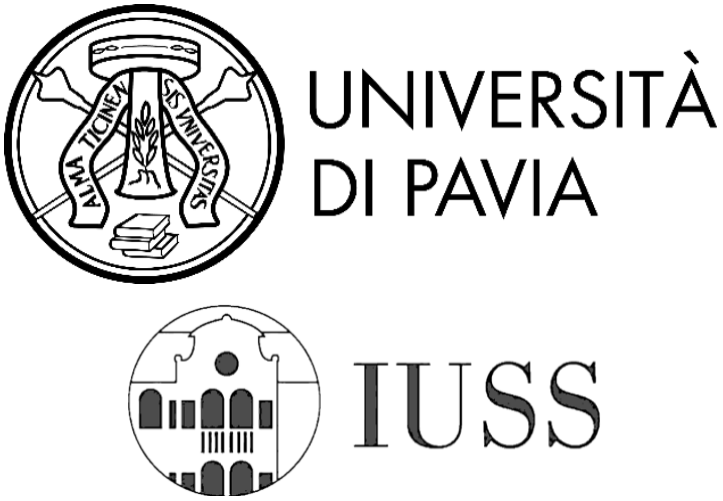
UNIVERSITY OF PAVIA – IUSS SCHOOL FOR ADVANCED STUDIES PAVIA

Department of Brain and Behavioral Sciences (DBBS)
MSc in Psychology, Neuroscience and Human Sciences



Examining the Efficacy of Probiotics as an Alternative Therapeutic Approach for Anxiety and Depression Symptoms: A Scoping Review

Supervisors:
Prof. Serena Barello

Thesis written by

Angelina Corpora

Academic year 2023/2024

ABSTRACT

In recent years, the gut-brain-axis has become an increasingly researched topic due to its role in health, stress reactivity, and the pathomorphosis of mood disorders. Probiotics are live microorganisms that, when ingested in adequate amounts, can pose a health benefit to the host through their improvement of beneficial gut-microbiota. The objective of this review is to conduct a comprehensive scoping review investigating the potential of probiotic interventions to modulate gut microbiota composition and their associated effects on anxiety and depression symptomology. Various probiotic strains were examined and analyzed comparing pathological versus healthy participants and the impact of natural interventions on validated psychological measures. The findings of this review underscore the promising role of probiotics in their ability to modulate brain function patterns, reduce stress reactivity, and improve emotional and cognitive processes through the enhancement of gut microbiota. The findings suggest that probiotic intervention for mental health disorders could become a valuable component of personalized, non-invasive treatment strategies, offering a safer alternative to traditional pharmacological approaches.

Key words: probiotics, prebiotics, mood disorders, microbiota, gut-brain axis

Introduction

Chapter 1: The Gut Microbiota

1.1 The Importance and Influence of Gut Microbiota

In recent years, the study of the gut-brain-axis has become an increasingly fascinating topic leading to further research and discussion due to the connection it plays with the entirety of the human body and mental health. Stemming from research on animal models, the importance of the microbiota in the central nervous system (CNS) function has contributed to investigations directly to brain and body systems in humans. The term 'microbiota' refers to "the collection of microorganisms in an ecosystem" as stated by the International Scientific Association for Probiotics and Prebiotics (ISAIPP), and the term 'microbiome' is used when individual "genetic elements are also considered" (Hill et al., 2014). The microbiota contains over 1 trillion different types of microbial cells including bacteria, archaea, fungi and protists, carrying more than 100 times the genes in our genome (Sender et al., 2016). The intestinal microbiota function fuels essential processes such as metabolism, vitamin production, hormone level regulation, peristalsis, and neurologic health enhancement (Snigdha et al., 2022b). The revised estimates of human cells to bacteria cells in the body have been reduced from a 10:1 ratio of cells to bacteria to a more accurate estimate of 1:1, reconfirming the biological importance of the microbiota for specific biological questions, for example, disease and viruses (Sender et al., 2016).

The main sites of this microbial colonization include the body's largest organ, the skin, along with the airways, the eyes, the oral microbiota and perhaps most importantly, the gastro-intestinal (GI) tract (Tan, 2023b) (Kilian et al., 2016). The GI tract itself harbors four main bacteria phyla, namely *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria*. The microbiota is a community of symbiotic and pathogenic fungi, bacteria and viruses that live in and on the body including the skin, mammillary glands, placenta, seminal fluid, lungs, saliva and oral mucosa. (Nestler et al., 2001). The greatest abundance of organisms is inside the GI tract,

and the diversity of the microbiota can be characterized by the richness and number of differential microbial species (α), and the abundance of species in a single sample (*β)* (Biasucci et al., 2010).

The gut microbiome begins developing during birth and early post-partum through exposure to the mother's vaginal cavity and continues developing throughout breastfeeding. While vaginally delivered infants benefit from contact with maternal vaginal and fecal matter, Cesarean-section (C-section) infants show a more simplified structure of gut microbiota (Neu & Rushing, 2011). This simplified structure includes less colonization of microorganisms like *Bifidobacterium* and *Bacteroides*, and these differences in gut microbiota can be detected in children as old as the age of seven. Additionally, the establishment of early infant gut microbiota is propelled by human milk, an intriguing example of host-microbe evolution where both mother and child benefit (Milani et al., 2017). The microbiome is a crucial component of brain development and continues to influence behavior into adolescence. In fact, alterations to the composition of the gut microbiota during neonatal life, a period from birth until 2 years, is extremely important due to the associations with pediatric disorders and onset of disease in adulthood (Rautava et al., 2012). The gut microbiota has also been implicated in various conditions such as anxiety, obesity, autism, schizophrenia and neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Tan, 2023a). In turn, there is increasing data supporting the long-term benefits to health in modulating risk factors stemming from infantile gut microbiota (Relman, 2012). The following sections delineate key factors influencing gut microbiota composition and function, including microbial dysbiosis, dietary interventions and pharmacological agents like antibiotics and antidepressants. These elements are elucidated due to their critical role in modulating the gut microbiome and their potential implications for mood disorder onset.

1.2 What can cause "Dysbiosis"?

The differential microbiota profile of an individual can provide valuable insights to their environmental history, including illness risk, disease and response to treatment (Foster & Neufeld, 2013b). Genetic and environmental factors, including early-life stress, have been linked to stress-related diseases and can even alter the function of the central brain network (Snigdha et al., 2022c). When "dysbiosis", a state of disruption of the homeostasis of gut microbiota, occurs, this can be associated with long-term effects potentially leading to disease and disorders later in life (Kim, 2015). Studies from animal models have suggested the influence of maternal behaviors, such as licking and grooming in rats, on the responsiveness of the Hypothalamic Pituitary Axis (HPA) axis during early life exposure (D. Liu et al., 1997b). These concerns have led to increasing research about the importance of gut-microbiota in early and adult life to prevent microbiota dysbiosis related pathologies.

1.2.2 Western Style Diet

Like environmental factors, diet is also a crucial influencer of the gut microbiome. In fact, acute shifts in diet can be observed within days of beginning an unhealthy diet (David et al., 2014), let alone years of improper nutritional intake. In Western societies, it is common to consume a diet high in simple carbohydrates, saturated fats, and salt, yet low in fiber and complex micronutrients, all of which contribute to accelerated weight and adipose gain (Kendig et al., 2021). As a result, increased adiposity throughout the human lifespan has been linked to not only impaired cognition, but impacts in memory, intelligence, cognitive flexibility and executive functioning (Farruggia & Small, 2019b). In a study on the influence of diet on microbiota, David et al. (2014) demonstrated that changes in microbial diversity (β) were observed after just two days of a shift in diet as compared to baseline. By comparing short-term consumption of animal or plant-based diets, researchers noticed a change in microbial gene expression from high animal

fat diets, specifically a promotion of bile acid production and an increase in the abundance of *Bilophila wadsworthia,* a bacteria capable of contributing to Irritable Bowel Disease (IBS) (David et al., 2014). Hence, the composition of microbiota is greatly determined by diet, namely the quality and quantity of carbohydrates essential in forming bacterial communities (G. D. Wu et al., 2011b). These associations have also been linked to the increasing rate of obesity and cancer in the worldwide population, as demonstrated in a 2015 Metagenomic survey of gut microbiota (Mandal et al., 2015).

1.3 Effects of Manipulating the Gut Microbiome

In addition to the role dietary factors play in the microbiome, medications such as antibiotics and antidepressants can cause disruption to the human microbiota, posing a serious health threat to the host (Patangia et al., 2022). Especially in Western societies, consequences of overprescription of antibiotics, antidepressants, and anti-anxiety medications have led to the search for alternative therapeutic techniques due to their disruptive properties and potential for addiction. More specifically, despite only accounting for 4.6% of the global population, the United States accounts for 46% of the global antibiotic market. Additionally, 1 in 10 doctors will write a prescription simply because it is asked for by the patient, and an upwards of 95% of clinicians prescribe antibiotics when they are not entirely sure if it is needed by the patient (Romm, 2021).

Recent studies have highlighted the implications of prolonged use of copious amounts of therapeutic drugs and their compromising effects on the microbial gastro-intestinal environment. A significant clinical challenge in response to Selective Serotonin Reuptake Inhibitors (SSRIs) treatment is that a substantial proportion of patients exhibit treatment resistance, despite receiving SSRIs at therapeutic doses for an adequate duration (Rush et al., 2006). SSRIs have been demonstrated to significantly alter the delicate balance and diversity of the gut microbiota, and chronic use can lead to persistent changes of microbiome composition. These antimicrobial

properties of antidepressants influence therapeutic and negative side effects in patients, impacting neurotransmitter production, immune response and overall mental health. The monoamine hypothesis of depression posits that the disorder is related to depletions of monoamine neurotransmitters in the brain, specifically serotonin, norepinephrine, and dopamine, due to the use of antidepressant medications like monoamine oxidase inhibitors (MAOi) and tricylic antidepressants (TCA) (Sjöstedt et al., 2021). In a review investigating the antimicrobial effects of commonly prescribed antidepressants, authors McGovern et al. (2019) reported that consequences of these drugs may impose inhibitory effects on the growth of significant bacterial strains. Given that approximately 95% of serotonin production occurs in the GI tract (Richard et al., 2009), SSRIs can significantly impact this process.

Chapter 2: The Gut-Brain Axis

2.1 The Enteric Nervous System

Did you know our gut has its own nervous system? The enteric nervous system (ENS), located in the gastrointestinal tract and member of the autonomic nervous system, is stimulated by nearly 200-600 million neurons that directly communicate with the CNS (Hyland & Cryan, 2016). Beginning during birth and continuing through the postnatal period, the microbiome influences the stress response system through colonization of bacteria in the intestines (Foster & Neufeld, 2013). With bacterial concentrations ranging from 10^1 to 10^12 cells per gram in the upper intestine and colon, the GI tract is the most densely populated area in the body (Hyland & Cryan, 2016) including its gut-associated lymphoid tissue (GALT) creating the largest immune organ of the human body (Rudzki et al., 2019). Along with these, the human gut is innervated with approximately 200-600 million intrinsic sensory neurons, motor neurons and enteric interneurons all communicating with microorganisms in the intestinal tract (Hyland & Cryan, 2016). Due to its functionality and

communication potential, it has even been termed the "second brain" and can act independently of the autonomic nervous system (Nestler et al., 2001).

The ENS is more important than it is given credit for and is instrumental in the activation of the HPA axis and neurotransmitter production. In recent years, we have gained clarity on the function the microbiome serves in interacting with the HPA axis and stress reactivity. During the stress response, signals from the ENS activate the HPA axis by releasing corticotropin-releasing hormone (CRH), responsible for elevating cortisol levels in our body. With the help of involvement from the hypothalamus, pituitary gland, and adrenal glands, the HPA axis is fundamental in regulating the body's response to stress. After subjection to a stressful experience, catecholamines adrenaline and noradrenaline are released, the fight-or-flight hormones responsible for escape tactics from potentially harmful stimuli.

This is a suggestion of the crucial role in the communication between stomach bacteria and the brain. The gut microbiota influences the host through various mechanisms, including cellular components, biosynthesis of unique molecules, and dietary modification, which facilitate direct and indirect communication between the microbiota and the ENS (Hyland & Cryan, 2016b). Known as a bidirectional communication route, interaction occurs from the ENS to the CNS via the Vagus nerve.

2.2 Communication & Synthesis

The gut microbiome is able to engage with the brain via bidirectional communication channels consisting of multiple pathways: the immune system, the Vagus nerve, the ENS and the process of tryptophan metabolism. The communication involves an exchange of microbial metabolites, such as short-chain fatty acids, branched-amino acids and peptidoglycans, which influence various physiological processes and signaling mechanisms parallelling the gut and brain (Tan, 2023). The gut microbiome is not only responsible for regulation of the immune system, but also

aids in production of neurotransmitter metabolites important for serotonin, dopamine, glutamate, gamma-aminobutyric acid (GABA) and acetylcholine (Pluta & Januszewski, 2022b). Furthermore, studies have estimated that roughly "95% of mammalian serotonin is found within the gastrointestinal tract" (Richard et al., 2009). Through direct precursors and metabolic pathways, neurotransmitters are released from gut bacteria and endocrine cells into the bloodstream and transported to the brain by the Vagus nerve (Chen et al., 2021). It is important to note that levels of neurotransmitters can be affected and modulated by gut microbiota in terms of availability of precursors, direct production and synthesis pathways.

An essential contributor to the pathophysiology of psychiatric disorders is the activation of inflammatory response, including pro-inflammatory cytokines, kynurenine catabolites (TRYCATs) and oxidative and nitrosative stress (Schwarcz et al., 2012). Known as the "kynurenine pathway", kynurenine is important for physiological processes such as generation of cell energy, immune response, inflammation, and its power of neurotransmission influences mental health and has been linked to depression (Schwarcz et al., 2012). One of the most important essential amino acids not synthesized by the body, but important for the synthesis of kynurenine, is L-Tryptophan. L-Tryptophan, or Tryptophan, is a protein synthesizer that needs to be supplemented through diet (ex. oats, banana, milk, tuna, chicken, peanuts), and is essential to the production of metabolites for neurotransmitters, serotonin and melatonin synthesis. By binding with Albumin, Tryptophan is one of the amino acids with the ability to cross the blood brain barrier (BBB) (Richard et al., 2009). Though it is estimated that only 1% of dietary tryptophan intake is utilized for serotonin synthesis (Richard et al., 2009), its impact is broad and has been associated in psychiatric conditions and numerous psychiatric properties. Evidence suggests that the diversion of gut-derived tryptophan into the immune-mediated kynurenine

pathway may significantly contribute to serotonin dysregulation and its associated physiological effects (Kennedy et al., 2017).

Chapter 3: Pathophysiology of Stress, Anxiety and Depression

3.1 Stress and the Microbiota

The gut microbiome plays a crucial role in the pathogenesis of depression. Gut dysbiosis, or disruptions to the microbiota's diversity and composition, is a potential risk factor in mood disorders such as depression and anxiety. This dysbiosis can lead to imbalance in production of neurotransmitters and systemic inflammation, both closely linked to the development and persistence of associative symptoms (Tarutani et al., 2022b). Stress is an inevitable consequence of human existence and can lead to anxiety and even depression in severe cases (Pittenger and Duman, 2008). In addition to diet, stress can significantly impact gut microbiota through changes in the Hypothalamic-Pituitary-Axis (HPA).

The HPA axis is critical for regulating the body's response to stress and influences symptoms of depression and anxiety (Bibbò et al., 2022b). When high chronic stress is experienced, elevated levels of cortisol can negatively impact brain health and cognition, potentially causing attentional difficulties, slowed thinking processing, and a reduction in memory (Teja, 2024b). In fact, excessive levels of cortisol can inhibit the formation of new neurons, a process known as "neurogenesis", and suppress the formation of new synapses resulting in atypical dendritic branching and development of axons. (Blankenship et al., 2019b). Long-term cortisol abundance has even been connected to reduced hippocampal volume, likewise, affecting other areas of the brain such as the amygdala and prefrontal cortex, all common areas associated with depression (Teja, 2024).

3.2 Gut Microbiota and Mood Disorders

Depression and anxiety are chronic psychiatric disorders and together are one of the leading causes of disability worldwide, with increasing prevalence rates each year. The World Health Organization (WHO) has detailed that nearly 280 million people suffer from depression worldwide, while approximately 301 million suffer from anxiety (World Health Organization, 2019). In 2019, a global risk factor burden of anxiety systematic analysis was calculated encompassing 204 countries and territories, evaluating specific risk factors (ex. sodium intake) and relative aggregates (ex. Nutrition quality). A 2018 survey conducted by the American Psychological Association (APA) on stress in America found that a significant portion of adults, approximately 75%, experienced at least one physical or emotional symptom of stress within the past month. Additionally, nearly 50% of respondents reported that their average stress levels were higher than what they considered healthy in the same period (American Psychological Association, 2018). The COVID pandemic in 2019 had extensive effects on these numbers. During the first year of the COVID-19 pandemic, an upsurge of 25% of cases resulted in approximately 76 million new cases of anxiety disorders (Delpino et al., 2022).

3.3 Depression

The pathophysiology of depression has many theories. From a neurobiological perspective, depression is linked to the dysregulation of neuroendocrine, neuroimmune, metabolic and transmitter systems, which are interconnected with the microbiota-gut-brain axis (Kelly et al., 2016). Evidence from animal models have demonstrated that depression is influenced by disruptions in gut microbiota (Kelly et al., 2016) (Dash et al., 2015) Depression can be sub-categorized into major depressive disorder (MDD), disruptive mood dysregulation disorder, premenstrual dysphoric disorder (PMDD), dysthymia, and depressive disorder in occurrence of previous medical conditions, according to the American Psychiatric Associations Diagnostic and

Statistical Manual of Mental Disorders (DSM-5). Major Depressive Disorder (MDD) is the most common mental disorder, affecting approximately 300 million people worldwide and accumulating nearly 800,000 deaths by suicide each year (World Health Organization [WHO], 2020). Treatment resistant depression, an insufficient therapeutic effect of antidepressant medication or an unattainment of remission, has gained a lot of traction in scientific research because of its rising rates of resistance (Fava, 2003). With rates of treatment resistant depression reaching over 30% of people with MDD, it is becoming necessary to seek alternative therapeutic techniques (Zhdanava et al., 2021). Patients with subthreshold depression (SD), a subclinical form of depression with increased risk of MDD, may be considered ineligible for antidepressant medication, or the use of can lead to adverse effects outweighing the positives (Ullah et al., 2022).

Chapter 4: Probiotics

4.1 Probiotics and How they Work

The substantial impact of antibiotics and SSRIs on gut microbiota composition and subsequent mental health outcomes has led to a pressing imperative within clinical practice to transition towards holistic interventions that encompass natural alternatives to restore dysbiosis and mitigate symptoms of depression and anxiety. In fact, gastrointestinal dysfunction, partially due to intake of SSRIs, is one of the most comorbid symptoms of depression (Steffens et al., 1997).

Given the significant challenges associated with treatment-resistant disorders, increasing rates of mental health concerns and decreasing nutritional intake, a new therapeutic approach is necessary to address these issues. As famously stated by Hippocrates, “Let food be thy medicine, and medicine thy food”, food as medicine has shifted in a promising direction for probiotics research. The definition of Probiotics, given by the Food and Agriculture Organization (FAO) and World Health Organization (WHO), delineates them as living microorganisms that, when

administered in sufficient quantities, bestow health benefits upon the host. Long before the term "probiotic" was coined, fermented dairy products were consumed by populations around the world (Prajapati et al., 2023). Advancing into the 1900s, Louis Pasteur and E. Metchnikoff recognized the role bacteria plays in the fermentation process and endorsed *Lactobacilli* as a possible countermeasure to the negative effects of gastrointestinal metabolism associated with diseases and aging (Prajapati et al., 2023). Since then, probiotics, also referred to as "functional foods" have been consumed as dietary supplements with increasing popularity for their ability to enhance intestinal communities, aid in digestion and improve human immunity (Wang et al., 2021) (Hemarajata & Versalovic, 2012).

In addition, probiotics have gained attention for their therapeutic potential due to their safety, efficacy, and tolerability. Before potential probiotic strains can be commercialized as food additives or health supplements, they must meet established probiotic criteria and obtain Generally Recognized as Safe (GRAS) status. (Forssten et al., 2020). This rigorous evaluation process ensures the safety and efficacy of probiotic strains for human consumption and therapeutic applications by meeting regulatory requirements.

4.1 Pre-biotics, Probiotics, & Psychobiotics

Probiotics are a type of bacteria naturally occurring in the body (Kumari et al., 2023b), and can be identified by their specific strain, including the genus, species, and subspecies (Gibson et al., 2017). There are seven core genera of these microbial organisms most used that include: *Lactobacillus, Bifidobacterium, Saccharomyces, Streptococcus, Enterococcus, Escherichia* and *Bacillus* that all play an important role in maintaining balance in the gut micro flora. They impact various microorganisms antagonistically through mechanisms like alteration of gut microbiota, competition for mucosal adhesion, enhancement of the gut epithelial barrier and modulation of the immune system (Prajapati et al., 2023). The gut flora is essential in aiding

digestion and promoting the immune system; therefore, an imbalance can lead to a variety of health concerns, which have proven effective treatment by probiotics (Prajapati et al., 2023).

Probiotic organisms exhibit health-improving effects through a variety of mechanisms, organized by nonspecific, species-specific, and strain-specific categories. Nonspecific mechanisms, which vary considerably among different probiotic strains, species and genera, extend several processes. These include inhibiting the proliferation of pathogenic microorganisms within the GI environment, enhancing intestinal mobility, and assisting in restoring disrupted microbiota. (Hill et al., 2014) Additionally, probiotics contribute to the production of bioactive compounds like short-chain fatty acids and aid in reducing colon pH levels. Species-specific mechanisms are more targeted and involve processes like vitamin synthesis, reinforcement of the gut barrier, enzymatic activities and neutralizing toxins. Strain-specific mechanisms, less common and limited to certain strains within a certain species, can modulate cytokine production, immunoregulation and influence endocrine and nervous system functions (*Office of Dietary Supplements - Probiotics*, n.d.) (Gibson et al., 2017). Through this array of mechanisms, probiotics have the potential to influence various aspects of human physiology and pathophysiology, modulating both neurological and emotional health outcomes.

A primary metabolic function of probiotics is their ability to generate short-chain fatty acids (SCFSs) by fermenting dietary fibers. SCFSs including butyrate, acetate, and propionate are essential in preserving the integrity of the intestinal barrier, decreasing inflammation and influencing immune responses. These acids activate particular receptors in the gut, overall prompting the release of hormones in control of metabolism and appetite (He & Shi, 2017) (T. Wu et al., 2024) (Wang et al., 2021b).

Psychobiotics, a recently defined category of probiotics that primarily target the gut-brain axis, have demonstrated potential efficacy in the management of psychiatric disorders. These

microorganisms have shown validated antidepressant-like effects in mice and are hypothesized to exert their effects through various mechanisms, including neurotransmitter modulation, immune regulation and hypothalamic-pituitary-adrenal axis influence (Tian et al., 2022). Psychobiotics are also live microorganisms that are able to modulate the gut-brain axis through immune, humoral, neural and metabolic pathways. They have the direct ability to communicate with the gut and microbiome and have emerged as novel therapeutic alternatives for treatment of psychiatric disorders due to their short-chain fatty acids (SCFAs) bacteria production (Cheng et al., 2021). These SCFAs-producing bacteria, namely *Lactobacillus*, *Bifidobacterium* and *Clostridium*, have specific functions and mechanisms in various psychological disorders, intriguing their novelty in modulating internal micro-ecological balance.

4.2 Incorporating Probiotics as a Therapeutic Approach

The concept of "strain specificity" in probiotic therapy is well-documented in scientific literature. For example, *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 have been shown to alleviate psychological distress and reduce anxiety-like behaviors in human subjects, highlighting their specific effects on mental health (Messaoudi et al., 2011). Additionally, *Lactobacillus rhamnosus* has been associated with modulating stress responses by influencing central GABA receptor expression, further emphasizing the importance of strain selection for targeted therapeutic outcomes (Bravo et al., 2011). Recent clinical studies have shown the significant improvement effects of mood and reductions of anxiety and depression symptoms through probiotics, making them a promising novel therapeutic technique. In a 2021 systematic review on the effects of probiotics on depression, anxiety and psychological stress, De Souza Minayo et al. (2021) found that out of nine randomized double/triple blind placebo-controlled trials, seven found positive results in at least one category. Another study on the effects of prebiotics in patients with depression, Tatutani et al. (2022) hinted at indirect effects of the

prebiotic Lactosucrose (LS) on factors related to depression such as self-efficacy and potentially suicidal ideation. While intake of LS did not directly improve depression symptoms, as measured by the Montgomery Asberg Depression Rating Scale (MARDS), treatment group showed a trend towards improved self-efficacy as measured by the General Self-Efficacy Scale (GSES) (Tatutani et al., 2022).

There is question as to which type of probiotics are most beneficial to symptoms of stress, anxiety and depression. This can also include the duration of treatment, selected bacterial strain, and the dosage of the probiotic. A systematic review and meta-analysis further support the efficacy of probiotics is both strain-specific and disease-specific, underscoring the need to consider specific strains when evaluating probiotic interventions (McFarland, 2014). It has been suggested that the effect of probiotics may take as long as an 8-week intervention period to see beneficial results (Chahwan et al., 2019). In a study on psychological stress in students with pre-exam anxiety, authors Karbownik et al. (2022) highlighted contrasting data that high consumption of fermented foods may be associated with more severe symptoms of depression and anxiety. This is because “pure probiotics”, those found in fermented food products opposed to probiotic dietary supplements and medications, may contain pathogenic microorganisms and viruses with potentially deleterious effects. Fermentation processes can lead to accumulation of undesired microbial byproducts, including bacterial toxins, mycotoxins, and biogenic amines. These issues have occasionally caused outbreaks of foodborne infections, potentially evoking low-level inflammation leading to an effect in mental wellbeing (Karbownik et al., 2022).

To test for dosage effects of multispecies probiotics on anxiety, authors Tran et al. (2019) tested five conditions varying in colony-forming units (CFU) and bacterial species count of a multispecies probiotic, and their effects on anxiety and related factors in healthy young adults. Results found that a high CFU, high species count probiotic and a high CFU, low species count

probiotic mixture generally showed the most significant improvements across the measured outcomes. A low CFU and high species count probiotic showed general improvements but were less effective than high CFU conditions. The main findings showed that probiotics improved several aspects of mental health, including panic anxiety, neurophysiological anxiety, negative affect, worry and negative mood regulation. Most importantly, this study found that CFU levels are more effective in comparison to species count in producing improvements in these domains. In addition, a ceiling effect was observed finding that participants with high distress levels reported greater improvement compared to normative distress level patients. These findings provided valuable insights in neuroscience research on the gut-brain axis due to the contribution of our understanding of the potential therapeutic applications of probiotics in anxiety and related mental health concerns (Tran et al., 2019). This study sets a precedent in probiotic research as it highlights the importance of dosage and consideration of CFU levels and species count.

METHODS AND MATERIALS

Due to the diverse natures of study populations, interventions, and outcomes in this field, a scoping review was used to provide an appropriate and in-depth framework to synthesize and present the current state of knowledge in this rapidly evolving area of research. This scoping review was conducted to systematically explore the current landscape of research using probiotics as a therapeutic intervention for anxiety and depression.

Design

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist for scoping reviews was used to guide this study. The investigation's parameters, literature review process and presentation of results were structured around a central research question:

Can probiotics serve as an alternative therapeutic approach in modulating gut microbiota to improve depression and anxiety symptoms? This approach ensured a systematic and comprehensive examination of the existing body of knowledge pertaining to probiotic interventions in anxiety and depressive symptoms.

Keywords: Gut microbiota, probiotics, prebiotics, gut-brain-axis, mood disorder, microbiome

Eligibility Criteria- PCC Framework

PARTICIPANTS

Studies involving adult participants over 18 with anxiety and/or depression symptoms; a diagnosis of anxiety and/or depression and healthy adults with no clinical diagnosis of a previous mental disorder.

CONCEPT

The use of probiotic interventions, including single-strain probiotics, multi-species probiotics, and probiotic/prebiotic combinations as an alternative therapeutic approach for managing symptoms of anxiety and depression. *Include which strands you evaluated.*

CONTEXT

Clinical settings utilizing probiotics as an intervention technique as a standalone treatment or in combination with antidepressant medications in the literature evaluated for this scoping review. Randomized Clinical Trials conducted worldwide published between the years 2015 and 2024 to ensure a current examination of the previous literature.

Search Strategy

Initially, 3,657 records were retrieved after the database search. Duplicates were removed, leaving a total of 2,737articles selected. The results were then screened in two phases: by title and

abstract, and by full text. After screening the full text of articles and disregarding irrelevant studies, 26 studies were eligible for inclusion.

Information Sources

A preliminary search of PUBMED, Scopus and PsycINFO was conducted using search terms:

SCOPUS: (TITLE-ABS-KEY (probiotic*) AND TITLE-ABS-KEY (“mental health”) OR TITLE-ABS-KEY (“psychological wellbeing”) OR TITLE-ABS-KEY (“mood disorder”) OR TITLE-ABS-KEY (“psychological well-being") OR TITLE-ABS-KEY (“psychological stress”) OR TITLE-ABS-KEY (depression) OR TITLE-ABS-KEY (anxiety))

Pubmed: ((“probiotics”[Mesh]OR(probiotic*[Title/Abstract])) AND (“mental health”[Mesh] OR “mental health” [Title/Abstract] OR “psychological well-being"[Mesh] OR “mood disorders”[Mesh] OR “stress, psychological”[Mesh] OR “depression”[Mesh] OR “anxiety”[Mesh]))

Psycinfo: noft(probiotic*) AND noft(“mental health” OR “psychological wellbeing” OR “psychological well-being" OR “mood disorder*” OR “psychological stress” OR depression OR anxiety)

Selection Process

Studies were considered eligible for inclusion if evaluation of alteration of psychological symptoms related to anxiety and depression using probiotics was involved. Inclusion criteria: healthy adults with symptoms of anxiety and depression (i.e. low mood, chronic stress, moderate stress), adults with clinical diagnosis of anxiety and/or depression

Full-text original research articles (including randomized controlled trials and clinical trials) were included published in the English language. Studies were excluded based on neurodegenerative diseases (Alzheimers, dementia), autism, schizophrenia, eating disorders ,

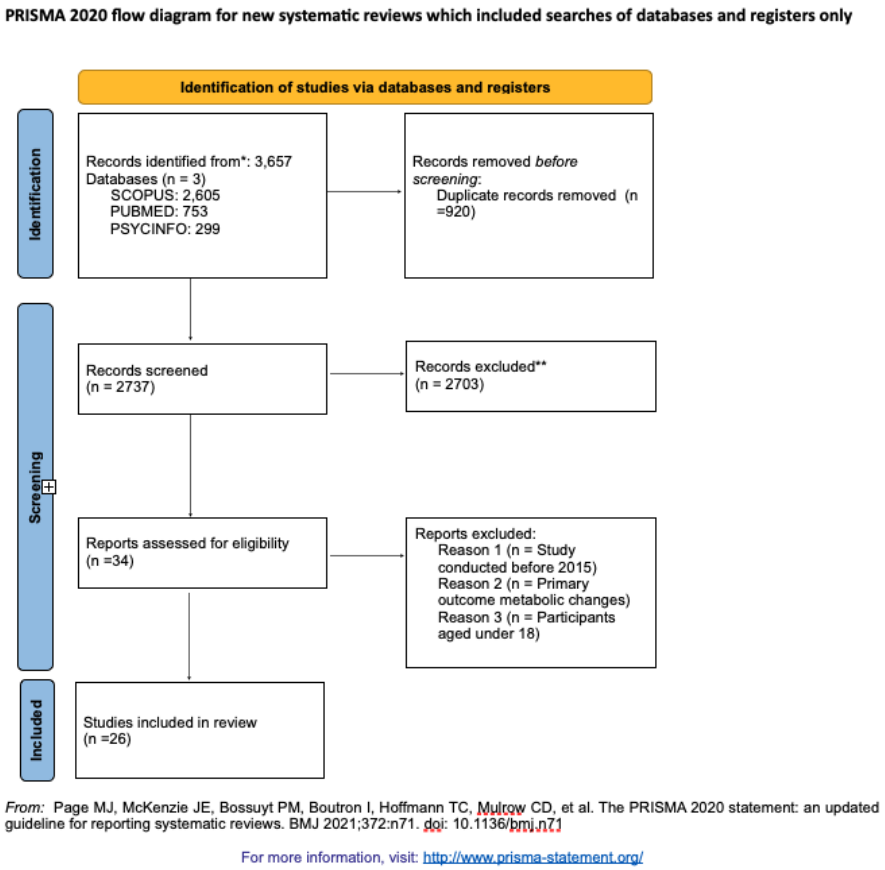
multiple sclerosis, cancer, obesity, epilepsy, neurodevelopment disorders, cardiovascular diseases, diabetes, Myocardial infraction, obsessive compulsive disorder, pediatrics, epilepsy, hypothyroidism, migraine, sleep disorders, acne, HIV, COVID-19, ADHD, poly-cystic ovarian syndrome (PCOS), infant colic, children, and animal studies.

In this study, clinical trials that focus exclusively on metabolic changes induced by probiotic administration were deliberately excluded. This decision aligns with the primary research objective of exploring the direct effects of probiotics on psychological symptoms of anxiety and depression via modulation of the gut-brain axis. While it is acknowledged that metabolic changes may indirectly affect psychological symptoms, including such studies would extend beyond the scope of this focused investigation. By adhering to this exclusion criteria, it is ensured that the data synthesis remains directly relevant to the research question, facilitating a more targeted analysis of the relationship between probiotic administration and mental health outcomes. This strategy enables us to draw a more specific conclusion regarding the potential efficacy of probiotics as an alternative therapeutic approach on anxiety and depression symptoms.

RESULTS

The results of this scoping review include a comprehensive analysis of randomized controlled trials (RCTs) investigating the efficacy of probiotic interventions in individuals with anxiety and/or depression symptoms. Each study includes a configuration of probiotic strains and treatment interventions. A total of 26 randomized clinical were (RCTs) were analyzed including a total of 1,857 participants conducted across various countries including China, New Zealand, Iran, Switzerland, Germany, the United Kingdom, the Netherlands, Italy, Japan, Poland, Indonesia, Australia and the United States. A total of ten RCTs assessed treatment interventions on clinical populations with diagnosis of depression (Akksaheh et al. (2015), Baiao et al. (2021),

Chahwan et al. (2019), Kazemi et al. (2018), Nikolova et al. (2023), Rudzki et al. (2018), Schaub et al. (2020), Tartutani et al. (2022), Tian et al. (2021), Ullah et al. (2022)), two studies analyzed clinical populations with a diagnosis of anxiety (Ezkandarzadeh et al. (2021), Meng et al. (2022)), and fourteen analyzed probiotic interventions on healthy participants dealing with mild to moderate stress (Chong et al. (2018), Frejy et al. (2023), Johnstone et al. (2021), Lew et al. (2018), Mutoh et al. (2023), Patterson et al. (2020), Rode et al. (2022), Steenbergen et al. (2015), Talbott et al. (2019), Theodora et al. (2017), Tran et al. (2019), Walden et al. (2023), Wang et al. (2019), Zhu et al. (2023)).



(FIGURE 1. PRISMA Flowchart of selected studies)

The image in Figure 1 shows a PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram. This diagram illustrates the process of identifying, screening, and including studies for this scoping review.

Figure 2- Probiotic Comparison Chart - SEE APPENDIX

The charts presented in the document (see appendecies) summarize the 26 various studies investigating the effects of a range of probiotic supplements on mental health, with specific focus on symptoms related to anxiety and depression. Each study is characterized by its population, intervention, duration, and outcome measures, highlighting the potential benefits of these dietary supplements as presumed treatments for mood disorders.

Microorganism Strains

Probiotics, prebiotics and psychobiotics were examined in this review. Single-strain and multispecies probiotic supplements were studied, including: *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, Bifidobacterium breve, Lactobacillus helveticus, Lactobacillus Plantarum.* Prebiotic strains including 4G-beta-D-Galactosylsucrose and Galacto-oligosaccharides (GOS) were concurrently examined. Two psychobiotic strains including PS128 in combination with citalopram (Meng et al., 2022) and *Bifidobacterium breve* CCFM1025 (Tian et al., 2021) were analyzed, and one study examined a coordinated pro-/pre-/phyto-biotic supplementation (Talbott et al., 2017).

Bio-Kult Advanced®, ADM Protexin Ltd: *Bacillus subtilis PXN® 21, Bifidobacterium bifidum PXN® 23, Bifidobacterium breve PXN® 25, Bifidobacterium infantis PXN® 27, B. longum PXN® 30, Lactobacillus acidophilus PXN® 35, Lactobacillus delbrueckii ssp. bulgaricus PXN® 39, Lactobacillus casei PXN® 37, Lactobacillus plantarum PXN® 47, Lactobacillus rhamnosus PXN® 54, Lactobacillus helveticus PXN® 45, Lactobacillus salivarius PXN® 57, Lactococcus lactis ssp. lactis PXN® 63, Streptococcus thermophilus PXN® 66*)

Ecologic®Barrier (2.5 × 109 CFU/g): *Bifidobacterium bifidum* W23, *Bifido- bacterium lacti*s W51, *Bifidobacterium lactis* W52, *L. acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19 and *Lactococcus lactis* W58

Vivomixx® (*Streptococcus thermophilus* NCIMB 30438, *Bifidobacterium breve* NCIMB 30441, *Bifidobacterium longum* NCIMB 30435, *Bifidobacterium infantis* NCIMB 30436, *Lactobacillus paracasei* NCIMB 30439, *Lactobacillus delbrueckii sibsp. Bulgaricus* NCIMB 30440)

BioCeuticals R © (12 billion colony forming units (CFU) per capsule, containing: *Bifidobacterium bifidum* (Bb-06): 2 billion CFU; *Bifidobacterium animalis* subsp. *lactis* (HN019): 1 billion CFU; *Bifidobacterium longum* (R0175): 1 billion CFU; *Lactobacillus acidophilus* (La-14): 2 billion CFU; *Lactobacillus helveticus* (R0052): 2 billion CFU; *Lactobacillus casei* (Lc-11): 2 billion CFU; *Lactobacillus plantarum* (Lp-115): 1 billion CFU; *Lactobacillus rhamnosus* (HN001): 1 billion CFU)

Assessment Tools Used to Measure Anxiety and Depression

The main assessment tools to measure symptomology were Beck Depression Inventory (BDI-II), Beck Anxiety Inventory (BAI), Depression, Anxiety and Stress Scale (DASS), Hospital Anxiety and Depression Scale (HADS), Hamilton Anxiety Rating Scale (HAMA), Hamilton Depression Rating Scale (HAMD), Profile of Mood States Survey (POMS), Quality of Life inventory (WOL), State Trait Anxiety Inventory (STAI), and Montgomery-Asberg Depression Rating Scale (MADRS).

Duration of Intervention

In this scoping review, a range of treatment durations appropriate for probiotic interventions aimed at alleviating the symptoms of anxiety, depression and stress were selected for exploration. By deciding to include studies with varying durations, a breath of research was analyzed to understand the impact of probiotic administration on mental health outcomes. Interventions ranged from the shortest duration of 28 days (about 4 weeks), to the longest of 24 weeks (about 5 and a half months). The main treatment durations from the studies selected were 4-week and 8-week interventions.

Single Strain Probiotics

Single-strain probiotic interventions provide valuable insights into the potential efficacy of specific bacterial strains as targeted therapeutic agents for psychological conditions. Six studies administered single-strain probiotic interventions including: *Lactobacillus Plantarum 299v (LP299v), Lactobacillus plantarum* DR7, *Lactobacillus plantarum* P8, *Lactobacillus helveticus* MCC1848, *Lacticaseibacillus paracasei* Lpc-37, and *Bifidobacterium longum 1714*™.

Single-strain probiotic bacteria *Lactobacillus Plantarum 299v* was administered to patients with MDD undergoing treatment with SSRIs where Rudzki et al. (2018) found improved cognitive performance in depressed patients and a decrease in KYN concentration. In comparison, a single-strain probiotic *Lactobacillus plantarum* DR7 was administered to healthy adults with moderate stress levels (based on PSS-10 questionnaire scores) for 8 weeks to examine the effects on symptoms of stress and anxiety in healthy populations. Chong et al. (2018) found that after 8 weeks supplementation with *Lactobacillus Plantarum* DR7 in stressed adults, symptoms of stress and anxiety were reduced, along with improved cognitive and memory pathway, and secondary results of reduced plasma cortisol levels and pro-inflammatory cytokines (Chong et al., 2018). Similarly, a single-strain *Bifidobacterium longum* 1714™ (Zenflore) was tested to examine

neural responses during social stress. Wang et al. (2019) reported the modulation of resting neural activity, accompanying improved vitality and fatigue through the alteration of neural oscillations (Wang et al., 2019).

Multi-Species Probiotics

Of the clinical trials accepted into this review, a total of 14 studies administered multi-species probiotics, seven examining the effects on pathological populations and seven investigating healthy, non-pathological, participants. To examine the effects of a multispecies probiotic on subjects with moderate depression, Baião et al. (2021) administered a probiotic supplement ((Bio-Kult® Advances, ADM Protexin Ltd)) consisting of 14 species of bacteria (*Bacillus subtilis PXN® 21, Bifidobacterium bifidum PXN® 23, Bifidobacterium breve PXN® 25, Bifidobacterium infantis PXN® 27, B. longum PXN® 30, Lactobacillus acidophilus PXN® 35, Lactobacillus delbrueckii ssp. bulgaricus PXN® 39, Lactobacillus casei PXN® 37, Lactobacillus plantarum PXN® 47, Lactobacillus rhamnosus PXN® 54, Lactobacillus helveticus PXN® 45, Lactobacillus salivarius PXN® 57, Lactococcus lactis ssp. lactis PXN® 63, Streptococcus thermophilus PXN® 66*) to 71 depressed volunteers for 4 weeks. Emotional processing and reward learning were measured as primary outcomes, along with secondary outcomes of mood, anxiety, positive and negative affect, salivary cortisol, sleep and serum C-reactive peptide. After a 4-week intake period, depression scores on the Patient Health Questionnaire-9 from the probiotic group saw a significant reduction (-50% from baseline, $p<0.05$, n=35), along with increased accuracy in recognizing facial emotions during emotional test battery (ETB) task (placebo = 51.8 ± 1.41, probiotic = 56.2 ± 1.43) (Baião et al., 2021).

Prebiotic Comparison

Tartutani et al. (2022) measured a sample of Japanese outpatients diagnosed with major depressive disorder and single strain probiotic (4G-beta-D-Galactosylsucrose (LS)) in

combination with prescribed antidepressants in a single-cite, double-blind, randomized controlled trial for a period of 24 weeks with a primary outcome goal of improvement in total MADRS scores. Long term consumption of LS contributed to significantly improved global self-efficacy scores (2.00 ± 4.24 vs -1.36 ± 4.15, $p = 0.091$) and demonstrated that prebiotics can aid in treatment of depression symptoms through moderation of internal tension, suicidal ideation and quality of life, however, these changes were not significant (Tartutani et al., 2022).

In a double-blind clinical trial investigating the effects of probiotic supplementation (*Lactobacillus helveticus* and *Bifidobacterium longum*) or prebiotic (galactooligosaccharide) intervention versus placebo on BDI scores in 81 patients with MDD, Kazemi et al. (2018) found a significant decrease in mean BDI scores from probiotic group compared to placebo. However, the decrease in mean BDI scores by prebiotic group was not significant in comparison to placebo and probiotic groups (Kazemi et al., 2018). The same prebiotic galacto-oligosaccharides (GOS) was administered to 64 healthy females to examine changes in mood and well-being in a 4-week double blind, placebo-controlled prebiotic intervention. Johnstone et al., (2021) found anxiolytic effects in highly anxious participants in self-reports of trait anxiety and emotional well-being.

Probiotics and Antidepressant Combination

In addition to antidepressant treatment, probiotics have been demonstrated effective in reducing depression symptoms. There has been a suggested interplay between the severity of depression (mild to severe), and outcome of treatment effects (Chahwan et al., 2019). Chahwan et al. (2019) found a significant correlation between *Ruminoccus gnavus* multi-species probiotic interventions in addition to antidepressant treatment showed improvement in psychological symptoms and cognitive improvement. Akkasheh et al., 2015 found similar findings in Iran during an 8-week multi-species probiotic trial in combination with antidepressants on patients with MDD with a key result of lowered BDI scores and insulin levels as compared to placebo

(Akkasheh et al., 2015). Multi-strain probiotic Bio-Kult Advanced was also tested in a single-center, double-blind, placebo-controlled RCT on outpatients with diagnosis of MDD currently taking anti-depressant medication, and findings suggested that after 8 weeks of intervention, probiotic group displayed greater improvement HAM-D symptoms of depression scores (Nikolova et al., 2023). Analysis from this study also suggested an improvement of anxious-somatic symptoms from probiotic supplementation.

Psychobiotics

Two studies selected for this review examined the effects of psychobiotics, live microorganisms that can confer a health benefit specifically on patients suffering from psychiatric illness when administered in sufficient amounts (Dinan et al., 2013). PS128, a novel psychobiotic strain, has been demonstrated to have an effect in neuropsychiatric disorders in animal models (Liao et al., 2020) (W. Liu et al., 2016). To investigate its clinical effects in humans, Meng et al., (2022) recruited 200 patients with a clinical diagnosis of anxiety according to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders and assessed the severity of anxiety using the Hamilton Anxiety (HAMA) Scale-14 and Self-Rating Anxiety Scale (SAS). After 2 months intervention with PS128 group receiving one capsule twice a day along with SSRI citalopram, HAMA score significantly decreased compared with pretreatment ($p<0.05$) along with a SAS score significantly lowered (Meng et al., 2022). The findings of this study suggest that the treatment of anxiety with citalopram in combination with PS128 is satisfactory clinically, along with a feasible application due to improvement of anxiety symptoms, increase in cure rate and reduction of adverse reactions (Meng et al., 2022).

Furthermore, Tian et al (2021) administered a single-strain freeze-dried psychobiotic powder *Bifidobacterium breve* CCFM1025 to patients with MDD for a total of 4 weeks and evaluated psychometric symptoms using the HDRS-24, MADRS and BPRS and serum serotonin

rates were investigated as a secondary outcome. After 4 weeks intervention with psychobiotic CCFM1025, an antidepressant-like effect was seen based on HDRS-24 (placebo: M=6.44, SD=5.44, CCFM1025: M=10.40), and MADRS (placebo: M=4.92, SD=7.15, CCFM1025: M=9.60) scores, as compared to placebo. This may be due to gut tryptophan metabolism during probiotic treatment, making CCFM1025 a promising candidate for depression and gastrointestinal improvement.

Anxiety

Two studies examined probiotic intervention with Generalized Anxiety Disorder (GAD), one with a psychobiotic (Meng et al., 2022) and the other with a multi-strain probiotic supplement in combination with an SSRI (Sertraline) (Eskandarzadeh et al., 2021). After 8-week administration of a multispecies probiotic capsule containing 18*10^9 CFU (*Bifidobacterium longom, Bifidobacterium bifidum, Bifidobacterium lactis* and *Lactobacillus acidophilus*) in addition to 25mg Sertraline, Eskandarzadeh et al. (2019) found that HAM-A scores decreased in the probiotic group along with reduction of BAI scores. Meng et al. (2022) administered a novel psychobiotic strain (*Lactobacillus plantarum PS128*) on the basis of citalopram intake to patients with diagnosed anxiety and measured HAMA and SAS scores after a 2-month treatment period. Citalopram is a selective serotonin reuptake inhibitor (SSRI) that can produce antidepressant effects and has been reported to effectively treat anxiety disorders (Kang et al., 2017). While HAMA scores decreased in both the treatment and control groups, the decrease rate of the PS128 group was significant ($p<.05$) compared with pretreatment (Meng et al., 2022).

Psychological Stress

Out of fourteen studies selected analyzing the effects of probiotics on healthy populations, all saw improvements of symptoms after supplementation. Single-strain probiotic *Lactobacillus plantarum* P8 (10 log CFU) intervention in stressed adults proved reduced scores of stresses and

anxiety as compared to placebo measured by the Perceived Stress Scale (PCC) questionnaire (Lew et al., 2018). Modulation of low chronic stress and high chronic stress were compared in findings by Patterson et al. (2020) after intervention with probiotic *Lacticaseibacillus paracasei* Lpc-37® and results proved a reduction in perceived stress as compared to placebo. Recent similar findings by Mutoh et al. (2023) found suggestion of maintaining a relaxed state after scores of friendliness were significantly higher post-intervention of heat-killed *Lactobacillus helveticus* MCC1848 in a study of 53 high-stress nursing students.

Adverse Events

Though probiotics are popular for their tolerability, one trial found that the most common side effect from the probiotic group was nausea (11/34 participants), followed by drowsiness (20.6%) (Chahwan et al., 2019). Adverse events, though rare, were also reported such as bloating, abdominal discomfort, headaches and changes in bowel movements (Frejy et al., 2023).

DISCUSSION

By selecting to compare a multitude of probiotic mixtures, including single strain, multi-species and add-on therapy, a thorough examination of diverse interventions currently being investigated in this field was examined and followed. While it is important to explore probiotics in conjunction with standard antidepressant medication, it is also pivotal to examine them as a standalone treatment to evaluate their potential independent efficacy. By incorporating a diverse array of probiotic interventions, the full spectrum of potential therapeutic alternatives, can be captured providing an in-depth understanding of their effects on neural function and behavior.

Clinical population interventions were examined following probiotic administration in combination with and without antidepressant medication. In comparison, a study population of healthy adult's was studied with varying degrees of stress levels, anxiety symptoms and stress-

induced situations moderated with probiotic intervention. By comparing these groups, we aimed to elucidate the potential stress-mitigating properties of probiotics in clinical and sub-clinical populations, potentially informing strategies for stress management before the onset of clinically significant symptoms.

Clinical Outcomes

Emerging evidence indicates that probiotics can serve as a valuable adjunctive therapy for individuals experiencing anxiety and depression. This study encompassed diverse clinical populations, including individuals diagnosed with varying severity levels of Major Depressive Disorder (MDD) and Generalized Anxiety Disorder (GAD), both with and without concurrent antidepressant pharmacotherapy. The findings suggest that probiotics interventions may offer viable and ethical therapy alternatives, demonstrating efficacy both as a monotherapy and an adjunctive treatment modality. In addition to an SSRI treatment Sertraline, a combination therapy with probiotics was superior for treating anxiety symptoms than Sertraline treatment alone, suggesting probiotics as an add-on therapy may be effective treatment in decreasing anxiety symptoms in patients with GAD (Eskandarzadeh et al., 2019). In comparison, similar results were found in a double-blind clinical trial performed by Akkasheh et al. (2015) while studying 40 patients with diagnosed depression receiving probiotic supplementation. Patients who received a freeze-dried, multi-species probiotic supplement of *Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium bifidum* alongside their standard antidepressant treatment for 8 weeks exhibited a notable reduction in BDI scores and improved mood symptoms (Akkasheh et al., 2015). This improvement of symptoms was accompanied by beneficial changes in metabolic parameters, including decreased serum insulin levels and reduced insulin resistance, along with lower C-reactive protein inflammatory levels (Akkasheh et al., 2015). These findings suggest that probiotics may enhance the efficacy of antidepressants by modulating inflammatory

and metabolic pathway and can be useful as an add-on therapy in combination with antidepressant medications to effectively modulate the progression of the disorder and enhance therapeutic outcomes.

From previous research, multispecies probiotics, consisting of both *Bifidobacteria* and *Lactobacilli* strains, have been proven more effective than single strains for the improvement of mood (R. T. Liu et al., 2019). In this scoping review, results indicated that multi-species probiotics demonstrated effective qualities in improving symptoms in healthy and clinical populations, with similar results. Chong et al. (2018) noted important evidence of administration of *Lactobacillus plantarum* DR7 being able to modulate serotonergic and dopaminergic pathways, demonstrating a broad impact on neurochemical regulation as compared to placebo. The administration of DR7 for 8 weeks to moderately stressed adults significantly alleviated stress, anxiety, and psychological distress as measured by the DASS-42 questionnaire. Participants receiving DR7 also exhibited reduced levels of plasma cortisol and pro-inflammatory cytokines, along with increased anti-inflammatory cytokines and improvements in cognitive and memory pathways (Chong et al., 2018). In a separate study administering *Lactobacillus plantarum* P8 to stressed adults, Lew et al. (2018) observed comparable findings of reduction in pro-inflammatory cytokines, enhanced memory and reduced scores of stress and anxiety (Lew et al., 2018). This is in line with previous research suggesting the role of inflammation in the pathogenesis of psychological disorders. In MDD, studies have consistently linked the role of elevated levels of pro-inflammatory cytokines to patients with depression, influencing neurotransmitter dysregulation and the severity of the disease (Rhie et al., 2020) (Bauer er al., 2021).

Reductions in cognitive reactivity were also seen in multiple studies, including altered brain regions involved in emotional, cognitive and facial processing (Rode et al., 2022) and

significant reductions of cognitive reactivity, particularly aggressive and ruminative thoughts after probiotic supplementation for 4 weeks (Steenbergen et al., 2015). Probiotics might be an effective form of alternative therapy due to their alterations in cognitive reactivity in pathological populations and healthy subjects dealing with stress. Patients with depressive disorders typically exhibit heightened neural responses to emotionally negative stimuli and diminished responses to emotionally positive stimuli, as compared to healthy controls (Surguladze et al., 2005). Neuroimaging studies have revealed altered neural activation patterns in individuals with depression when processing emotional stimuli. More specifically, Baião et al. (2021) suggested that the intake of multispecies probiotic Bio-Kult® Advances is effective in altering emotional processing in moderately depressed people (Baião et al., 2021). Depressed individuals are traditionally more likely to interpret neutral or positive stimuli as negative, these biases contributing to the etiology of depression (Baião et al., 2021). After only 28 days of intervention, probiotic patients were more accurate in depicting facial expressions as less negative and showed lower sensitivity to win and loss outcomes. Overall, data indicated that repeated administration of a multispecies probiotic altered emotional processing in moderately depressed individuals, differently than antidepressant therapy in healthy adults and those diagnosed with MDD (Baião et al., 2021).

Furthermore, after 4 weeks of short-term, high-dose (900 billion CFU/day), add-on, multi-strain probiotic supplement Vivomixx® in addition to treatment as usual (TAU), Schaub et al. (2022) found an improvement of depression symptoms and modulation of gut microbiota after probiotic supplementation. Changes in brain structure were examined and a significantly higher activation in the putamen region was observed, indicating its relevance in depression (Schaub et al., 2022). The importance of this finding is highlighted by the fact that the putamen was considerably more activated in depressive patients during facial emotion processing, to which the

reduced activation in the putamen in the probiotics group suggests a shift in emotional valance, a predisposing factor of depression (Surguladze et al., 2005).

This scoping review also examined the effects of probiotic interventions on stress responses in two distinct "healthy" populations: individuals with self-reported moderate stress levels and those undergoing an acute stress task. Prolonged exposure to stressors is recognized as a significant contributor to the etiology of affective disorders and stress-related psychopathologies (Patterson et al., 2020). In highly anxious adults, prebiotic Galacto-oligosaccharides (GOS) showed anxiolytic effects in self-reports of trait anxiety and emotional wellbeing (Johnstone et al., 2021). Trait anxiety, which can be characterized by a persistent state of doubts, fear and worry, can determine emotion regulation abilities and set a trajectory for lifelong behaviors (Johnstone et al., 2021) (Haller et al., 2015). These findings further support previous studies where GOS was also observed to lower secretion of cortisol in healthy adults, leading to a reduction of stress response systems and improved emotional processing (Schmidt et al., 2014). The observed effects of GOS suggest its potential to attenuate negative attentional biases, a characteristic cognitive pattern in individuals with anxiety disorders, indicating a possible role for prebiotics in modulating anxiety-related psychological mechanisms.

Prebiotics, dietary ingredients with the ability to enhance growth of favorable intestinal bacteria (Barile & Rastall, 2013), have demonstrated their effectiveness in modifying anxiety-related psychological mechanisms by lowering secretion of cortisol levels and modulating emotional processing including bias towards negative information (Johnstone et al., 2021). In non-clinical populations, Frejy et al. (2023) found that a prebiotic rich diet may be useful for promoting mental health and reducing mood disturbance in adults with moderate psychological distress, however found no evidence that synbiotic combinations had benefit (Frejy et al., 2023).

Stress-induced interventions were included as well to investigate the effect of probiotic supplementation on healthy, non-clinical populations. Functional brain responses in regions involved in emotional and facial processing were affected by a multispecies probiotic product (*Bifidobacterium longum* R0175, *Lactobacillus helveticus* R0052, and *Lactiplantibacillus plantarum* R1012) during an emotional attention task in healthy adults with no clinical diagnosis (Rode et al., (2022). Similarly, healthy participants subject to social stress during the "Cyberball game" task showed altered resting neural activity after intervention with B. longum 1714™, resulting in diminished mental fatigue and increased vitality (Wang et al., 2019). During this task, subjects who had received probiotic supplementation were able to mitigate stress and anxiety better than placebo group during exposure to a controlled stressor. Comparable to cognitive reactivity and emotional processing, modulated neural responses may play a role in coping mechanisms when regulating negative emotions (Wang et al., 2019).

Studies indicate that medical students tend to have moderate depression, potentially due to lack of sleep, academic stress, workload and psychological pressure stemming from student abuse and bullying. (Rines et al., 2017) (Sobowale et al., 2014). Two studies selected medical students as their target population due to their susceptibility to depression. In test-anxious college students, psycho supplementation of *Lactobacillus plantarum* JYLP-326 twice a day for 3 weeks showed significantly improved outcomes related to depression and anxiety, measured by HDRS-17 and HAMA scores. *L. plantarum* JYLP-326, an isolate of fermented sticky rice in China able to bind to Caco-2 cells, has been previously proven to be effective in improving sleep disorders like insomnia in animal models (Zhu et al., 2023). Results demonstrated that probiotic intervention partially normalized the gut microbiome composition in subjects experiencing anxiety, evidenced by significant shifts in specific bacterial taxa. The treatment modulated anxiety-induced dysbiosis, characterized by decreased relative abundances of Bacteriodes and

Roseburia genera, concurrent with increased populations of Prevotella and *Bifidobacterium*. These alterations suggest a potential mechanism by which probiotics may influence the gut-brain axis in anxiety-related conditions (Zhu et al., 2023). Furthermore, administration of probiotics demonstrated the potential to modulate the diversity and compositional profile of the gut microbiome disturbed by stressful stimuli. Talbott et al. (2019) found similar results of increased populations of good bacteria *Lactobacillus* and *Bifidobacterium* after supplementation with a natural multi-ingredient supplement containing probiotic, prebiotic and phytobiotic fibers. These findings suggest that the composition and relative abundance of specific gut microbial species can influence resilience against stress-induced pathologies.

Dosage and length of duration are important factors when examining probiotic mixtures as treatment options. To compare the efficacy between colony forming units (CFU) and species count, Tran et al. (2019) examined the benefits of probiotics in healthy populations with no psychological disorders. The findings indicate that the efficacy of probiotics in modulating anxiety and anxiety-related factors may be primarily dependent on the concentration of colony-forming units (CFU) rather than the diversity of bacterial species present. A weak but significant correlation between gender and BAI neurophysiological anxiety and PSWQ total scores was also found, suggesting that gender might affect symptom improvement (Tran et al., 2019).

CONCLUSION

This scoping review was grounded in the evolving body of literature that connects gut microbiota with mental well-being and investigated the efficacy of probiotic, prebiotic and psychobiotic interventions in enhancing mental health. The bidirectional communication network between the GI tract and the CNS has emerged as a central focus in understanding the etiology of depression and other mental health disorders, as explored in this paper. This axis supports the complex interaction between gut microbiota, intestinal permeability, neuroendocrine pathways and neural

signaling, with influences on mood, cognition and behavior. Studies have shown that the gut microbiome composition varies between healthy individuals and those with depression and anxiety, suggesting the role of dysbiosis in the pathophysiology of mood disorders.

Anxiety and depression represent distinct yet interconnected neuropsychological states with anxiety being a potential risk factor of depression. Anxiety can manifest itself in the form of heightened autonomic arousal, increased muscle tension, and persistent feelings of unease. Stress generally presents itself with a lower level of acute symptoms in comparison to anxiety. Stress-related psychological dysfunctions, elevated nervous system action and increased emotional reactivity may reflect the underlying differences in the neurobiological mechanisms and neural circuits involved in each condition.

Depression is a complex neuropsychological disorder that can develop due to chronic exposure to anxiety and stress. It is characterized by a range of symptoms, including persistent low mood, anhedonia, feelings of hopelessness, diminished self-worth, and negative self-perception.

By utilizing a scoping review for this thesis, a variety of populations dealing with mood symptoms were studied and compared to examine the results of diverse probiotic mixtures and their effect on mood. The study populations varied significantly, ranging from patients diagnosed with Major Depressive Disorder (MDD), to those diagnosed with Generalized Anxiety Disorder (GAD), to healthy participants dealing with mild/moderate/high stress, and healthy participants in stress-induced situations. These populations are important for comparison due to the differences in efficacy of the type of probiotic used, dosage, treatment duration and after-intervention findings.

After synthesizing the current body of literature, this review underscores the comprehensive role of probiotics as an adjunctive treatment for mood disorders, highlighting their potential to modulate emotional processing and enhance the efficacy of traditional antidepressant therapies. In severe clinical populations affected by anxiety and depression, probiotics as an add-on therapy in combination with antidepressant medications proved beneficial in not only reducing affective symptoms, but also improving gut microbiota. While probiotics may not be viable as a monotherapy in all severe populations, their administration can still confer beneficial effects on the host compared to no probiotic intervention. These benefits may include modulation of the gut-brain axis, improvements in metabolic parameters, and potential enhancement of conventional treatment efficacy when used as an adjunctive therapy.

Key findings include the ability of specific probiotic strains to alter brain activation patterns, reduce cortisol levels, and normalize gut microbiome composition disturbed by stress in both healthy and clinical populations. In moderate and subclinical populations, probiotics have demonstrated emotional, cognitive and metabolic improvement from probiotic supplementation. In patients with manifestations of low mood or mild depression, a food supplement based on S-Adenosyl-L-methionine in combination with probiotics resulted in a change from mild depression to an absence of depression symptoms (Ullah et al., 2022). Specific probiotic strains, like *Lactobacilllus plantarum* DR7, demonstrated ability in modulating serotonergic and dopaminergic pathways, indicated an impact on neurochemical regulation and modulation of stress and anxiety systems. Anti-inflammatory effects were consistently shown to lead to reductions in pro-inflammatory cytokines and increases in anti-inflammatory cytokines. This is particularly significant given the previously established link between inflammation and the pathogenesis of psychological disorders, especially MDD.

Cognitive and emotional processing revealed compelling evidence after probiotic intervention with multiple studies reporting improvements in cognitive reactivity, emotional processing and memory function. Most notably, probiotics altered brain activation patterns in regions involved in emotional and facial processing, suggesting their benefit in minimizing symptoms pre diagnosis. Additionally, the ability to attenuate stress responses was evidenced by reduced cortisol levels and improvement of coping mechanisms during acute stressors in both healthy and clinical populations.

In conclusion, these findings contribute towards a growing body of research advocating for the potential use of probiotics to influence mental health outcomes, namely depression and anxiety, through modulation of the gut microbiome. As the understanding of the gut-brain axis continues to evolve, probiotics may become an integral part of personalized and non-invasive treatment strategies for mental health disorders, offering a safer and more accessible alternative in pharmacological medicine.

Limitations

There are a few limitations that were incurred during the duration of this study. First, the study population was a bit large, including pathological and non-pathological populations, the use of and without anti-depressant medications, and a variation of probiotic supplementations like multi-species, single-strain, probiotic vs prebiotic intervention and psychobiotics. The large number of variables incurred in this study can be considered a limitation because they did not allow the review to focus on a specific factor; however, it was also a strength to study and compare a variety of treatment interventions on varying populations. Second, patients with high chronic stress as well as individuals experiencing low mood may not have met the requirements for a clinical diagnosis, and therefore are secluded from the clinical study population. This could

have been avoided by creating stricter inclusion and exclusion criteria to specifically monitor the measurement of symptomology.

Future Directions

The efficacy of probiotic interventions is also influenced by factors such as dosage, duration of treatment, and individual dietary habits. Future research should focus on optimizing probiotic formulations, controlling for cofounding variables like diet and medication use, and investigating the long-term effects of probiotic supplementation on mental health outcomes. Dietary measures could also diminish the efficacy of probiotic supplementation during clinical intervention, as well as antibiotic usage. Limitations from a randomized clinical trial testing the effects of psychobiotics on MDD highlighted the importance in future studies of controlling diet and medication, as results from probiotic-induced changes may be obscured (Tian et al., 2021). Factors like high sugar intake and low nutrient diversity can also lead to altered gut pH, leading to increased intestinal permeability and systemic inflammation, making it difficult for probiotic components to thrive (Satokari, 2020). Therefore, it is crucial for future research to consider dietary habits and medication use to accurately evaluate the effects of probiotics and minimize confounding variables.

An interesting emerging niche of research in this field would be the study of probiotic intervention in early-life stress and its outcomes of strategic influence into adulthood. This area of research could potentially pose benefit for infants born C-section and premature. The administration of probiotic supplementation could work as an influencing factor to grow and maintain beneficial intestinal microflora and therefore, prevent dysbiosis related health effects.

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APPENDICIES

Figure 2 Probiotic Comparison Chart

Reference	Country/Year	Study Sample	Probiotic Type	Study Design	Duration of Intervention	Measurement	Key Findings
STUDY POPULATIONS WITH CLINICAL DIAGNOSIS							
Akksaheh et al.	Iran/2015	40 patients with diagnosis of MDD ages 20-55yr	freeze-dried *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum*	randomized, double-blind, placebo-controlled clinical trial	8 weeks	BDI	In addition to antidepressant drug treatment, probiotic supplementation lowered BDI scores and insulin levels, decreased oxidative stress
Baiao et al.	United Kingdom/2021	71 adults with moderate depression	Bio-Kult Advanced (14 species: *Bacillus subtilis PXN® 21, Bifidobacterium bifidum PXN® 23, Bifidobacterium breve PXN® 25, Bifidobacterium infantis PXN® 27, B. longum PXN® 30, Lactobacillus acidophilus PXN® 35, Lactobacillus delbrueckii ssp. bulgaricus PXN® 39, Lactobacillus casei PXN® 37, Lactobacillus plantarum PXN® 47, Lactobacillus rhamnosus PXN® 54, Lactobacillus helveticus PXN® 45, Lactobacillus salivarius PXN® 57, Lactococcus lactis ssp. lactis PXN® 63, Streptococcus thermophilus PXN® 66*)	Randomized, double-blind, placebo-controlled study	4 weeks	PHQ-9, PANAS, STAI, ETB, dot probe task, tests of reward learning	DR7 reduced symptoms of stress and significantly reduced depression scores on PHQ-9; reduced plasma cortisol levels; reduced plasma pro-inflammatory cytokines
Chahwan et al.	Australia/2019	71 participants with mild to severe depression	Ecologic®Barrier (2.5 × 10⁹ CFU/g): *Bifidobacterium bifidum* W23, *Bifido- bacterium lactis* W51, *Bifidobacterium lactis* W52, *L. acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19 and *Lactococcus lactis* W58	Triple-blind, parallel, placebo-controlled ransomized clinical trial	8 weeks	BDI, DASS, BAI	Probiotic group showed lower cognitive reactivity after intervention; no significance of probiotics altering microbiota in depressed group; signigicant correlation found between *Ruminoccus gnavus* and one metric of depression
Eskandarzadeh et al.	Iran/2021	48 patients drug-free patients with GAD diagnosis	Probiotic capsule: 18*109 CFU: *Bifidobacterium longom, Bifidobacterium bifidum, Bifidobacterium lactis* and *Lactobacillus acidophilus* + sertraline (PS)	Double blind, randomized, placebo-controlled trial	8 weeks	QOL, HAM-A, BAI, SAS-A	Combination of probiotics and sertraline (an SSRI) was more effective than sertraline alone in reducing anxiety symptoms than placebo; no significant impact on quality of life
Kazemi et al.	Iran/2018	81 Mild to moderate MDD patients ages 18-50 with history of anti-depressant drugs	Probiotic: *Lactobacillus helveticus* and *Bifidobacterium longum,* Prebiotic: galactooligosaccaride	Three-arm parallel design, placebo-controlled, double-blind Randomized Controlled Trial (RCT)	8 weeks	BDI	Significant decrease in mean BDI score from probiotic group compared to placebo, decrease in mean BDI score by prebiotic was not significant compared to placebo and probiotic group
Meng et al.	China/2022	200 patients with diagnosed anxiety	Psychobiotic strain PS128 combined with citalopram	two groups: control group (citalopram only) and PS128 group (citalopram and PS128)	2 months	HAMA, SAS	The combined effect of citalopram and PS128 is significantly decreased HAMA and SAS scores in patients with anxiety
Nikolova et al.	United Kingdom/2023	50 outpatients ages 18-55 with diagnoses MDD currently taking antidepressant medication	Multi-strain probiotic:(8 billion CFU) Bio-Kult Advanced; ADM Protexin	single-center, double-blind, placebo-controlled pilot randomized clinical trial	8 weeks	HAMD-17, IDS, HAMA, GAD-7	Probiotic group displayed greater improvement in symptoms of depression (reduction of 1 severity grade on depression rating scales), analysis suggests anxious-somatic symptoms may have been improved by probiotic supplementation,
Rudzki et al.	Poland/2018	79 Patients with MDD undergoing treatment with SSRIs	Probiotic bacteria *Lactobacillus Plantarum 299v (LP299v)*	Randomized, double-blind, placebo-controlled trial	8 weeks	HAM-D 17, SCL-90, PSS	Probiotic *Lactobacillus Plantarum 299v* in combination with SSRI treatment improved cognitive performance in depressed patients and decreased KYN concentration

Schaub et al.	Switzerland/2020	60 MDD patients	Multi-strain probiotic supplement Vivomixx® (*Streptococcus thermophilus* NCIMB 30438, *Bifidobacterium breve* NCIMB 30441, *Bifidobacterium longum* NCIMB 30435, *Bifidobacterium infantis* NCIMB 30436, *Lactobacillus paracasei* NCIMB 30439, *Lactobacillus delbrueckii sibsp. Bulgaricus* NCIMB 30440) and treatment as usual	Double-blind RCT high-dose, short-term probiotic add-on therapy	4 weeks	HAM-D	Short-term, high-dose add-on probiotic treatment proved to relieve symptoms of depression, but not anxiety in patients with IBS, showing potential of probiotics as add-on treatment for MDD. High-dose probiotic mixture also modulated gut microbiota and decreased activation in Putamen during stress task
Tarutani et. al.	Japan/2022	20 outpatients with depressive episodes	Prebiotic 4G-beta-D-Galactosylsucrose (Lactosucrose, LS) with prescribed antidepressant	Single cite, double-blind, randomized controlled trial	24 weeks	MADRS, QHO/QOL-26, GSES	Prebiotic group showed trend towards improvement in self-efficacy and moderation of suicidal ideation measured by GSES; No significant improvement in total MADRS scores as compared to placebo,
Tian et. al.	China/2021	45 MDD Patients over the age of 18	Psychobiotic freeze-dried *Bifidobacterium breve* CCFM1025 powder	Double-blind Randomized Controlled trial, placebo controlled	4 weeks	HDRS-24, MADRS, BPRS, GSRS	CCFM1025 significantly reduces the patients serotonin turnover rate in circulation; reduced psychiatric cymptoms potentially through change in gut microbiome and tryptophan metabolism
Ullah et. al.	Italy/2022	80 subjects with mild-to-moderate and subthreshold depression	Food supplements based on combination of SAMe and probiotics (*lactobacillus helveticus rossel-52, Bifidobacterium longim-175)*	monocentric, randomised, double-blind, placebo-controlled, cross-over clinical trial	3 months	HAM-D and PHQ-9	Significant decrease of PHQ-9 and HAM-D scores, reduced SD and MDD symptoms compared to placebo, daily supplement of probiotic strains is effective in the improvement of quality of life
STUDY POPULATIONS NOT HAVING CLINICAL DIAGNOSIS							
Chong et al.	Malaysia/2018	111 adults with moderate stress levels based on PSS-10 questionnaire	*Lactobacillus plantarum* DR7	Randomized, double-blind, placebo-controlled trial	8 weeks	PSS-10, DASS-42	*L. planatarum* DR7 in stressed adults resulted in reduced symptoms of stress and anxiety; improved cognitive and memory pathway; reduced plasma cortisol levels and pro-inflammatory cytokines
Frejy et al.	Australia/2023	119 non-clinical adult participants with moderate psychological stress and low prebiotic intake	12 billion colony forming units (CFU) per capsule, containing: *Bifidobacterium bifidum* (Bb-06): 2 billion CFU; *Bifidobacterium animalis* subsp. *lactis* (HN019): 1 billion CFU; *Bifidobacterium longum* (R0175): 1 billion CFU; *Lactobacillus acidophilus* (La-14): 2 billion CFU; *Lactobacillus helveticus* (R0052): 2 billion CFU; *Lactobacillus casei* (Lc-11): 2 billion CFU; *Lactobacillus plantarum* (Lp-115): 1 billion CFU; *Lactobacillus rhamnosus* (HN001): 1 billion CFU	2x2 factorial, randomized, placebo-controlled trial	8 weeks	Primary outcomes: total mood disturbance scores; Secondary outcomes: BAI, BDI, PSS, WHO-5, SF-36	High-prebiotic diet improved symptoms of TMD (total mood disturbance) compared to placebo and improved anxiety, perceived stress and sleep; probiotic results showed preliminary improvement in wellbeing; no benefits in response to synbiotic combination; in conclusion: prebiotic-rich diet may be useful for promoting mental health and reducing self-reported mood disturbance in non-clinical populations
Johnstone et al.	United Kingdom/2021	64 Late Adolescent (18-25) Female Volunteers	Prebiotic: Galacto-oligosaccharides (GOS)	Double-blind, placebo-controlled prebiotic supplement intervention	4 weeks	STAI, SAS-A, MFQ, BDI-II, TCAQ, ERQ-CA, PSQI, Attentional dot-probe task	GOS administration to young adult women showed anxiolytic effects in high anxious participants in self-reports of trait anxiety and emotional wellbeing; reduced negative emotional bias; increased positive bias in dot-probe task

Lew et. al.	China/2018	103 stressed adults (PSS-10 questionaire criteria of moderate stress diagnosis)	Probiotic *Lactobacillus plantarum* P8 (10 log CFU daily)	Randomized, double-blind, placebo-controlled study	12 weeks	PSS-10 questionnaire and DASS-42 questionnaire	Reduced scores of stress and anxiety compared to placebo. Higher reduction in pro-inflammatory cytokines, enhanced memory and cognitive traits
Mutoh et al.	Japan/2023	58 healthy 3rd year nursing students	Heat-killed *Lactobacillus helveticus* MCC1848 powder	Randomized, double-blind, placebo-controlled, parallel-group clinical trial	4 weeks	POMS 2, STAI, SF-36v2, sleep and fatigue	Significantly higher scores for friendliness at week 4 after ingestion of *L. helveticus* compared to placebo; Significant improvement in vigour-activity scores; suggestion of effect on maintaining relaxed state according to VAS score
Patterson et al.	Germany/ 2020	117 participants with low chronic stress (LCS) or high chronic stress (HCS) based on TICS score	*Lacticaseibacillus paracasei* Lpc-37	Randomized, double-blind, placebo-controlled, two-arm and parallel groups clinical trial	5 weeks	Primary objective: heart rate effect after TSST, secondary objective: biomarkers and self report scales	Primary outcome: effect of Lpc-37 dependent on chronic stress, heart rate increase after TSST was lower in patients with LCS but higher in patients with HCS; Lpc-37 decreased acute stress induced blood pressure in females and HCS group, increase in perceived exhaustion in LCS group significantly lower vs control after TSST
Rode et al.	Sweden/2022	22 Healthy subjects	Probiotic product: *Bifidobacterium longum* R0175, *Lactobacillus helveticus* R0052 and *Lactiplantibacillus plantarum* R1012	Randomized, double-blinded, placebo-controlled crossover design	4 weeks	fMRI, EAT paradigm	Altered brain response in brain regions involved in emotional, cognitive and face processing; sig reduction in task-related fynctional connectivity after probiotic intervention vs placebo
Steenbergen et al.	The Netherlands/2015	40 Healthy participants without mood disorder	Multi-Species Probiotic Ecologic® Barrier (2.5 109 CFU/g): B*ifidobacterium bifidum* W23, B*ifidobacterium lactis W* 52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei*	Triple-blind, placebo-controlled, randomized, pre- and post-intervention assessment design	4 weeks	LEIDs-r, BDI-II, BAI	Significant reduction of cognitive reactivity to depression, particularily agressive and ruminative thoughts in probiotics group
Talbott et al.	United States/2019	32 healthy subjects with "moderate" levels of psychological stress	Coordinated pro-/pre-/phyto-biotic supplementation		1 month	POMS for Global Mood State and six sub-scales (depression, tention, fatigue, anger, confusion and vigor)	Significant increase in "good" bacteria (+28% Lactobacillus; +30% Bifidobacterium) after probiotic administration vs placebo, Significant improvement in psychological indicies for positive mood state parameters (Global mood, Vigor) and decrease in negative mood parameters (Fatigue, Depression, Anger, Tension, Confusion) vs placebo
Theodora et al.	Indonesia/2017	90 medical students	*Lactobacillus rhomnosus* and *Lactobacillus helveticus* 2x10^9 CFU	Quasi-experimental, single-blind, pre-posttest with control group design	28 days	BDI-II	Probiotic group had significantly loser BDI-II score after 28 day administration
Tran et al.	USA/2019	86 Healthy college students age 18-31 with no serious psychological disorder	A: high CFU andf high species count probiotic, B: high CFU and low species count probiotic, C: sugar pill	double-blind, randomized, placebo-controlled design	29 days	BAI, ACQ-R, PANAS, NMR, PSWQ	Results suggest significant reduction in panic anxiety and negative affect, high-level CFU may be determining factor in probiotic efficacy
Walden et al.	United States/2023	70 healthy men and women	MSP capsule containing: (1x109CFU) *Limosilactobacillus fermentum* LF16, *Lacticaseibacillus rhamnosus* LR06, *Lactiplantibacillus plantarum* LP01, *and Bifidobacterium longum* 04	Randomized, double-blind, placebo-controlled trial	6 weeks followed by 3-week washout period	BDI-II, STAI, LEIDS-R	Probiotic supplementation significantly improved outcomes related to depression, anxiety and mood including lower scores of hopeless, aggression and rumination; significant reductions in state anxiety compared to baseline; significant increase in plasma serotonin concentration levels
Wang et al.	United States/2019	40 healthy volunteers	*Bifidobacterium longum* 1714™	Randomized, double-blind, placebo-controlled trial	4 weeks	Brain activity was measured using magnetocephalography and 36-item short-form health survey	*B. longum 1714* altered brain activity during social stress tasl, influenced resting neural activity that may be involved in counter-regulation of negative emotions *mitigated stress and anxiety during exposure to controlled stressor*
Zhu et al.	China/2023	60 Test anxious college students	Psychobiotic: *Lactobacillus plantarum* JYLP-326	Randomized control design	3 weeks	HDRS-17, HAMA-14, AIS-8	Probiotic supplementation twice a day for 3 weeks sig relieved anxiety/depression in college students, partial restoration of disordered fecal metabolome

List of Abbreviations

ACQ-R: Anxiety Control Questionnaire-revised

BAI: Beck Anxiety Inventory

BDI: Beck Depression Inventory

CCL: Coping Checklist

CGI: Clinical Global Impression Scale

DASS-42: Depression, Anxiety, and Stress Scale

EAT: Emotional Attention Task

ERQ-CA: Emotion Regulation Questionnaire for Children and Adolescents

ETB: Emotional Test Battery

GAD-7: General Anxiety Disorder 7-item

GAF: Global Assessment of Functioning

GSES: Global Self Efficacy Score

HADS: Hospital Anxiety and Depression Scale

HAMA: Hamilton Anxiety Rating Scale

HAMD-17: Hamilton Depression Rating Scale

HSCL-90: Hopkins Symptom Checklist-90

IDS: Inventory of Depressive Symptamology

LEIDS-R: Leiden Index of Depression Sensitivity

MADRS: Montgomery-Asberg Depression Rating Scale

MFQ: Mood and Feelings Questionnaire: Short Version

NMR: Negative Mood Regulation

PANAS: Positive and Negative Affect Schedule

PHQ-9: Patient Health Questionnaire-9

POMS: Profile of Mood States Survey

PSQI: Pittsburgh Sleep Quality Index

PSS: Perceived Stress Scale 10-Item Questionnaire

PSWQ: Penn State Worry Questionnaire

QIDS-SR16: Quick Inventory of Depressive Symptoms

QOL: Quality of Life

SAS-A: State-Trait Anxiety Inventory

SF-36: Short Form Survey-36 on wellbeing and fatigue

STAI, State-Trait Anxiety Inventory

TCAQ: Thought Control Ability Questionnaire

TICS, Screening Subscale for Chronic Stress

QHO/QOL-26, World Health Organization QOL

WHO-5, Wellbeing Index