Ho CYX, *et al*. A meta-analysis of the use of probiotics to alleviate depressive symptoms. J Affect Disord 2018; 228: 13–19.
91. Jackson-Koku G. Beck depression inventory. Occup Med 2016; 66(2): 174–175.
92. Mohebbi M, Dodd S, Dean O, *et al*. Patient centric measures for a patient centric era: agreement and convergent between ratings on the Patient Global Impression of Improvement (PGI-I) scale and the Clinical Global Impressions–Improvement (CGI-S) scale in bipolar and major depressive disorder. Eur Psychiatry 2018; 53: 17–22.
93. Morrens J, Mathews M, Popova V, *et al*. Use of Clinical Global Impressions-Severity (CGI-S) to Assess Response to Antidepressant Treatment in Patients with Treatment-Resistant Depression. Neuropsychiatr Dis Treat 2022; 18: 1127.
94. Forkmann T, Scherer A, Boecker M, *et al*. The clinical global impression scale and the influence of patient or staff perspective on outcome. BMC Psychiatry 2011; 11(1): 1-7.
95. T-TEST IS. Independent Samples T-test. Scatterplot Dependent Variable: VAR00008.
96. Ross A and Willson VL. *Paired samples T-test*, in *Basic and advanced statistical tests*. 2017, Brill. p. 17–19.
97. Lenhard W and Lenhard A. Computation of effect sizes. 2022 [Accessed 20 September]; Available from: https://www.psychometrica.de/effect_size.html.
98. Cohen J. *Statistical power analysis for the behavioral sciences*. 2013: Academic press.
99. Sullivan GM and Feinn R. Using effect size—or why the P value is not enough. J Grad Med Educ 2012; 4(3): 279–282.
100. Bakker A, Cai J, English L, *et al*. Beyond small, medium, or large: Points of consideration when interpreting effect sizes. Educ Stud Math 2019; 102: 1–8.
101. Bolyen E, Rideout JR, Dillon MR, *et al*. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019; 37(8): 852–857.
102. Callahan BJ, McMurdie PJ, Rosen MJ, *et al*. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 2016; 13(7): 581–583.
103. Katoh K and Standley DM. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol Biol Evol 2013; 30(4): 772–780.
104. Price MN, Dehal PS, and Arkin AP. FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS One 2010; 5(3): e9490.
105. Lozupone C and Knight R. UniFrac: A new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 2005; 71(12): 8228–8235.
106. McMurdie PJ and Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 2013; 8(4): e61217.
107. Oksanen J, Kindt R, Legendre P, *et al*. The vegan package. Community Ecology Package 2007; 10.
108. Bokulich NA, Kaehler BD, Rideout JR, *et al*. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 2018; 6(1): 1–17.
109. Yoon S-H, Ha S-M, Kwon S, *et al*. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. International journal of systematic and evolutionary microbiology 2017; 67(5): 1613–1617.
110. Chahwan B, Kwan S, Isik A, *et al*. Gut feelings: A randomised, triple-blind, placebo-controlled trial of probiotics for depressive symptoms. J Affect Disord 2019; 253: 317–326.

111. Reininghaus E, Platzer M, Kohlhammer-Dohr A, *et al*. Provit: Supplementary probiotic treatment and vitamin b7 in depression—a randomized controlled trial. Nutrients 2020; 12(11): 3422.
112. Chen H-M, Kuo P-H, Hsu C-Y, *et al*. Psychophysiological effects of *Lactobacillus plantarum* ps128 in patients with major depressive disorder: a preliminary 8-week open trial. Nutrients 2021; 13(11): 3731.
113. Zhang X, Chen S, Zhang M, *et al*. Effects of fermented milk containing *Lacticaseibacillus paracasei* strain Shirota on constipation in patients with depression: a randomized, double-blind, placebo-controlled trial. Nutrients 2021; 13(7): 2238.
114. Tian P, Chen Y, Zhu H, *et al*. *Bifidobacterium breve* CCFM1025 attenuates major depression disorder via regulating gut microbiome and tryptophan metabolism: A randomized clinical trial. Brain Behav Immun 2022; 100: 233–241.
115. Schaub A-C, Schneider E, Vazquez-Castellanos JF, *et al*. Clinical, gut microbial and neural effects of a probiotic add-on therapy in depressed patients: a randomized controlled trial. Translational psychiatry 2022; 12(1): 227.
116. Radford-Smith DE and Anthony DC. Prebiotic and Probiotic Modulation of the Microbiota–Gut–Brain Axis in Depression. Nutrients 2023; 15(8): 1880.
117. Ng QX, Lim YL, Yaow CYL, *et al*. Effect of probiotic supplementation on gut microbiota in patients with major depressive disorders: A systematic review. Nutrients 2023; 15(6): 1351.
118. Knuesel T and Mohajeri MH. The role of the gut microbiota in the development and progression of major depressive and bipolar disorder. Nutrients 2021; 14(1): 37.
119. Maslennikov R, Ivashkin V, Efremova I, *et al*. Gut dysbiosis is associated with poorer long-term prognosis in cirrhosis. World J Hepatol 2021; 13(5): 557.
120. Hao W, Ma Q, Wang L, *et al*. Gut dysbiosis induces the development of depression-like behavior through abnormal synapse pruning in microglia-mediated by complement C3. Microbiome 2024; 12(1): 34.
121. Yun S-W, Shin Y-J, Ma X, *et al*. *Lactobacillus plantarum* and *Bifidobacterium longum* Alleviate High-Fat Diet-Induced Obesity and Depression/Cognitive Impairment-like Behavior in Mice by Upregulating AMPK Activation and Downregulating Adipogenesis and Gut Dysbiosis. Nutrients 2024; 16(22): 3810.
122. Jang H-M, Kim J-K, Joo M-K, *et al*. Enterococcus faecium and Pediococcus acidilactici deteriorate Enterobacteriaceae-induced depression and colitis in mice. Sci Rep 2022; 12(1): 9389.
123. Wang Y, Jiang R, Wu Z, *et al*. Gut microbiota is involved in the antidepressant-like effect of (S)-norketamine in an inflammation model of depression. Pharmacol Biochem Behav 2021; 207: 173226.
124. Logan NA and De Vos P. Family Bacillaceae. Bergey's Manual of Systematic Bacteriology 2009; 3: 20.
125. Nikolova V, Cleare A, Young A, *et al*. Probiotics as Adjunctive Treatment in Major Depressive Disorder: Estimates of Treatment Effect and Underlying Mechanisms From a Double-Blind Placebo-Controlled Randomised Pilot Trial. BJPsych Open 2023; 9(S1): S64–S65.
126. Gao M, Wang J, Liu P, *et al*. Gut microbiota composition in depressive disorder: a systematic review, meta-analysis, and meta-regression. Transl Psychiatry 2023; 13(1): 379.
127. Jiang Y and Fan L. The effect of Poria cocos ethanol extract on the intestinal barrier function and intestinal microbiota in mice with breast cancer. J Ethnopharmacol 2021; 266: 113456.
128. Duan D-M, Wang Y-C, Hu X, *et al*. Effects of regulating gut microbiota by electroacupuncture in the chronic unpredictable mild stress rat model. Neuroscience 2024; 557: 24–36.
129. Takahashi K, Kurokawa K, Hong L, *et al*. Antidepressant effects of Enterococcus faecalis 2001 through the regulation of prefrontal cortical myelination via the enhancement of CREB/BDNF and NF-κB p65/LIF/STAT3 pathways in olfactory bulbectomized mice. J Psychiatr Res 2022; 148: 137–148.

130. Joo M-K, Ma X, Yoo J-W, *et al*. Patient-derived *Enterococcus mundtii* and its capsular polysaccharides cause depression through the downregulation of NF-κB-involved serotonin and BDNF expression. Microb Infect 2023: 105116.

131. Jang H-M, Kim J-K, Joo M-K, *et al*. Enterococcus Faecium is a Risk Factor for The Outbreak of Anxiety and Depression in Patients With Inflammatory Bowel Disease. 2020.

132. Mass M, Kubera M, and Leunis J-C. The gut-brain barrier in major depression: intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. Neuroendocrinology Letters 2008; 29(1): 117–124.

133. Ma F, Song Y, Sun M, *et al*. Exopolysaccharide produced by Lactiplantibacillus plantarum-12 alleviates intestinal inflammation and colon cancer symptoms by modulating the gut microbiome and metabolites of C57BL/6 mice treated by azoxymethane/dextran sulfate sodium salt. Foods 2021; 10(12): 3060.

134. Yan Shuang YS, Yang Bo YB, Zhao JiChun ZJ, *et al*. A ropy exopolysaccharide producing strain *Bifidobacterium longum subsp. longum* YS108R alleviates DSS-induced colitis by maintenance of the mucosal barrier and gut microbiota modulation. 2019.

135. Chung Y-CE, Chen H-C, Chou H-CL, *et al*. Exploration of microbiota targets for major depressive disorder and mood related traits. J Psychiatr Res 2019; 111: 74–82.

136. Fontana A, Manchia M, Panebianco C, *et al*. Exploring the role of gut microbiota in major depressive disorder and in treatment resistance to antidepressants. Biomedicines 2020; 8(9): 311.

137. Otaka M, Kikuchi-Hayakawa H, Ogura J, *et al*. Effect of Lacticaseibacillus paracasei strain Shirota on improvement in depressive symptoms, and its association with abundance of Actinobacteria in gut microbiota. Microorganisms 2021; 9(5): 1026.

138. Kremer S, Wiesinger T, Bschor T, *et al*. Antidepressants and Social Functioning in Patients with Major Depressive Disorder: Systematic Review and Meta-Analysis of Double-Blind, Placebo-Controlled RCTs. Psychother Psychosom 2023; 92(5): 304–314.

139. Song A, Cheng R, Jiang J, *et al*. Antidepressant-like effects of hyperoside on chronic stress-induced depressive-like behaviors in mice: Gut microbiota and short-chain fatty acids. J Affect Disord 2024; 354: 356–367.

140. Jiang H, Ling Z, Zhang Y, *et al*. Altered fecal microbiota composition in patients with major depressive disorder. Brain, behavior, and immunity 2015; 48: 186–194.

141. Chen Z, Li J, Gui S, *et al*. Comparative metaproteomics analysis shows altered fecal microbiota signatures in patients with major depressive disorder. Neuroreport 2018; 29(5): 417–425.

142. Messaoudi M, Lalonde R, Violle N, *et al*. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. Br J Nutr 2011; 105(5): 755–764.

143. Ait-Belgnaoui A, Colom A, Braniste V, *et al*. Probiotic gut effect prevents the chronic psychological stress-induced brain activity abnormality in mice. Neurogastroenterol Motil 2014; 26(4): 510–520.

144. Arseneault-Bréard J, Rondeau I, Gilbert K, *et al*. Combination of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 reduces post-myocardial infarction depression symptoms and restores intestinal permeability in a rat model. Br J Nutr 2012; 107(12): 1793–1799.

145. Mohammadi G, Dargahi L, Naserpour T, *et al*. Probiotic mixture of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 attenuates hippocampal apoptosis induced by lipopolysaccharide in rats. Int Microbiol 2019; 22(3): 317–323.

146. Partrick KA, Rosenhauer AM, Auger J, *et al*. Ingestion of probiotic (*Lactobacillus helveticus* and *Bifidobacterium longum*) alters intestinal microbial structure and behavioral expression following social defeat stress. Sci Rep 2021; 11(1): 1–12.
147. Kazemi A, Noorbala AA, Azam K, *et al*. Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: A randomized clinical trial. Clin Nutr 2019; 38(2): 522–528.
148. Ait-Belgnaoui A, Payard I, Rolland C, *et al*. *Bifidobacterium longum* and *Lactobacillus helveticus* synergistically suppress stress-related visceral hypersensitivity through hypothalamic-pituitary-adrenal axis modulation. J Neurogastroenterol Motil 2018; 24(1): 138.
149. Asgharian M, Gholizadeh P, Kafil HS, *et al*. Correlation of inflammatory biomarkers with the diversity of *Bacteroidaceae*, *Bifidobacteriaceae*, *Prevotellaceae* and *Lactobacillaceae* families in the intestinal microbiota of patients with end stage renal disease. Adv Med Sci 2022; 67(2): 304–310.
150. Youssef O, Lahti L, Kokkola A, *et al*. Stool microbiota composition differs in patients with stomach, colon, and rectal neoplasms. Dig Dis Sci 2018; 63: 2950–2958.
151. Li J, Wang J, Wang M, *et al*. *Bifidobacterium*: a probiotic for the prevention and treatment of depression. Front Microbiol 2023; 14: 1174800.
152. Hidalgo-Cantabrana C, Delgado S, Ruiz L, *et al*. *Bifidobacteria* and their health-promoting effects. Bugs as Drugs: Therapeutic Microbes for the Prevention and Treatment of Disease 2018: 73–98.
153. Bangsgaard Bendtsen KM, Krych L, Sørensen DB, *et al*. Gut microbiota composition is correlated to grid floor induced stress and behavior in the BALB/c mouse. PLoS One 2012.
154. Bagot RC, Labonté B, Peña CJ, *et al*. Epigenetic signaling in psychiatric disorders: stress and depression. Dialogues Clin Neurosci 2022.
155. Berding K, Bastiaanssen TF, Moloney GM, *et al*. Feed your microbes to deal with stress: a psychobiotic diet impacts microbial stability and perceived stress in a healthy adult population. Mol Psychiatry 2022: 1-10.
156. Stilling RM, Dinan TG, and Cryan JF. Microbial genes, brain & behaviour–epigenetic regulation of the gut–brain axis. Genes, Brain and Behav 2014; 13(1): 69–86.
157. Huang Y, Shi X, Li Z, *et al*. Possible association of Firmicutes in the gut microbiota of patients with major depressive disorder. Neuropsychiatr Dis Treat 2018; 14: 3329.
158. Cepeda MS, Katz EG, and Blacketer C. Microbiome-Gut-Brain Axis: Probiotics and Their Association With Depression. J Neuropsychiatry Clin Neurosci 2017; 29(1): 39–44.
159. Garcia-Cebrian A, Bauer M, Montejo AL, *et al*. Factors influencing depression endpoints research (FINDER): study design and population characteristics. Eur Psychiatry 2008; 23(1): 57–65.
160. Rahmannia M, Poudineh M, Mirzaei R, *et al*. Strain-specific effects of probiotics on depression and anxiety: a meta-analysis. Gut Pathog 2024; 16(1): 46.
161. Kurilshikov A, Medina-Gomez C, Bacigalupe R, *et al*. Large-scale association analyses identify host factors influencing human gut microbiome composition. Nat Genet 2021; 53(2): 156–165.
162. Su Q and Liu Q. Factors affecting gut microbiome in daily diet. Front Nutr 2021: 218.
163. Kurilshikov A, Wijmenga C, Fu J, *et al*. Host genetics and gut microbiome: challenges and perspectives. Trends Immunol 2017; 38(9): 633–647.
164. Fernandes BS, Williams LM, Steiner J, *et al*. The new field of 'precision psychiatry'. BMC Med 2017; 15(1): 1–7.
165. Passos IC, Ballester P, Rabelo-da-Ponte FD, *et al*. Precision psychiatry: the future is now. Can J Psychiatry 2022; 67(1): 21–25.

166. Williams LM. Precision psychiatry: a neural circuit taxonomy for depression and anxiety. Lancet Psychiatry 2016; 3(5): 472–480.

167. Salazar de Pablo G, Studerus E, Vaquerizo-Serrano J, *et al.* Implementing precision psychiatry: a systematic review of individualized prediction models for clinical practice. Schizophr Bull 2021; 47(2): 284–297.

168. Lau AWY, Tan LT-H, Ab Mutalib N-S, *et al*. The chemistry of gut microbiome in health and diseases. Prog Microbes Mol Biol 2021; 4(1).

169. Elshaghabee FM, Rokana N, Gulhane RD, *et al*. Bacillus as potential probiotics: status, concerns, and future perspectives. Front Microbiol 2017; 8: 1490.

170. Li P, Gu Q, and Zhou Q. Complete genome sequence of *Lactobacillus plantarum* LZ206, a potential probiotic strain with antimicrobial activity against food-borne pathogenic microorganisms. J Biotechnol 2016; 238: 52-55.

171. Nowak A, Paliwoda A, and Błasiak J. Anti-proliferative, pro-apoptotic and anti-oxidative activity of *Lactobacillus* and *Bifidobacterium* strains: A review of mechanisms and therapeutic perspectives. Crit Rev Food Sci Nutr 2019; 59(21): 3456–3467.

172. Dinan TG and Quigley EM. Probiotics in the treatment of depression: science or science fiction? Aust N Z J Psychiatry 2011; 45(12): 1023–1025.

173. Mörkl S, Butler MI, Holl A, *et al*. Probiotics and the microbiota-gut-brain axis: focus on psychiatry. Curr Nutr Rep 2020; 9(3): 171–182.

174. Kelesidis T and Pothoulakis C. Efficacy and safety of the probiotic Saccharomyces boulardii for the prevention and therapy of gastrointestinal disorders. Therap Adv Gastroenterol 2012; 5(2): 111–125.

175. De Oliveira FL, Salgaço MK, de Oliveira MT, *et al*. Exploring the Potential of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 as Promising Psychobiotics Using SHIME. Nutrients 2023; 15(6): 1521.

176. Kang Y and Cai Y. Gut microbiota and depression: from pathogenesis to new therapeutic strategies. Rev Res Med Microbio 2017; 28(2): 56–62.

177. Kazemi A, Noorbala AA, Azam K, *et al*. Effect of prebiotic and probiotic supplementation on circulating pro-inflammatory cytokines and urinary cortisol levels in patients with major depressive disorder: a double-blind, placebo-controlled randomized clinical trial. J Funct Foods 2019; 52: 596–602.

178. Dehghani F, Abdollahi S, Shidfar F, *et al*. Probiotics supplementation and brain-derived neurotrophic factor (BDNF): a systematic review and meta-analysis of randomized controlled trials. Nutr Neurosci 2022: 1–11.

179. Heidarzadeh-Rad N, Gökmen-Özel H, Kazemi A, *et al*. Effects of a psychobiotic supplement on serum brain-derived neurotrophic factor levels in depressive patients: a post hoc analysis of a randomized clinical trial. J Neurogastroenterol Motil 2020; 26(4): 486.

180. Romijn AR, Rucklidge JJ, Kuijer RG, *et al*. A double-blind, randomized, placebo-controlled trial of *Lactobacillus helveticus* and *Bifidobacterium longum* for the symptoms of depression. Aust N Z J Psychiatry 2017; 51(8): 810–821.

181. McVey Neufeld K-A, Kay S, and Bienenstock J. Mouse strain affects behavioral and neuroendocrine stress responses following administration of probiotic *Lactobacillus rhamnosus* JB-1 or traditional antidepressant fluoxetine. Front Neurosci 2018; 12: 294.

182. Ross K. Psychobiotics: Are they the future intervention for managing depression and anxiety? A literature review. EXPLORE 2023.

183. Bravo JA, Forsythe P, Chew MV, *et al*. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci 2011; 108(38): 16050–16055.

184. Janik R, Thomason LA, Stanisz AM, *et al*. Magnetic resonance spectroscopy reveals oral *Lactobacillus* promotion of increases in brain GABA, N-acetyl aspartate and glutamate. NeuroImage 2016; 125: 988–995.

185. Foster L, Tompkins T, and Dahl W. A comprehensive post-market review of studies on a probiotic product containing *Lactobacillus helveticus* R0052 and *Lactobacillus rhamnosus* R0011. Benef Microbes 2011; 2(4): 319–334.

186. Strawbridge R, Young AH, and Cleare AJ. Biomarkers for depression: recent insights, current challenges and future prospects. Neuropsychiatr Dis Treat 2017: 1245–1262.

187. Evans M, Salewski RP, Christman MC, *et al*. Effectiveness of Lactobacillus helveticus and Lactobacillus rhamnosus for the management of antibiotic-associated diarrhoea in healthy adults: a randomised, double-blind, placebo-controlled trial. Br J Nutr 2016; 116(1): 94–103.

188. Talbott SM, Talbott JA, Stephens BJ, *et al*. Effect of coordinated probiotic/prebiotic/phytobiotic supplementation on microbiome balance and psychological mood state in healthy stressed adults. Funct Food Health Dis 2019.

189. Hempel S, Newberry S, Ruelaz A, *et al*. Safety of probiotics used to reduce risk and prevent or treat disease. Evidence report/technology assessment 2011(200): 1–645.

190. Dore MP, Bibbò S, Fresi G, *et al*. Side effects associated with probiotic use in adult patients with inflammatory bowel disease: a systematic review and meta-analysis of randomized controlled trials. Nutrients 2019; 11(12): 2913.

191. Sotoudegan F, Daniali M, Hassani S, *et al*. Reappraisal of probiotics' safety in human. Food Chem Toxicol 2019; 129: 22–29.

192. Akkasheh G, Kashani-Poor Z, Tajabadi-Ebrahimi M, *et al*. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial. Nutrition 2016; 32(3): 315–320.