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Psychobiotics: Are they the future intervention for managing depression and anxiety? A literature review

Kim Ross [1]

Sonoran University of Health Sciences, 2140 E. Broadway Rd. Tempe, AZ 85282, United States

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ABSTRACT

Mental health is a public health concern among professional organizations, clinicians, and consumers alike, especially in light of the COVID-19 pandemic. Indeed, the World Health Organization has identified mental health as an epidemic of the 21st century contributing to the global health burden, which highlights the urgency to develop economical, accessible, minimally invasive interventions to effectively manage depression, anxiety, and stress. Nutritional approaches, including the use of probiotics and psychobiotics to manage depression and anxiety, have elicited interest in recent years. This review aimed to summarize evidence from studies including animal models, cell cultures, and human subjects. Overall, the current evidence suggests that 1) Specific strains of probiotics can reduce depressive symptoms and anxiety; 2) Symptoms may be reduced through one or more possible mechanisms of action, including impact on the synthesis of neurotransmitters such as serotonin and GABA, modulation of inflammatory cytokines, or enhancing stress responses through effects on stress hormones and the HPA axis; and 3) While psychobiotics may offer therapeutic benefits to manage depression and anxiety, further research, particularly human studies, is needed to better characterize their mode of action and understand optimal dosing in the context of nutritional interventions.

Introduction

The Center for Disease Control (CDC) indicates that as of 2020, 11.3% of Americans suffer from anxiety and 4.5% from depression. Of those suffering, 8.8% are on disability, and another 11.9% have missed six or more workdays due to their symptoms [1]. In 2018, 10.6% of all doctor visits had depression noted on the medical record [1]. The World Health Organization (WHO) has reported that more than 280 million people worldwide are affected by depression, making it a leading cause of disability [2]. Additionally, the WHO has identified mental wellness issues "as the leading cause of 'global disease burden' and as the 'health epidemic of the 21st century'…" [3], highlighting the urgency to develop interventions to effectively manage depression, anxiety, and stress. Mental health is further highlighted as a concern resulting from the COVID-19 pandemic. It is reported that depressive symptoms in the U.S. were more than 3-fold higher during the COVID-19 pandemic than before (8.5% increased to 27.8%) [4]. Social isolation, overburdened healthcare systems, short-term and long-term symptoms, death, media panic, boredom, food insecurity, limited outdoor exposure, financial losses, and changes to dietary patterns, including consuming more comfort foods or fast foods, are some of the reported stresses impacting the mental health of many [5,6].

More than 2000 years ago, Hippocrates stated, "all disease begins in the gut" [7]. Since this time, the recognition of the Gut-Brain Axis has shifted the thinking of how the microbiome has a synergistic effect on the human body, including the bidirectional signaling that occurs (brain-gut and gut-brain) [8–11]. The connection of altered microbiota is not only associated with digestive disorders but also mental health. For example, irritable bowel syndrome (IBS) affects 10–20% of the U.S. population, and 70–90% of those individuals report having mood and

Abbreviations: 5-HIAA, 5- Hydroxyindoleacetic acid; 5-HT, Serotonin; ACTH, adrenocorticotropic hormone; BAI, Beck's Anxiety Inventory; BDI, Beck's Depression Inventory; BDNF, Brain-derived neurotrophic factor; FOS, fructooligosaccharides; GABA, gamma-aminobutyric acid; GAD-7, General Anxiety Disorder-7 (questionnaire); GOS, galactooligiosacchardies; HADS, Hospital Anxiety and Depression Scale; HSCL, Hopkins Symptoms Checklist-90; HPA, Hypothalamus-Pituitary-Adrenal; LEIDS-r, Leiden Index of Depression Sensitivity-revised; MADRS, Montogemery-Asberg Depression Rating Scale; MDD, Major Depressive Disorder; POMS, Profile of Mood States; PSS, Perceived Stress Scale; PSQI, Pittsburgh Sleep Quality Index; STAI, State-Trait Anxiety Inventory; SSRI, Selective Serotonin Reuptake Inhibitor.

E-mail address: k.ross@sonoran.edu.
[1] 315-794-4049.

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anxiety disorders [12]. It has been reported that 84.1% of individuals with GI disease have anxiety, and 27% have depression [13]. Additionally, many gastrointestinal symptoms have been established as comorbidities in several neurological conditions, including, but not limited to, Parkinson's disease, autism spectrum disorder, and schizophrenia [14,15]. The "leaky gut" hypothesis has been expanded to encompass "leaky brain," meaning that increased intestinal permeability seems to impact brain function and moods [10].

The relationship between the gastrointestinal tract and the brain is bidirectional, with the gut microbes communicating to the brain via the central nervous system [16]. There are several proposed mechanisms through which the gut microbiota communicates with the central nervous system, contributing to the development or inhibition of chronic diseases and human behavior. This includes immunological (gut-immune system), biochemical, and neuro-endocrine (HPA axis) mechanisms, as well as gut microbiota metabolism system, intestinal mucosal barrier, and the blood-brain barrier, with the vagus nerve being identified as a key route of communication [15,17–22].

Despite early discoveries of mood improvement with *Lactobacillus,* [23] research has been lacking until recently to understand the intimate relationship between the gut microbes and their impact on brain function, mental health, and overall mood. As of February 2017, it was reported that only 142 articles on microbes and mental health were listed on PubMed, with only 23 relating to mental illness and 35 relating to psychobiotics [24]. This topic continues to be an emerging area of interest, with nearly 140 articles on psychobiotics and mental health as it relates to depression and anxiety alone. This literature review aims to summarize evidence on the relationship between psychobiotics and mental health outcomes, namely depression and anxiety. The potential therapeutic role of psychobiotics in these conditions, through their contribution to the production of the neurotransmitters serotonin and GABA and the reduction of cortisol via the hypothalamus-pituitary-adrenal (HPA) axis, will be discussed.

Methods

A review was conducted by searching PubMed. Search terms included "Psychobiotics and mental health", "Psychobiotics and anxiety", "Psychobiotics and depression", "Psychobiotics and serotonin", and "Psychobiotics and GABA". Studies were considered eligible if they met the following inclusion criteria: 1) published in the last ten years (2013–2022), 2) included animal or human subjects, 3) used validated questionnaires to measure depression, anxiety and/or stress, 4) measured one or more neurotransmitters including GABA and serotonin 5) measured cortisol. This resulted in 243 articles. Articles were excluded if they were 1) A duplicate article, 2) Mini-review, systematic review, or meta-analysis, 3) Non-specific, including biological mechanism of action of psychobiotics, an overview of microbiota, an overview of the gut-brain axis, 4) For a health condition other than depression or anxiety 5) Were a commentary or opinion. The literature search resulted in 48 articles reviewed and included in this review.

Introduction

Depression and anxiety are complex and multi-factorial. Diet, stress, medication, genetics, and the microbiome play a role in the presentation or absence of symptoms [25,26]. It is reported that certain bacteria have an influence on the production of neurotransmitters, including *Lactobacillus* and *Bifidobacterium* on the production of GABA [11,15,17,23,27,28]; *Lactobacillus* and *Bacillus* on the production of acetylcholine [27–29]; *Bacillus, Escherichia*, and *Saccharomyces* on the production of norepinephrine [11,17,23,27,28]; *Escherichia, Lactobacillus, Bacillus, Lactococcus,* and *Serratia* on the production of dopamine [11,17,23,27–29]; and *Streptococcus, Achromobacter xylosoxidans, Lactococcus, Lactobacillus, Escherichia, Enterococcus* and *Candida* on the production of serotonin [17,23,27–30]; and *Bifidobacterium* on the production of tryptophan,[29] the precursor for serotonin. Further, in individuals with depression, multiple alterations in the gut microbiota have been reported [22].

GABA is a primary inhibitory neurotransmitter that helps regulate sleep, memory, locomotor activity, muscle relaxation, and acts as an anti-inflammatory [8,15,31–34]. Dysfunction in GABA has been associated with anxiety, depression, autism, panic attacks, epilepsy, Parkinson's disease, Amyotrophic lateral sclerosis (ALS), Huntington's disease, headaches, cognitive impairment, and Alzheimer's disease, as well as affecting several aspects of health such as reducing blood pressure, suppressing appetite and easing PMS [8,15]. Like other neurotransmitters, the gut microbiota can produce GABA [8,15].

Serotonin (5-HT) is one of the metabolites of the essential amino acid tryptophan [35]. It is an inhibitory neurotransmitter that helps regulate sleep, appetite, memory and learning, temperature, mood or emotional state, behaviors, gut motility, intestinal fluid secretion, and muscle contraction while also acting as an anti-inflammatory resulting in its importance in the immune system [8,15,18,33,36]. Serotonin is synthesized (90–95%) in the GI tract [14,18,22]. As a major signaling molecule, mucosal serotonin levels play a role in leaky gut, IBS, and other physiological functions [37]. Serotonin deficiency can occur when tryptophan is metabolized via the kynurenine pathway rather than the serotonin pathway [38]. Probiotics support tryptophan's metabolism to the serotonin pathway [35,38].

Stress negatively affects serotonin levels, suggesting a connection between the HPA axis and the gut-brain axis [17,39,40]. Under acute and chronic stress, cortisol levels increase, leading to impaired sleep, increased heart rate, increased hunger, and mood changes. Animal models have shown that chronic stress (as measured by cortisol levels) can directly impact the microbiome, decreasing the presence of beneficial bacteria while increasing the harmful bacteria [14,17,39]. Research shows that social and maternal prenatal stress is associated with changes in the microbiome in as little as two hours [14]. When under acute or chronic stress, cortisol levels were decreased when given certain *Lactobacillus* or *Bifidobacterium* strains [3,41,42].

Probiotics, psychobiotics, depression, and anxiety

Probiotics are "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host."[43] The most commonly known probiotics are *Lactobacillus* and *Bifidobacterium.*[44]

In 2013, psychobiotics were defined as "a live microorganism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness" [45]. This definition has been further refined to include non-living (heat-activated) microbes, which have been shown to have a positive impact on markers of stress and immune function [26], as well as prebiotics, including galactooligosaccharides (GOS) and fructooligosaccharides (FOS), that enhance the growth and population of probiotics in the gut, creating a symbiotic effect [20,44,46].

Multiple studies have been carried out in cellular models, animals, and, more recently, humans to explore the potential benefits of psychobiotics relating to depression and anxiety. The potential mechanisms for their therapeutic effects, such as increased production of GABA, serotonin, or other neurotransmitters, regulation of the HPA axis (including a decrease in cortisol production), and decreased inflammatory markers, have also been the target of research interest [19,44]. Tables 1-4 summarize the studies included in this review.

Summary of the research on rodent models

This summary highlights some of the research on select psychobiotics used in animal models, including but not limited to *Bifidobacterium infantis, Lactobacillus rhamnosus, Lactobacillus paracasei*, and *Lactobacillus plantarum.* Overall, the evidence suggests a significant reduction in depressive and anxiety-like behaviors, multiple possible

Table 1
Animal studies and human trials of Lactobacillus strains impact on serotonin, GABA, or other markers and the clinical outcomes.

Microbe	Model	Impact on serotonin, GABA, or other markers	Symptom/Clinical Outcome	Notes	Reference
L. casei Shirota	Human ($N = 132$)	Not measured	Reduced depressive moods, as reported in POMS questionnaires	3 weeks 6×10^9 CFU Probiotic milk drink was consumed	[79]
L. casei Shirota	Human ($N = 39$)	Not measured	Decreased anxiety in those with CFS, as reported with the BDI and BAI.	2 months 24 billion CFU	[80]
L. casei Shirota YIT 9029	Human ($N = 47$)	Reduced cortisol and proposed influence on tryptophan metabolism	Reduced feeling of stress and stress-induced GI symptoms	8 weeks 1×10^{11} CFU of *L. casei* Shirota given as fermented milk product	[81]
L. fermentum NS9	Sprague-Dawley rats	Restored corticosterone levels that were increased by ampicillin administration	Reduced anxiety-like behaviors induced by ampicillin	41 days 1×10^9 CFU/mL Increased lactobacillus in stool by 20%	[67]
L. gasseri CP2305	Human ($N = 29$)	Decrease in cortisol levels (saliva)	Significant reduction in anxiety and depression scores as observed in validated questionnaires	24 weeks 1×10^{10} CFU (2 tablets daily)	[95]
L. helveticus NS8	Sprague-Dawley rats with hyperammonemia	Serotonin was reduced in the cerebellum and hippocampus, but metabolite (5-HIAA) was not affected; lower inflammatory markers (IL-1beta, PGE2)	Decreased anxiety-like behaviors; slowed cognitive decline	2 weeks 10^9 CFU/mL^{-1}	[58]
L. helveticus NS8	Male specific pathogen-free (SPF) Sprague-Dawley rats	Increased serotonin; decreased cortisol and ACTH; modulated some inflammatory markers; increased BDNF	Anxiolytic and antidepressant effects	21 days 10^9 CFU/mL Compared to SSRI with similar outcomes	[57]
L. paracasei PS23	Mouse-corticosterone induced depression	Reversed corticosteroid-induced decrease of serotonin and dopamine levels	Anxiety decreased with live and heat-killed; depression reduced more effectively with heat-killed bacteria	6 weeks 10^8 CFU Compared to fluoxetine, performing better	[39]
L. paracasei PS23	Senescence accelerated mouse prone 8 (SAMP8) mice	Increased serotonin and dopamine; increased BDNF; modulation of some inflammatory markers	Live bacteria decreased anxiety and slowed age-related cognitive decline	12 weeks 5×10^9 CFU/mL SAMP8 mice were used due to their rapid aging after 4 months of age.	[52]
L. paracasei PS23	Maternally separated mouse	Increased serotonin and dopamine	Reduced stress, anxiety, and depressive traits	4 weeks 5×10^9 CFU/mL Live and heat-killed bacteria	[53]
L. plantarum 299v	Human ($N = 42$)	Reduced salivary cortisol	Decrease in stress-related anxiety relating to academic exams	14 days 1×10^{10} CFU	[41]
L. plantarum 299v (Sanprobi IBS®)*	Human ($N = 60$)	Decrease in kynurenine	No significant changes in depressive or anxiety symptoms as reported in multiple validated questionnaires; Significant improvement in cognitive function as reported on two validated questionnaires.	8 weeks 10×10^9 CFU	[92]
L. plantarum PS128	Germ-Free Mouse	Increased dopamine and serotonin in striatum	Reduced anxiety-like behaviors	16 days 1×10^9 CFU No adverse or toxic effects noted	[55]
L. plantarum PS128	Naïve adult mice and early life stress (ELS) mice	Increased serotonin in naïve adult; reduced corticosterone in ELS mice; increased dopamine in both mice	Reduced anxiety in naïve adult mice and reduced depression in ELS mice	28 days 5×10^9 CFU/mL Results from live-bacteria, not heat-killed bacteria	[56]
L. plantarum PS128	Human ($N = 32$)	Reduced cortisol levels	Reduction in anxiety, depression, and stress, as recorded by multiple validated questionnaires	8 weeks 300 mg/10 billion CFU	[94]
L. reuteri 3	Male C57BL/6 mice	Increased expression of genes involved in 5-HT metabolism in colon and prefrontal cortex. Increased blood and colon serotonin levels	Reduction on depressive like symptoms	28 days 10^{10} CFU/mL	[62]
L. rhamnosus JB-1	BALB/c Mouse	Reduced $GABA_A$ mRNA expression in the prefrontal cortex and amygdala and decreased $GABA_{B1b}$ in the amygdala and subareas of the hippocampus.	Reduced stress-induced anxiety and depressive behaviors	28 days 5×10^9 CFU/mL Same results were not found in vagotomized mice	[49]
L. rhamnosus JB-1	BALB/c mouse	Increased glutamate in 2 weeks, remained elevated for 6 weeks, GABA peaked after 4 weeks of treatment and remained elevated for 4 weeks ceasing administration on JB-1	Not assessed	4 weeks 1×10^9 CFU	[50]

(*continued on next page*)

Table 1 (*continued*)

Microbe	Model	Impact on serotonin, GABA, or other markers	Symptom/Clinical Outcome	Notes	Reference
L. rhamnosus JB-1	Human ($N = 29$)	No significant impact on markers measured.	No impact on stress-induced anxiety.	8 weeks 10^9 CFU Demonstrates that animal models may not translate to human application	[84]

Key: CFU = colony forming units; *=Risk of bias due to the use of a commercially available probiotic supplement.

mechanisms of action, including the impact on GABA, serotonin, and tryptophan production, a decrease in inflammatory cytokines, and an improved stress response.

Bifidobacterium infantis 35624 has been shown to decrease early life stress and anxiety in rats separated from their mothers [47]. In this study, researchers compared *B. infantis* 35624 (dose 1×10^{10} CFU of live bacteria per mL of drinking water) to citalopram (a commonly prescribed medication for anxiety and depression). IL-6, an inflammatory cytokine known to contribute to depression, was increased upon maternal separation. After administration of *B. infantis* 35624, it was reported that IL-6 was reduced, and a significant increase ($P < 0.05$) in 5-hydroxyindoleactic acid- 5-HIAA (a serotonergic precursor) in the amygdaloid occurred [47]. Further, another animal study conducted for 14 days, using a dose of 1×10^{10} CFU of the same live bacteria per 100 mL of drinking water, reported a significant increase in tryptophan ($P < 0.005$) and kynurenic acid ($P < 0.05$) as well as a reduction in 5-HIAA and inflammatory cytokines, including IFN-γ, TNF-α and IL-6 ($P < 0.05$) [48]. No direct depression or anxiety symptoms were measured in this study (Table 2).

With the use of *L. rhamnosus* JB-1, mice showed fewer depressive symptoms and anxious behaviors [49,50]. The administration of *L. rhamnosus* JB-1 (1×10^9 CFU per day) in healthy mice over four weeks results in changes of GABA-A and B receptors in the brain, accompanied by reducing anxiety and depressive-like behaviors [50]. While *L. rhamnosus* JB-1 was shown to increase GABA production, GABA concentrations appear to have only remained elevated for four weeks following treatment, whereas the increase of glutamate lasted six weeks following administration [50]. Further, the specific strain, *L. rhamnosus*

Table 2
Animal studies and human trials of Bifidobacterium strains impact on serotonin, GABA, or other markers and the clinical outcomes.

Microbe	Model	Impact on serotonin, GABA, or other markers	Symptom/Clinical Outcome	Notes	Reference
B. breve CCFM1025	C57Bl/6 J Mice	Increased serum 5-HTP and hippocampal 5-HT levels	Significant reduction in depression and anxiety-like behaviors	5 weeks 1×10^9 CFU/mL	[61]
B. breve CCFM1025	Human ($N = 45$)	Significant reduction in 5-HT turnover and 5-HIAA; upregulation of tryptophan and 5-HTP in gut microbiota	Significantly reduced depression	28 days 10^{10} CFU	[77]
B. infantis 35624	Sprague-Dawley rats	Affected serotonin, but not to statistical significance; no change in corticosterone or tryptophan	Reduced depression caused by maternal separation	45 days 1×10^{10} CFU/100mL Compared to citalopram	[47]
B. infantis 35624	Sprague-Dawley rats	Increased circulating tryptophan and kynurenic acid (plasma), lowered 5 HIAA in the frontal cortex; decrease in pro-inflammatory markers	It did not elicit antidepressant effects (the aim of the study)	14 days 1×10^{10} CFU/100mL	[48]
B. longum NCC3001	AKR mice	Not measured	Reduced inflammation-induced anxiety behavior	31 days 10^{10} mL^{-1} Reduction in anxiety behaviors requires vagal integrity.	[64]
B. longum NCC3001	Human ($N = 44$)	No changes in serotonin, inflammatory markers, or BDNF were identified	64% of participants had reduced depression, no impact on anxiety; reduction in depression was observed 4 weeks post-treatment.	6 weeks 1.0E + 10 CFU/1 gram powder Participants presented with mild-moderate depression and anxiety and IBS	[90]
B. longum 1714	Human ($N = 22$)	Decrease in cortisol	Reduced stress-induced anxiety and improved memory, as reported in the Cohen Perceived Stress Scale	4 weeks 1×10^9 CFU Subtle improvements in memory were also noted	[42]
B. longum 1714, *B. breve* 1205	BALB/c Mice	Proposed impact on serotonin, GABA, acetylcholine, and/or glutamate	B longum 1714: Improved learning and memory and decreased anxiety *B. breve* 1205: Lowered stress response, decreased anxiety-like behaviors; less impact than *B. longum* 1714	11 weeks 1×10^9 CFU mL^{-1} BALB/c chosen for innate anxiety	[65]
B. longum 1714, *B. breve* 1205	BALB/c Mice	Proposed impact on serotonin and/or GABA	Both strains reduced stress, anxiety, and compulsive behaviors; no impact on depression; *B. breve* 1205 also induced weight loss/improved metabolism	6 weeks 1×10^9 CFU/mL Compared to citalopram and placebo groups	[66]
B. pseudocatenulatum CECT 7765	Wild type C57BL-6 Mice	Restored serotonin levels in hippocampus; reduced dopamine; reduced adrenaline	Reduced depression related to obesity	13 weeks 1×10^9 CFU High-fat diet-induced obesity	[63]

Key: CFU = colony forming units.

JB-1 (5×10^9 CFU/mL), significantly reduced $GABA_A$ mRNA expression in the prefrontal cortex ($P < 0.001$) and the amygdala ($P < 0.05$), reducing fear and anxiety responses, and reduced $GABA_{B1b}$ in the amygdala ($P < 0.01$) and subareas of the hippocampus ($P < 0.001$), assisting in the modulation of behavior [49]. However, the changes in anxiety and depression were not found in vagotomized mice, stressing the importance of the gut-brain-vagal nerve connection [49]. Despite this positive finding, another mouse study found that the treatment of *L. rhamnosus* JB-1 could have a detrimental effect (enhanced fear) when given as an early intervention (within 48 h of the event) for Post-Traumatic Stress Disorder (PTSD), suggesting that the relationship between the time of the stress and exposure to the bacterium may influence the effectiveness of its possible therapeutic use. It is noteworthy that the same study found similar detrimental results when the mouse model was treated with sertraline (a commonly prescribed SSRI) in the early onset of PTSD [51].

L. paracasei PS23 (LPPS23) normalized or increased serotonin and dopamine levels in the hippocampus contributing to a decrease in anxiety, depression, and age-related cognitive decline [39,52,53]. An animal study by Wei et al. showed both live and heat-killed *L. paracasei* PS23 had antidepressant activity on mice treated with corticosterone, which induced anxiety and depression. This study supports the use of specific probiotics for mood regulation, and their impact on the microbiome may be similar with live or heat-killed strains [39]. The authors reported that depression was improved equivalently with heat-killed LPPS23 compared to fluoxetine (a prescription drug used for managing depression), suggesting the potential clinical use. Additionally, LPPS23 has shown a positive impact on reducing anxiety-like behaviors and improving age-related cognitive decline in animal models [52]. Huang et al. reported that the live LPPS23 bacteria decreased anxiety-like behaviors and slowed age-related cognitive and memory decline. It is thought that the improvements in serotonin, dopamine, and brain-derived neurotrophic factor (BDNF) levels that occurred after the administration of the probiotics may be the reason for this improvement [52]. Further, live and heat-killed *L. paracasei* PS23 improved anxiety and depression caused by maternal separation in mouse models. The authors suggest the modulation of the stress response along the dopaminergic pathways of the brain is the potential reason for these outcomes [53].

Lactobacillus plantarum 286 (Lp286), derived from cocoa fermentation, had an antidepressant and anti-anxiolytic effect on Swiss male mice when administered for 30 days. Though specific neurotransmitters were not measured, the authors speculated that this may have resulted from changes in GABA production [54] (Table 3).

Live (not heat-killed) *L. plantarum* PS128 reduced anxiety-like behaviors, increased serotonin and dopamine levels in the striatum [55], increased dopamine in the prefrontal cortex in early life stress mice [55,56], increased serotonin in adult mice,[56] and normalized the release of corticosterone [56].

L. helveticus NS8 administration resulted in multiple effects, including reduced anxiety, slowing cognitive decline, improving cognitive function, and lowering inflammatory markers. The use also resulted in lower serum corticosterone, adrenocorticotropic hormone (ACTH), and the restoration of hippocampal serotonin and norepinephrine [57,58]. For depression induced by chronic stress, *L. plantarum* NS8 was compared against citalopram, a Selective Serotonin Reuptake Inhibitor (SSRI), resulting in decreased depression and anxiety, decreased stress hormones, increased serotonin and decreased cortisol, and decreased inflammatory markers [57].

In addition, researchers have reported several other strains for their benefits to depressive or anxiety-like behaviors in animal models. The research found reduced depressive behaviors following a myocardial infarction after administering *L. helveticus* R0052 and B. *longum* R0175 [59]. This combination also decreased dopamine and norepinephrine after ten weeks of administration in a mouse model [60]. *B. breve* CCFM1025 increased serum 5-HTP and hippocampal 5-HT levels after five weeks, resulting in a significant reduction in depressive and anxiety-like behaviors in chronically stressed mice [61]. *L. reuteri* 3 was administered for 4 weeks to mice that had been exposed to chronic social defeat stress. *L. reuteri* 3 increased the expression of genes that metabolize 5-HT in the colon and prefrontal cortex, as well as increased blood and colon serotonin levels, accompanied by a reduction in depressive like behaviors [62]. Adult male mice showed reduced anxiety associated with learning a new task when given *Mycobacterium vaccae.*[44] *B. pseudocatenulatum* CECT7765, through the increased production of serotonin in the intestine and hippocampus, reduced depressive behaviors related to obesity while also reducing hyperleptinemia and decreasing fat mass [63]. *B. longum* NCC3001 was shown to help normalize anxiety-like behavior in those with colitis. However, in comparison, mice with a vagotomy did not experience the same impact, suggesting that the vagus nerve may modulate behavior associated with anxiety [64]. *B. longum* 1714 and *B. breve* 1205 were both shown to decrease anxiety and lower the stress response, with a proposed impact on serotonin and/or GABA [65,66]. (Table 2) One study reported the use of *L. fermentum* NS9 to reduce anxiety-like behaviors, restore serum corticosterone levels and reduce colonic inflammation that resulted from the administration of ampicillin (an antibiotic) [67]. Though neurotransmitters were not measured, a six-strain probiotic formula derived from Thai fermented foods (pickled cabbage, kefir, and Pak-Sian Dong) reduced anxiety and compulsiveness and improved memory and locomotor function in a male rat model, suggesting the importance of the synergy of multi-strain probiotics versus a single strain bacteria [29] (Table 3).

A summary of the research on human studies

Evrensel et al. point out that the successful use of *Lactobacillus* for depression was first published in early 1910 [23]. Since then, several human studies have replicated the animal models, showing a decrease in anxiety, depression, and stress after administration of specific *Lactobacillus* and *Bifidobacterium* strains, as will be discussed.

When considering the microbiome of humans, it is important to recognize that beneficial and potentially harmful bacteria are present. It has been found that microbiota diversity is altered in those with depression and anxiety. This includes an increased abundance of *Bacteroidetes, Alistipes,* and *Proteobacteria* and a decreased amount of *Firmicutes, Lactobacillus, Bifidobacterium, Lachnospiraceae, and Faecalibacterium* [23,46,68–70]. *Alistipes* strains have been found in abundance in fecal microbiota in those with depression and anxiety. *Alistipes'* presence alters tryptophan availability, though this strain can be reduced with diet therapy [69]. On the contrary, a decreased amount of *Faecalibacterium*, which is important for butyrate production, may contribute to bipolar depression [70]. The gut-brain connection is made apparent when exploring some drugs used for depression, which have been shown to have an antibiotic effect, increasing *Firmicutes* while decreasing *Proteobacteria* and *Actinobacteria* [23]. Further, antibiotics can induce colonic inflammation and alter microbial diversity, such as decreasing *Lactobacillus* and *Bacteroides* while increasing *Firmicutes* [29,67]. This is important as the use the antibiotics before the age of one has been associated with depression in adulthood [23].

Research of psychobiotics used in humans includes, but is not limited to: *L. helveticus, B. longum*, and *L. casei* Shirota. Overall, the evidence suggests that single or combination strains of psychobiotics can reduce depression and anxiety in humans, measured by validated questionnaires, in as little as 30-days when consumed as a supplement or food source. There are several possible mechanisms of action, including the impact on neurotransmitter production, specifically GABA and serotonin, favorably influencing the HPA axis function and reducing inflammatory cytokines, though more research is needed to confirm these suggestions.

The combination of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 has decreased anxiety and depression in several

Table 3
Animal studies and human trials of the combination of Lactobacillus and Bifidobacterium strains impact on serotonin, GABA, or other markers and the clinical outcomes.

Microbe	Model	Impact on serotonin, GABA, or other markers	Symptom/Clinical Outcome	Notes	Reference
Bifidobacterium bifidum W23, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, and *Lactococcus lactis* (W19 and W58)- Commercially known as Ecologic®Barrier*	Human (*N* = 20)	Proposed impact on tryptophan and serotonin or leaky gut	Reduced rumination, negative thoughts, and aggressive thoughts associated with sad mood, as measured by the LEIDS-r questionnaire.	28 days One 2-gram sachet (2.5×10^9 CFU) Healthy individuals not diagnosed with a mood disorder.	[86]
Bifidobacterium bifidum W23, *Bifidobacterium lactis* W52, *Lactobacillus. acidophilus* W37, *Lactobacillus. brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, and *Lactococcus lactis* (W19 and W58)- Commercially known as Ecologic®Barrier*	Human (*N* = 34)	No biomarkers were measured	Lower cognitive reactivity towards sad mood, but no statistical difference in depression or anxiety in those who experience mild to moderate depression	8 weeks Two 2-gram sachets (2.5×10^9CFU) Low attrition rate (34%)	[87]
Bifidobacterium bifidum W23, *Bifidobacterium lactis* W52, *Lactobacillus. acidophilus* W37, *Lactobacillus. brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, and *Lactococcus lactis* (W19 and W58)- Commercially known as Ecologic®Barrier*	Human (*N* = 83)	No biomarkers measured	Significant reduction in depression and anxiety; Improved quality of life, as measured by validated questionnaires	2 months One 2-gram sachet (2.5×10^9CFU) Some (<5%) experienced mild symptoms of fullness, diarrhea, or sleep complaints	[88]
B. longum R0175 and *L. helveticus* R0052- Commercially known as Probio'Stick®*	Human (*N* = 25)	Decrease in urinary cortisol output; proposed impact on the vagal nerve, lowering inflammation and increasing overall bifidobacteria in gut	Alleviated psychological distress	30 days Dose unspecified	[71]
B. longum R0175 and *L. helveticus* R0052 Commercially known as Probio'Stick®*	Sprague-Dawley rats	*B. longum* on GABA and *L. helveticus* on serotonin	Decreased depression, increased social interaction post-myocardial infarction	14 days Dose unspecified	[59]
B. longum R0175 and *L. helveticus* R0052 Commercially known as Probio'Stick®*	Flinders Sensitive Line rats	Decreased dopamine and norepinephrine	Decreased depression	10 weeks 1×10^{10} CFU Rats were chosen due to their predisposition to depression	[60]
B. longum R0175 and *L. helveticus* R0052- Commercially known as CEREBIOME®*	Human (*N* = 10)	Not measured	Decrease in depression as measured by MEDRS; decreased anxiety in 4 weeks as measured by GAD-7; improved sleep in 8 weeks as measured by PSQI	8 weeks 3×10^9 CFU Human participants were diagnosed with MDD	[74]
B. longum R1075 and L. helveticus R0052	Human (*N* = 81)	A significant decrease in the kynurenine/tryptophan ratio	Reduction in depressive symptoms as measured by BDI	8 weeks 10×10^9 CFU (in 5-gram sachet) Participants were diagnosed with MDD	[38]
B. longum R0175 and *L. helveticus* R0052 Commercially known as Probio'Stick®*	Wister rats and Human (*N* = 66)	No measure of neurotransmitters	Reduced anxiety-like behaviors in rats; Decrease in anxiety and depression in humans as measured by multiple questionnaires	30 days 3×10^9 CFU for human 1×10^9 CFU for rats	[72]
B. longum R1075 and L. helveticus R0052	Human (*N* = 79)	No impact on inflammatory or other biomarkers (No neurotransmitters were analyzed)	No effect on mood as reported by multiple validated questionnaires	8 weeks 3×10^9 CFU	[76]
L. helveticus R0052, *B. longum* R0175, and *L. rhamnosus* R0011(with prebiotic and phytonutrients	Human (*N* = 32)	Not measured	Significant improvement in mood as measured by POMS	30 days 3×10^9 CFU	[3]
L. plantarum 286, *L. plantarum* 81	Swiss male mouse	Neurotransmitters were not analyzed; it hypothesized that LP286 could block kynurenine metabolism	L. *plantarum* 286 decreased anxiety and depression; L. *plantarum* 81 did not have an impact on moods	30 days 1×10^9 CFU of each PS286 and PS81	[54]
L. reuteri NK 33 *and B. adolescentis* NK98 Known as NVP-1704*	Human (*N* = 156)	Reduction in IL-6	Significant reduction in depression and anxiety, as measured by validated questionnaires	8 weeks 500 mg capsule (2.5×10^9 CFU *L. reuteri* and 0.5×10^9 CFU *B. adolescentis*	[89]
P. pentosaceus WS11, *L. plantarum* SK 321, *L. fermentum brevis* TRBC 3003, *B. adolescentis* TBRC 7154, *L. lactis* TBRC 375	Wistar rats	Not measured	Significant reduction in anxiety	14 days 6×10^9 CFU/day Strains from Thai fermented foods	[29]
S. thermophilus (CNCM strain number I-1630), *L. bulgaricus* (CNCM strain numbers I-1632 and I-1519), *L. lactis* subsp. lactis	Human (*N* = 30)	Not measured	Decreased anxiety in 3 weeks, using Hamilton Anxiety rating score- decreased 5 points with	3 weeks 3 gs (1.5×10^{10} CFU of each strain)	[97]

(*continued on next page*)

Table 3 (*continued*)

Microbe	Model	Impact on serotonin, GABA, or other markers	Symptom/Clinical Outcome	Notes	Reference
(CNCM strain number I-1631); *L. acidophilus; S. thermophiles; L. plantarum; B. lactis* (CNCM I-2494); *L. reuteri* (DSM 17,938)			probiotics and 9.5 points when combined with a balanced diet.		

Key: CFU = colony forming units; * = Risk of bias due to the use of a commercially available probiotic supplement.

randomized control trial studies [3,38,71,72]. *B. longum* impacts GABA production and has been shown to decrease anxiety, partly due to the impact the bacteria has on the myenteric neurons [3,23,72], whereas *L. helveticus* impacts serotonin, decreasing depressive behaviors and anxiety [3,71,72].

Messaoudi et al. conducted a study to examine the anxiolytic effects on rats of two strains, *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175, commercially known as Probio'Stick®, as well as its effects on moderately stressed human volunteers ($N = 55$). In the human arm of the double-blind, placebo-controlled study conducted for 30 days, they utilized validated measurement tools, including the Hopkins Symptoms Checklist-90 (HSCL), Hospital Anxiety and Depression Scale (HADS), Perceived Stress Scale (PSS), and the Coping Checklist. The study found that *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 had several benefits in psychological behaviors in humans, including decreased anger/hostility ($P < 0.01$), depression ($P < 0.05$), anxiety ($P < 0.05$), obsessive compulsive behaviors ($P < 0.05$) and paranoid ideation ($P < 0.05$), as well as anxiolytic effects in rats. The authors proposed that decreasing inflammatory cytokines and harmful gut bacteria may have improved neurotransmitter production or function [72]. Analyzing data from the same group of volunteers, the authors reported that participants who would be considered low-stressed individuals, measured by urinary cortisol levels, found *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 were effective for lowering anxiety and depression [71].

In 2019, Kazemi et al. conducted a randomized controlled trial (RCT) comparing the effectiveness of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 to a prebiotic supplement in individuals who had been diagnosed with major depressive disorder and were taking antidepressant medication for at least three months prior to the study. Using the Beck's Depression Inventory (BDI) as the primary outcome measurement, in 8 weeks, a significant decrease ($P = 0.042$) in BDI scores occurred for those taking the probiotic formula compared to the placebo group. There were no significant findings for the prebiotic group. A secondary outcome measure included a decrease in the kynurenine to tryptophan ratio for the group taking the probiotic formula. This was noted as the possible mechanism for a decrease in depressive symptoms [38] (Table 3).

A *post hoc* analysis of the RCT conducted by Kazemi et al [38]. included 78 of the 110 individuals from the original RCT. Based on recent animal model studies, the author aimed to analyze the levels of BDNF as it correlates to depressive symptoms after the administration of *B. longum* R0175 and *L. helveticus* R0052. Depressive symptoms were measured using BDI validated questionnaire. BDNF levels were significantly increased in the probiotic group as compared to the prebiotic group ($P < 0.001$) and the placebo group ($P = 0.021$). BDI scores were significantly reduced in the probiotic group compared to the placebo ($P = 0.012$) [73]. While neurotransmitter production was not the focus of this study, BDNF does serve as a neurotransmitter modulator and may be a mechanism of action to explore further.

The combination of *L. helveticus* R0052 (90%) and *B. longum* R0175 (10%), commercially known as CEREBIOME®, was used in an eight-week, open-label pilot study in males and females diagnosed with Major Depressive Disorder (MDD). There was a significant improvement in overall mood and anxiety in 4 weeks, as measured by validated questionnaires including the General Anxiety Disorder-7 questionnaire (GAD-7) ($P = 0.031$) and the State-Trait Anxiety Inventory (STAI) ($P = 0.008$) and in sleep in 8 weeks, as measured by the Pittsburgh Sleep Quality Index (PSQI) ($P = 0.005$). It is possible that the improvements, in part, are due to the bacteria's impact on tryptophan and serotonin. The study did not find a complete resolution to depressive symptoms, though they did significantly improve from a moderate to mild rating, as measured by the Montgomery-Asberg Depression Rating Scale (MADRS) ($P < 0.001$) [74] (Table 3).

A randomized, placebo-controlled, double-blind trial explored the use of a combination supplement consisting of probiotics (*L. helveticus* R0052, *B. longum* R0175, *L. rhamnosus* R0011), prebiotics (fibers including galactooligosaccharides, galactomannan, partially hydrolyzed guar gum, and phytobiotics (containing L. Theanine, ginger root extract, and various polyphenols). It was given to 32 adults for 30 days and the Profile of Mood States (POMS) validated questionnaire was used as the assessment tool. The study reported the following results: a 55% reduction in depression, 45% reduction in tension, 64% reduction in fatigue, 43% reduction in confusion, and 54% reduction in anger, with a 44% improvement in vigor and 25% improvement in overall mood vs. placebo in 30 days ($P < 0.05$) [3]. A limitation in this study is that the combination product makes it challenging to know if the synergy of the combination product contributed to the favorable results or if one or more ingredients had a bigger impact. For example, one ingredient in this combination supplement was L-theanine which is a supplement and food source that has been shown to reduce depression and therefore is used by some clinicians to manage symptoms [75].

Contrary to the above studies, Romijn et al. found no significant difference in the probiotic groups compared to the placebo group on low mood after eight weeks when administering the same probiotic strains (*B. longum* R0175 and *L. helveticus* R0052), [76] demonstrating the need for further studies to be completed (Table 3).

Following the success in an animal model using *B. breve* CCFM2015 for depressive symptoms [61], (Table 2) Tian et al. conducted a double-blind, randomized controlled trial of 45 individuals diagnosed with major depressive disorder (MDD). In 28 days, there was a significant reduction in depression as measured by the validated Hamilton Depression Rating Scale-24 (HDRS-24) questionnaire ($P < 0.001$), reduced turnover of 5-HT ($P < 0.01$), and reduced 5-HIAA ($P < 0.05$). Based on the significance of 5-HT turnover, the researchers examined tryptophan metabolism in the gut microbiome. Eight of the 14 metabolites of tryptophan were up-regulated, including tryptophan ($P < 0.030$) and 5-HTP ($P < 0.004$), which, unlike serotonin, can cross the blood-brain barrier [77].

While the measurement of neurotransmitters was not the primary outcome of the subsequent studies on *L. casei* Shirota, the findings may help guide future studies. *L. casei* Shirota has been shown to increase mood in individuals with anxiety or depression [23,26,44,78–80]. (Table 1) *L. casei* Shirota was also beneficial for lowering cortisol levels in individuals experiencing academic stress [81], reducing physical symptoms related to academic stress and improving serotonin biosynthesis [82], while also increasing overall gut microbe diversity [81]. In a randomized, double-blind, placebo-controlled trial, *L. casei* Shirota also significantly reduced depression in 9 weeks, as measured by the Hamilton Depression Rating Scale and Beck Depression Inventory (BDI) ($P < 0.05$). It is thought that the reduction in inflammatory cytokines, specifically IL-6 ($P < 0.05$), may be the reason for this improvement [78].

Another randomized, double-blind, placebo-controlled trial utilized a combination of *L. acidophilus, L. casei, and B. bifidum* (specific strains were not identified) and reported a significant decrease in BDI in individuals diagnosed with major depressive disorder in 8 weeks ($P = 0.001$) [83].

The success of the mouse model with decreased depressive symptoms and anxious behaviors using *L. rhamnosus* JB-1 did not translate into therapeutic benefits in healthy male human volunteers; after an 8-week trial, there was no difference between the treatment group and placebo [84]. The fact that the study included healthy participants without depression or anxiety is an important limitation. Overall, the encouraging findings of animal studies suggest that the use of *L. rhamnosus* JB-1 may represent a potential therapeutic strategy for anxiety and depression, but more findings from adequately designed human studies are needed [85].

Three clinical trials have demonstrated that depression was improved with the mixture of *B. bifidum* W23, *B. lactis* W52, *L. acidophilus* W37, *L. brevis* W63, *L. casei* W56, *L. salivarius* W24, and *L. lactis* W19 and W58, commercially known as Ecologic®Barrier.[86–88] (Table 3) In a triple-blind, placebo-controlled, randomized, pre and post-intervention assessment design, participants rated their mood using the Leiden Index of Depression Sensitivity-revised (LEIDS-r) questionnaire, Beck Depression Inventory (BDI), and Beck Anxiety Inventory (BAI). The significant findings were regarding less aggression ($P < 0.001$) and rumination ($P < 0.001$) when in a sad mood, as reported on the LEIDS-r. The authors note that individuals with ruminating thoughts often have a harder time recovering from a depressive episode. While specific mechanisms were not identified or measured in this study, the authors note that the findings may provide a basis for future studies [86]. Another triple-blind parallel, placebo-controlled, randomized controlled trial was completed using the same combination (Ecologic®Barrier). This study found an improvement in the cognitive reactivity scores (which may suggest one's vulnerability to depression), but depressive symptoms, as measured by validated questionnaires (BAI, BDI, DASS), did not significantly change. Some limitations of this trial include the lack of saliva, blood, or urine measures that could have been considered and an attrition rate of only 34% [87]. The third study, a single-center uncontrolled trial ($N = 83$), using the same combination probiotic, found a significant reduction ($P < 0.001$) in anxiety and depression as reported in the Hospital Anxiety and Depression Scale (HADS) for individuals who also were diagnosed with irritable bowel syndrome (IBS) [88].

The combination of *L. reuteri* NK33 and *B. adolescentis* NK98, known as NVP-1704, was used in an 8 week randomized, double-blind, placebo-controlled trial of 156 participants presenting with symptoms of depression, anxiety, and insomnia. The use of validated questionnaires (BDI-II, BAI, PSQI, and Insomnia Severity Index (ISI)), as well as biomarkers (IL-6, TNF-α, ACTH, and cortisol), were used to measure outcomes. Specific neurotransmitters were not measured in this study. A significant improvement in depression and anxiety was observed at 4 weeks ($P = 0.006$) and 8 weeks ($P = 0.037$). Sleep improved significantly at 8 weeks ($P = 0.068$), though not at 4 weeks ($P = 0.123$). IL-6, a pro-inflammatory cytokine, was the only biomarker of significance ($P = 0.041$), suggesting a decrease in inflammation [89]. These findings were in alignment with previous animal studies that were conducted [62].

B. longum NCC3001 was given for ten weeks to adults ($N = 44$) in a randomized, double-blind, placebo-controlled, single-center pilot study presenting with depression and anxiety along with IBS. At ten weeks, there was a significant reduction in depression ($P = 0.04$), as measured by HAD-D, without a significant impact on anxiety, as measured by the HAD-A and STAI, in individuals with IBS [90]. (Table 2)

Given the potential effect of genetic variation on response to psychobiotics, a growing number of studies are focusing on this topic. One interventional randomized placebo-controlled trial examined carriers of a variant in the *IL1B* gene (rs16944), A allele (34 of the 65 subjects included in the study), which has been linked to an elevated risk of depression and anxiety in select populations. After 12 weeks of using a nine-strain combination probiotic supplement (including strains of *Streptococcus, Bifidobacterium, Lactobacillus,* and *Lactococcus*), those with the A allele experienced significantly less anxiety than the non-carriers of the variant and placebo group ($P = 0.02$) [91].

An 8-week randomized, double-blind placebo-controlled trial was conducted in individuals diagnosed with major depressive disorder who were being treated with an SSRI. The primary outcome measures of this study, a reduction of depressive and anxiety symptoms, did not result in a significant change. However, the group ($N = 30$) receiving *L. plantarum* 299v showed a decrease in kynurenine concentrations and improvement in cognitive function (secondary outcome measures) compared to the placebo group ($N = 30$). Kynurenines are believed to play a role in depression and can have neurotoxic and neurodegenerative effects on the brain [92].

Additionally, some strains of probiotics were administered and studied in relation to HPA function and responses to stress. For example, *B. longum* 1714 reduced salivary cortisol levels ($P = 0.05$) and stress as rated by the Cohen Perceived Stress Scale ($P < 0.01$) [42]. On the contrary, a double-blind, randomized, placebo-controlled, repeated measures, cross-over design found that while *B. longum* 1714 improved sleep quality, measured by PSQI ($P < 0.05$), and did not impact cortisol levels or depression when under stress during academic exams [93]. *L. plantarum* 299v also reduced cortisol levels ($P < 0.05$) when individuals were under examination stress (classified as acute stress) [41]. An open-label, single-arm, baseline-controlled pilot study by Wu et al. utilized *L. plantarum* PS128 for eight weeks in adults classified as high-stressed Information Technology (IT) specialists. There was a statistically significant decrease in self-perceived stress ($P < 0.001$), state and trait anxiety ($P < 0.001$), overall job stress ($P = 0.003$), job burden ($P = 0.037$), insomnia severity ($P < 0.001$), depression ($P < 0.002$), negative emotions ($P < 0.01$) while increasing overall positive emotions and satisfaction with the quality of life ($P < 0.001$). The authors noted that the significant decrease in salivary cortisol ($P < 0.05$) did not correlate to the changes in depression or perceived stress, suggesting that the PS128 mechanism of action may not occur through the HPA axis [94]. *L. gasseri* CP2305 was administered to 29 healthy adults who were preparing for a national exam for medical providers. This resulted in decreased salivary cortisol levels ($P = 0.039$) and significantly reduced anxiety and depression scores as measured by validated questionnaires ($P = 0.014$, $P = 0.041$, $P < 0.001$) after 24 weeks of use [95].

Ghorbani et al. used a blend of probiotics (*L. casei, L. acidophilus, L. bulgaricus, L. rhamnosus, B. breve, B. longum, S. thermophilus*) and prebiotic (FOS) as an adjuvant therapy with a commonly prescribed antidepressant (fluoxetine) in 40 adults in a double-blind, multicenter trial. After four weeks, the groups treated with the blend of probiotics and prebiotics significantly reduced symptoms ($P = 0.024$) as reported on the Hamilton rating scale for depression [96]. Though the specific strains of each probiotic were not specified, this study may provide useful insight for further research.

With an increased understanding of the link between body composition, obesity status, and anxiety, a blend of 8 probiotic strains (Table 3) was administered to a group of individuals in a prospective intervention study. In addition to significant reductions in weight and body composition, the two groups treated with the probiotic blend also realized a significant reduction in anxiety, as measured by the Hamilton Anxiety Rating Scale ($P = 0.01$ and $P = 0.04$) [97].

Summary of the research of microbes' effects on GABA production in cell culture

While lactic acid bacteria are considered the main microbial producers of GABA [98], *Lactobacillus* and *Bifidobacterium* are both known to increase the production of GABA in cell cultures, animal models, and human models. Specific strains of *Lactobacillus* and *Bifidobacterium* have been studied to measure the amount of GABA produced by these bacteria

and its effect on mental health [31,49]. Utilizing the knowledge that GABA is synthesized by glutamate decarboxylase (GAD) from MSG, multiple studies have focused on the amount of GABA that can be produced from MSG.

A cell culture study was completed to assess the conversion of MSG to GABA using 91 strains of *Lactobacillus* and *Bifidobacterium*. Five strains were identified as GABA producers with a conversion rate of 22–100% [31], as shown in Table 4.

Yunes et al. examined 135 human-derived strains of *Lactobacillus* and *Bifidobacterium*, finding that 58 strains were able to produce GABA, including *L. plantarum, L. brevis, B. adolescentis, L. angulatum*, and *B. dentium* with the *Bifidobacterium* species being reported as the "most efficient" resulting in 2500–6000 g/L of GABA. The genome of these bacteria includes *gad*B and *gad*C, which are necessary for synthesizing GABA [98]. (Table 4)

Another cell culture study identified 7 of the 57 food-derived lactic acid bacteria to produce GABA, with *L. lactis* LP-68 producing the highest amount. Interestingly, the authors observed that GABA was not produced when MSG was not present, suggesting the importance of MSG in the production of GABA [34]. (Table 4)

L. brevis is considered one of the most efficient GABA-producing bacteria, with all species of *L. brevis* being reported to produce GABA from food-derived *Lactobacillus* from fermented foods such as yogurt or kefir. [98] *L. brevis* CRL1942, isolated from food (amaranth, quinoa, and sourdough), was effective at producing GABA from MSG [99]. *L. brevis* TCCC13007, extracted from Chinese pickled vegetables, also produced high amounts of GABA [100]. Additionally, *L. brevis* NCL912, extracted from Chinese poacai, also showed a high production of GABA, using L-glutamic acid as the substrate instead of MSG in cell cultures [101]. *L. brevis* FPA3709 from MSG-cooked black soybeans produced the highest amount of GABA, which was then used in an animal model study. *L. brevis* K203, isolated from kimchi, resulted in a 99.7% conversion from glutamate to GABA within 72 h [102]. The use of *L. brevis* FPA3709 resulted in an antidepressant effect in rats similar to fluoxetine, a commonly prescribed antidepressant [103].

Several additional strains of *Lactobacillus* were highly effective at producing GABA from other food sources, including Italian cheeses (*L. brevis, L. lactis*) and Chinese adzuki beans (*L. rhamnosus* GG), and fermented milk products [33]. In cell culture, *L. delbrueckii* subsp. *bulgaricus* produced "0.3uM GABA on milk-containing medium", the equivalent of the amount of GABA produced in human cerebrospinal fluid [33]. Specific strains of *L. reuteri* (DSM 17938 and ATCC PTA 6475) produced significantly higher concentrations of GABA in fermented barley (0.05 mM of GABA) compared to heat treated barley (0.01 mM of GABA) [104]. Lastly, *L. reuteri,* isolated from human breast milk, has been shown to be a beneficial strain to produce GABA-enriched functional foods and psychobiotics [105].

Table 4
Cell culture studies-Conversion of monosodium glutamate (MSG) to GABA.

Microbe	Impact	Strain source	Reference
B. adolescentis DPC6044	22% conversion of MSG to GABA	Infant feces	[31]
B. adolescentis 150	3001–6000 mg/L GABA produced-considered an effective GABA producer	Feces, saliva, or vagina of humans	[98]
B. angulatum GT102	2616–3469 mg/L GABA produced-considered an effective GABA producer	Feces, saliva, or vagina of humans	[98]
B. dentium DPC6333	52.5% conversion of MSG to GABA	Infant feces	[31]
B. dentium NCFB2243	60.9% conversion of MSG to GABA	Dental caries	[31]
B. infantis UCC35624	34.6% conversion of MSG to GABA	Ileal-cecal region	[31]
L. brevis DPC6108	100% conversation of MSG to GABA	Infant feces	[31]
L. brevis CRL 1942	~90% conversion of MSG to GABA	Amaranth, quinoa, and sourdough source	[99]
L. *brevis* TCCC13007	92.2%–98.6% conversion of MSG to GABA	Chinese pickled vegetable source	[100]
L. brevis NCL912	L-glutamic acid to GABA was the most effective medium; up to 205 g/L of GABA produced	Chinese paocai food source	[101]
L. *brevis K203*	44.4 g/L GABA produced/ 99.7% conversion	Kimchi food source	[102]
L. buchneri WP2001	70 g/L of GABA produced	Food derived	[98]
L. lactis LP-68	Four-fold increase in GABA production	Culture of MRS broth. After 96 h of incubation. Can be considered for use as a starter culture for foods or supplements	[34]
L. reuteri DSM 17938 and ATCC PTA 6475	0.05 mM GABA produced	Fermented barley	[105]

Conclusion

The increasing economic and societal burden of depression and anxiety highlights the urgency to effectively develop dietary interventions to manage depression, anxiety, and stress. While psychobiotics for clinical use in managing depression and anxiety in humans remain in their infancy, the findings of these studies are promising. In addition to their use for depression and anxiety, extensive research is also being done to explore their use for other neurological and mental health conditions, including autism spectrum disorder, ADHD, schizophrenia, Alzheimer's disease, Parkinson's disease, and alcohol use disorder, to name a few areas of research interest [15,20,70,106–108].

At present, more research is still needed in humans to understand the mechanism of action psychobiotics have on mental health, specific strains that could produce the most beneficial effects, and how psychobiotics could be used as a clinical therapy for anxiety and depression. For example, Karakula-Juchnowicz et al. have a trial registered to examine the use of *B. longum* R0175 and *L. helveticus* R0052 with a gluten-containing or gluten-free diet called the SANGUT study, to be conducted on 120 individuals with major depressive disorder over 12 weeks. This study will aim to determine how the psychobiotics, diet, or combination of the two will impact mental state, inflammatory markers, and gut permeability markers [109].

Further, the data collected from animal models may not translate to human studies and therefore warrants more human clinical trials to be conducted [24]. Clinicians should continue to stay informed and educated on the doses and strains of psychobiotics that can be used for depression and anxiety, as well as the many other neurological conditions for which this research is emerging, as it is estimated that the probiotic market could reach over $66 billion by 2024 [110]. Consumers and clinicians should be cautious with the plethora of probiotic and prebiotic supplements readily available on the market today not to assume that the findings reported in the literature for one psychobiotics will translate into clinical utility for another strain. In supplementation, the label may not always clearly identify which strains of probiotics are being used, as we also observe that not all studies include the specific strains used. For example, a label may indicate *Bifidobacterium longum* but may not indicate the specific strain of R0175, NCC3001 or 1714, which may or may not be critical in the proper dosing and administration for mental health disorders. On the contrary, others, like the combination of *B. longum* R0175 and *L. helveticus* R0052, which has been extensively researched in animal and human studies, can be found in multiple over-the-counter and professional supplements labeled as such. Sharma et al. report on several commercially available

probiotic/psychobiotics products available to consumers, including strains highlighted in this review [107].

The inclusion of psychobiotics produced in foods or used as a supplement may prove to be an important treatment option for anxiety and depression. The term "Nutritional Psychiatry" was coined to recognize the relationship between mental health and diet, including psychobiotics, omega-3 fatty acids, B vitamins, vitamin D, and a diet rich in nutrients and fiber [111]. Butler et al. provide practical clinical tips for buying probiotics and dietary sources to include, such as fermented foods [110]. Clinically, including probiotic and prebiotic-rich foods as a daily staple of a healthy food plan, may be the ideal recommendation to help inoculate the GI tract with an abundance of beneficial bacteria.

Standard pharmaceutical options for depression and anxiety carry safety concerns and side effects are common. Psychobiotics have a low likelihood of adverse events and have been found to be safe for long-term use [26].

In summary, despite some limitations listed earlier, the existing evidence suggests that personalized psychobiotics as food or supplement may have a therapeutic role in the management of depression and anxiety.

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