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Systematic Review

Role of Probiotic Supplements in Decreasing Anxiety: A Systematic Review

Seyyed Mohammad Jalal Hakimi, Ariyo Movahedi*

Department of Nutrition, Science and Research Branch, Islamic Azad University, Tehran, Iran

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ABSTRACT

Background and Objectives: Anxiety is the feeling of worry or nervousness that may manifest as physical, cognitive and behavioral symptoms. When these symptoms persist and affect performance, anxiety can be regarded as a disorder. Several anxiety disorders have been described, including generalized anxiety disorder (GAD) and panic disorder. Maintaining a healthy diet is critical for physical and mental well-being. Recent studies have shown that the brain can be affected by dietary components and nutrients. Probiotics have become increasingly popular as part of a healthy diet due to their potential to regulate brain health through the microbiota-gut-brain axis. This review was designed to identify effective probiotic species in decreasing anxiety.

Materials and Methods: A systematic search was carried out in Google Scholar, Science Direct, PubMed and Web of Science for studies published until January 2024. The search was carried out with terms of "probiotic supplements" AND anxiety and "probiotic supplements" AND "anxiety disorders". First, duplicated studies were removed and 2024 studies were screened. Then, 23 studies met the selection criteria.

Results: Based on the results obtained from the 23 selected studies, 16 studies showed that supplementation with probiotics improved anxiety symptoms, although the improvement was small in cases**.**

Conclusions: Based on the results, probiotics are sometimes effective in decreasing anxiety and can be an adjunctive treatment in persons with psychiatric disorders (e.g. depression and GAD). However, there is a need of further clinical trials with larger sample sizes. Furthermore, *Lactobacillus plantarum* and *Bifidobacterium longum* are more effective than other probiotics in decreasing anxiety.

Keywords: Probiotics, Probiotic supplements, Anxiety, gut-brain axis

Highlights

- *Lactiplantibacillus plantarum HEAL9* can improve the mood of healthy males and females with moderate stress.
- *Bifidobacterium longum NCC3001* can improve stress and anxiety in healthy males and females with moderate stress.
- The probiotic supplements containing some *Bifidobacterium* species can be used as an adjunctive treatment in patients with GAD.
- Concomitant consumption of probiotic supplements containing a mixture of some *Bifidobacterium* and *Lactobacillus* species along with selective serotonin reuptake inhibitor drugs (SSRIs) can decrease anxiety in adults with major depressive disorder (MDD).

Introduction

Anxiety is a term used to describe the feeling of worry, discomfort or nervousness that can be accompanied by physical, cognitive and behavioral symptoms. It is normal to experience anxiety from time to time and it may help prepare for new or unfamiliar situations. However, if these symptoms persist, become excessive or start to affect performance negatively, it can be recognized as a disorder that needs treatment [1]. Several anxiety disorders have been described. Excessive worry is associated with various domains and physical symptoms associated with generalized anxiety disorder (GAD), which can lead to clinical distress or dysfunction. Panic disorder involves unexpected and

***Address for correspondence:** Ariyo Movahedi, Prof, Department of Nutrition, Science and Research Branch, Islamic Azad University, Tehran, Iran
E-mail address: amm35@mail.aub.edu

frequent panic attacks. In addition, it involves at least one month of constant worry about another panic attack or significant behavioral changes associated to the attack. Social anxiety disorder is characterized by feelings of anxiety and fear of social situations; in which, a person is likely to be negatively evaluated by others. Therapeutic approaches often used in the management of anxiety disorders are psychotherapy and pharmacotherapy [1].

Maintaining a healthy diet is important for physical and mental welfares throughout all stages of life. Recent research has shown that a healthy diet can help preserve brain health by affecting the brain through dietary components and nutrients. Probiotics have become increasingly popular in healthy diets due to their ability to adjust brain health through the microbiota gut-brain axis (GBA) [2]. Probiotics are described as live microorganisms that when administered in adequate quantities, provide health profits to the hosts. Fermented foods such as yogurt and kefir are the major sources of probiotics [3,4]. Probiotic bacteria may help maintain healthy microbial dynamics and homeostasis in the gut by inducing the physiology of the gut and other organs, including the brain. However, most findings are resulted from animal studies and it is difficult to assess whether these results can be generalized to humans. Therefore, it is important to assess clinical characteristics and effects of probiotics on human gut and brain health, especially cognitive and psychological disorders [2].

The Gut-Brain Axis

In recent years, extensive studies have been carried out on the concept of the GBA. These studies show a link between gut health and mental health. The GBA states bilateral communication between the central nervous system (CNS) and the intestinal nervous system that links the emotive and cognitive centers of the brain with peripheral gut functions. Nineteenth-century models of the GBA claimed that indigestion caused complications in feelings. In addition, scientists turned to a reductionist approach by the end of the 19th century. They understood they could not explain clinical phenomena such as stomach problems caused by violent or depressing proceedings. It has been shown that the role of the GBA is in monitoring and integrating gut function and the relationship between the emotional and cognitive centers of the brain and peripheral intestinal functions and mechanisms [3].

Effects of Probiotics on Mental Health

A novel group of probiotics or psychobiotics is used to improve cognitive function in patients with mental disorders. Various species of intestinal microbes produce a variety of neuromolecules that play a role in regulating mood and affect the host's physiology. For example, various strains of *Bifidobacterium* and *Lactobacillus* can produce gamma-aminobutyric acid (GABA), while *Enterococcus*, *Escherichia* and *Streptococcus* spp. can synthesize serotonin and *Bacillus* species can produce dopamine [4]. Several studies have shown that beneficial gut microbes (probiotics) play a significant role in a person's feelings and behavior and may play a role in mental disorder treatment, including attention deficit hyperactivity disorder (ADHD) [5].

This systematic review was designed to report and assess studies carried out on the effectiveness of probiotic supplements in treating anxiety.

Materials and Methods

Search Strategy and Study Selection Criteria

A systematic search was carried out in Google Scholar, Science Direct, PubMed and Web of Science to find relevant studies. The following terms were used in the search in Google Scholar and Science Direct: "probiotic supplements" AND anxiety, "probiotic supplements" AND "anxiety disorders". In PubMed and Web of Science, terms were searched as probiotic supplements AND anxiety, probiotic supplements AND anxiety disorders. The search filter on all databases was to limit the time from January 1, 2018, to January 1, 2024. The authors screened the studies using catchii.org website [6]. Exclusion criteria included animal studies, studies on pregnant and post-menopausal women, studies on people with physical health conditions, book chapters, comments, review articles, study protocols, studies that assessed effects of probiotics in combination with other nutrients and studies with methodological flaws.

Study Quality Assessment

Quality of the selected studies was assessed using critical appraisal checklists (CASP) for eligibility [7].

Data Extraction

Data were extracted from each study as authors and years, study designs, numbers of participants, sample characteristics, durations of intervention, bacterial species and doses (CFU), probiotic product forms, psychological symptoms, measurement scales, biomarkers and outcomes or major findings.

Results

As shown in Figure 1, 3127 studies were initially detected. Before the first screening, 1103 duplicate studies were excluded. Then, 2024 studies were screened based on the title and abstract, the full text of 529 studies was reviewed and 23 studies met the selection criteria. Therefore, this review includes the analysis of 23 studies, whose characteristics are described in Table 1.

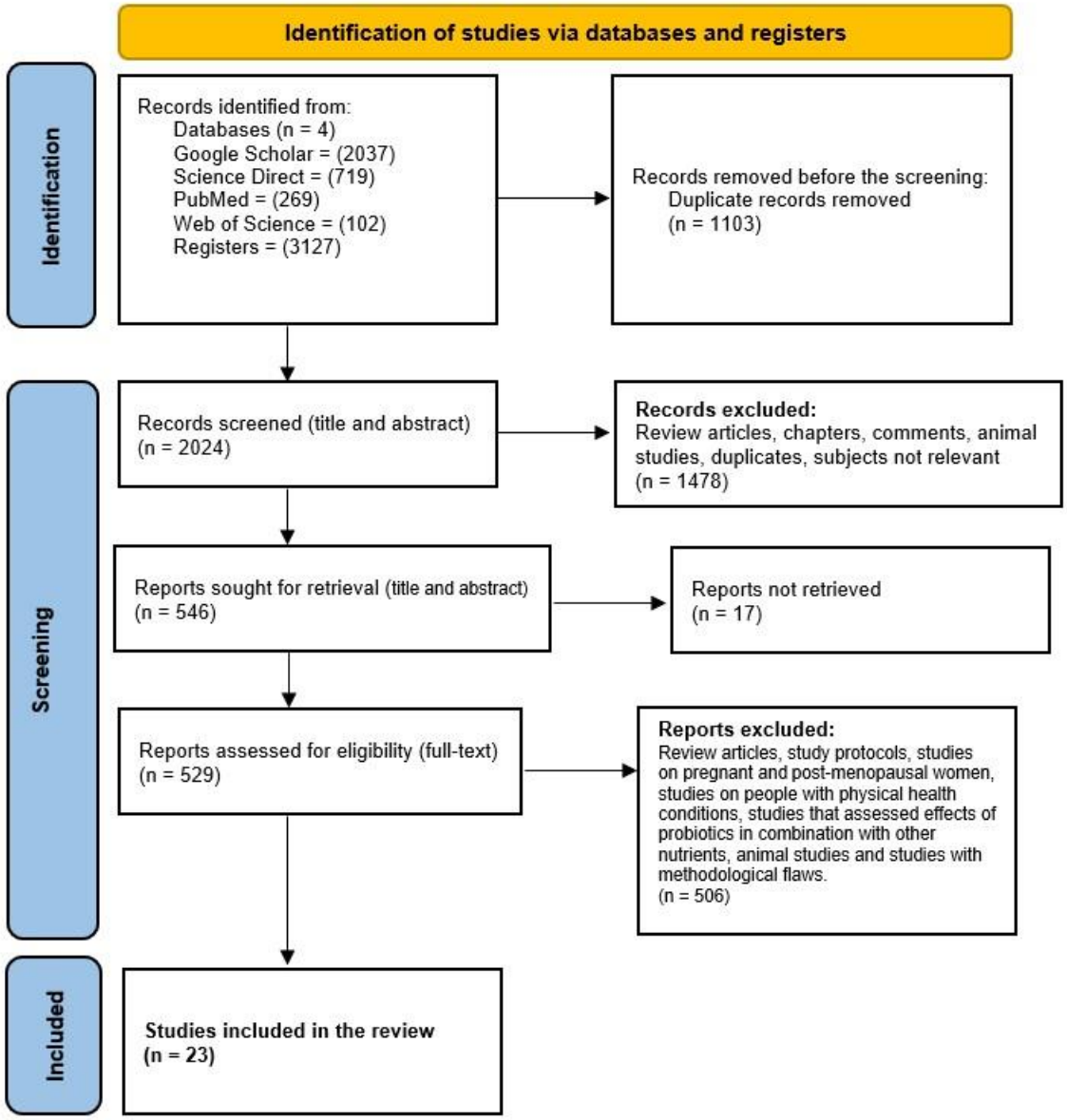

Figure 1. Flowchart of the systematic search and selection process based on the PRISMA 2020 recommendation

Table 1. Characteristics of the included studies

Authors, year	Sample characteristics	Number of participants	Study design	Duration of intervention	Bacteria species and dose (CFU)	Product form used	Psychological symptoms/ scales*/ biomarkers	Results*or major findings
Walden et al, 2023 [8]	Healthy men and women (18-50 years)	70	Randomized, double-blind, placebo-controlled, parallel groups trial	6 weeks	1×10^9 CFU of each of the 4 strains: *Limosilactobacillus fermentum* LF16 (DSM 26956), *Lacticaseibacillus rhamnosus* LR06 (DSM 21981), *Lactiplantibacillus plantarum* LP01 (LMG P-21021) and *Bifidobacterium longum* 04 (DSM 23233)	Capsule	Anxiety/STAI/serotonin, cortisol, dopamine, CRP	STAI was significantly reduced after supplementation. Serotonin increased and there was no change in plasma dopamine, CRP, or cortisol.
Mäkelä et al, 2023 [9]	Healthy students at the university (18-40 years)	190	Randomized, triple-blind, placebo-controlled, two-arm clinical trial (the ChillEx study)	10 weeks	1.56×10^{10} CFU of *Lacticaseibacillus paracasei* Lpc-37	Capsule	Anxiety and stress/STAI, HADS, PSS, DASS-21/CAR	The probiotic Lpc-37 was safe but did not affect stress, mood, or anxiety in healthy university students. There was no change in the CAR levels.

Authors, year	Sample characteristics	Number of participants	Study design	Duration of intervention	Bacteria species and dose (CFU)	Product form used	Psychological symptoms/ scales*/ biomarkers	Results*or major findings
Önning et al, 2023 [10]	Healthy males and females with moderate stress (21-50 years)	132	Randomized, double-blind, placebo-controlled, parallel-designed study	12 weeks	*Lactiplantibacillus plantarum* HEAL9 (LPHEAL9, HEAL9™, DSM 15312) at a dose of 10^{10} CFU (10B CFU)	Capsule	Anxiety and stress/PSS, POMS/CAR, BDNF, Hs-CRP, tryptophan	There was no change in PSS. POMS was significantly improved after supplementation. There was no change in blood biomarkers.
Boehme et al, 2023 [11]	Healthy adults with mild-to-moderate stress (25-65 years)	45	Randomized, placebo-controlled, 2-arm, parallel, double-blind exploratory clinical trial	6 weeks	1×10^{10} CFU of *Bifidobacterium longum* NCC3001	Sachet	Anxiety and stress/PSS-14, STAI-6, HADS-A/salivary cortisol	*B. longum* NCC3001 can improve stress and anxiety, therefore providing a hopeful result for use in people without mood disorders. No significant changes were detected in salivary cortisol levels.
Mutoh et al, 2023 [12]	Healthy nursing students (20-64 years)	58	Randomized, double-blind, placebo-controlled, parallel-group clinical trial	4 weeks	5×10^{9} CFU of heat-killed *Lactobacillus helveticus* MCC1848	Stick	Anxiety/POMS 2, STAI, VAS	Heat-killed *L. helveticus* MCC1848 intake had no significant effects on negative mood state items (e.g. anger and nervousness) measured by the shortened POMS 2, STAI and VAS versions.
Nikolova et al, 2023 [13]	Adults with major depressive disorder (MDD) (18-55 years)	49	Single-center, double-blind, placebo-controlled pilot randomized clinical trial	8 weeks	The probiotic supplement consisted 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)*, encapsulated at 2×10^{9} CFU/capsule.	Capsule	Anxiety/GAD-7, HAM-A	HAM-A improved and there was no change in GAD-7. Adjunctive treatment with a multi-strain probiotic is suitable and supportable for adults with MDD.
Akhgarjand et al, 2022 [14]	Adults with mild and moderate Alzheimer's disease (AD) (50-90 years)	90	Randomized, parallel, double-blind, placebo-controlled clinical trial	12 weeks	*[Lactobacillus rhamnosus* HA-114 (10^{15} CFU) or *Bifidobacterium longum* R0175 (10^{15} CFU) 2 times a day]	Capsule	Anxiety/GAD-7	The GAD-7 scale significantly improved after supplementation. adjuvant treatment with probiotics in these patients is helpful and can increase the drug's efficacy.
Ascone et al, 2022 [15]	Healthy adults (18-40 years)	59	Randomized, double-blind, placebo-controlled trial	4 weeks	450×10^{9} CFU per dose; The probiotic supplement, which was composed of eight bacterial strains, including *L. paracasei* [NCIMB 30439], *L. plantarum* [NCIMB 30437], *L. acidophilus* [NCIMB 30442], *L. helveticus* [NCIMB 30440], *B. lactis* [NCIMB 30435] & [NCIMB 30436], *B. breve* [NCIMB 30441], and *S. thermophilus* [NCIMB 30438].	Sachet	Anxiety and stress/BSI, PSS, Brain scan (MRI)	No significant interaction effects were identified in psychiatric symptoms.

Authors, year	Sample characteristics	Number of participants	Study design	Duration of intervention	Bacteria species and dose (CFU)	Product form used	Psychological symptoms/ scales*/ biomarkers	Results*or major findings
Wauters et al, 2022 [16]	Healthy female or male students (20-30 years)	92	Randomized, double-blind, placebo-controlled trial	4 weeks	*Lactobacillus rhamnosus* CNCM I-3690 (10^{11} CFU/100 g)	Non-commercialized fermented dairy product	Anxiety and stress/ STAI, PSS/salivary cortisol	Subjective (STAI) but not objective (salivary cortisol) stress markers were reduced with *L. rhamnosus* versus placebo, indicating anxiolytic effects.
Rode et al, 2022 [17]	Healthy adults (6 m/16 f; 24.2 ± 3.4 years)	22	A double-blinded, randomized, placebo-controlled crossover study	4 weeks	A probiotic mixture (in total 3×10^9 colony-forming units (CFU) per day at the end of shelf-life) containing *Lactobacillus helveticus* R0052 (CNCM-I-1722; 2×10^9 CFU), *Lactiplantibacillus plantarum* R1012 (CNCM-I-3736; 8×10^8 CFU) and *Bifidobacterium longum* R0175 (CNCM-I-3470; 7×10^7 CFU)	Sachet	Anxiety and stress/ HADS, STAI, PSS, Brain scan (MRI) /CAR, BDNF, Hs-CRP, serotonin	HADS, STAI and PSS decreased with probiotic intervention compared to placebo, but it was insignificant. The probiotic intervention did not significantly affect CAR, BDNF, Hs-CRP and serotonin in serum.
Slykerman et al, 2022 [18]	Healthy undergraduate students	483	A randomized, double-blind, placebo-controlled trial with two parallel arms	8-13 weeks depending on when participants enrolled in the study	*Lacticaseibacillus rhamnosus* HN001 (6×10^9 CFU)	Capsule	Anxiety and stress/STAI6, PSS	No significant differences were found between the groups in the mean alteration of stress, anxiety, or mental well-being.
Baião et al, 2023 [19]	Depressed adults (18-55 years)	71	A randomized double-blind, placebo-controlled study	4 weeks	The probiotic supplement consisted 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)*, encapsulated at 2×10^9 CFU/capsule.	Capsule	Anxiety/STAI/ salivary cortisol, CRP	No significant change in anxiety was observed. Supplementation did not change salivary cortisol or circulating CRP concentrations.
Watanabe et al, 2022 [20]	Healthy adults (aged ≥ 20 years)	66	Randomized, double-blind, placebo-controlled, parallel-group study	4 weeks	*Lactiplantibacillus plantarum* SNK12 (SNK) 0.05 g (100 billion bacteria cells) of SNK + 0.95 g of dextrin (SNK-L) per package and 0.15 g (number of bacteria: 300 billion cells) of SNK + 0.85 g of dextrin (SNK-H) per package	Granule	Anxiety and stress/ POMS2/salivary cortisol	Taking either 50 mg (100 billion cells) or 150 mg (300 billion cells) of SNK can help reduce stress caused by temporary strain from work or study. This includes negative feelings such as anxiety, tension, embarrassment, confusion, anger and hostility. Compared to the placebo group, both the SNK-H and SNK-L groups had lower levels of salivary cortisol, with the SNK-L group showing a significant change.

Authors, year	Sample characteristics	Number of participants	Study design	Duration of intervention	Bacteria species and dose (CFU)	Product form used	Psychological symptoms/ scales*/ biomarkers	Results*or major findings
Lee et al, 2021 [21]	Healthy adults with subclinical symptoms of anxiety or depression (19-65 years)	156	Randomized, double-blind, placebo-controlled parallel study	8 weeks	Probiotic NVP-1704 contained: 2.5×10^9 colony-forming units of microorganisms (2.0×10^9 CFU for *Lactobacillus reuteri* NK33 and 0.5×10^9 CFU for *Bifidobacterium adolescentis* NK98)	Capsule	Anxiety and stress/SRI, BAI/BDNF, TNF-alpha, ACTH, cortisol	Probiotic NVP-1704 may help alleviate subclinical symptoms of depression and anxiety in healthy adults. No significant difference was detected in the change of serum BDNF levels and other biomarkers between the two groups.
Sepehrmanesh et al, 2021 [22]	Children with ADHD (8-12 years)	34	Randomized, double‐blinded, placebo-controlled, clinical trial	8 weeks	8×10^9 CFU/day probiotic sachet with *Lactobacillus reuteri*, *Lactobacillus acidophilus*, *Lactobacillus fermentum* and *Bifidobacterium bifidum* (each 2×10^9)	Sachet	Anxiety/HAM-A/serum Hs‐CRP, plasma TAC	Taking probiotics during an 8-week interval by children with ADHD showed a positive effect on HAM-A. Supplementation also significantly reduced serum Hs-CRP, while plasma TAC was significantly augmented compared to placebo.
Venkataraman et al, 2021 [23]	Healthy students facing examination stress (18-24 years)	80	A randomized, double-blind, placebo-controlled study	4 weeks	Multi-strain probiotic (*Bacillus coagulans* Unique IS2, *Lactobacillus rhamnosus* UBLR58, *Bifidobacterium lactis* UBBLa70, *Lactobacillus plantarum* UBLP40 (each of 2 billion CFU); *Bifidobacterium breve* UBBr01, *Bifidobacterium infantis* UBBI01 (each of 1 billion CFU))	Capsule	Anxiety and stress/PSS, DASS, STAI/serum cortisol level	PSS, DASS and STAI, significantly improved. The serum cortisol levels in the probiotic group were significantly reduced compared to placebo.
Gualtieri et al, 2020 [24]	150 volunteers from the people who came to the University of Rome during their regular medical check-up visits. (18-65 years)	142	Randomized, placebo-controlled clinical trial (double-blind)	12 weeks	*Streptococcus thermophiles* (1.5×10^{10} colony-forming unit (CFU), CNCM strain number I-1630), *Bifidobacterium animalis subsp. Lactis* (1.5×10^{10} colony-forming unit (CFU)), *Bifidobacterium bifidum* (1.5×10^{10} colony-forming unit (CFU)), *Streptococcus thermophiles* (1.5×10^{10} colony-forming unit (CFU)), *Lactobacillus bulgaricus* (1.5×10^{10} colony-forming unit (CFU), CNCM strain numbers I-1632 and I-1519), *Lactococcus lactis subsp. Lactis* (1.5×10^{10} colony-forming unit (CFU), CNCM strain number I-1631), *Lactobacillus acidophilus* (1.5×10^{10} colony-forming unit (CFU)), *Lactobacillus plantarum* (1.5×10^{10} colony-forming unit (CFU)), *Lactobacillus reuteri* (1.5×10^{10} colony-forming unit (CFU), DSM 17938)	Oral suspension	Anxiety/HAM-A	HAM-A significantly reduced . The consumption of probiotics can alleviate anxiety symptoms, particularly in healthy adults who have the minor A allele of rs16944 as a risk factor.
Eskandarzadeh et al, 2021 [25]	Patients with GAD (18-65 years)	48	Double-blinded, randomized, placebo-controlled trial	8 weeks	18×10^9 CFU *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium lactis* and *Lactobacillus acidophilus*	Capsule	Anxiety/HAM-A, STAI, BAI/plasma ACTH, serum cortisol	The score of HAM-A and BAI decreased more in the (probiotics + sertraline group) than the (sertraline alone group) after 8 weeks.

Authors, year	Sample characteristics	Number of participants	Study design	Duration of intervention	Bacteria species and dose (CFU)	Product form used	Psychological symptoms/ scales*/ biomarkers	Results*or major findings
								The plasma ACTH, serum cortisol level and STAI did not significantly change in either group.
Chahwan et al, 2019 [26]	Adults with depressive symptoms (>18 years old)	71	Triple-blind parallel, placebo-controlled, randomized clinical trial	Over 8 weeks	The probiotic supplement (2.5×10^9 CFU/g) constituted of the following nine bacterial strains: *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Bifidobacterium lactis* W52, *L. acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19 and *Lactococcus lactis* W58 (total cell count 1×10^{10} CFU/day)	Sachet	Anxiety/DASS-21, BAI	There was no significant main effect on anxiety (BAI), or the depression, anxiety, stress, scale scores on the DASS-21. There was no significant change among the groups in depression, anxiety, or stress scales at any level of severity.
Nishida et al, 2019 [27]	Healthy young adults (medical students)	60	Randomized, double-blind, placebo-controlled, parallel-group clinical trial	24 weeks	*Lactobacillus gasseri* CP2305 (1×10^{10} bacterial cells per 2 tablets)	Tablet	Anxiety/STAI, HADS/salivary cortisol and chromogranin A (CGA)	During the intervention period, taking CP2305 resulted in a significant decrease in STAI-trait anxiety scores compared to the placebo. The HADS questionnaire revealed that CP2305 intake also reduced anxiety and depressive moods in comparison to the placebo. Additionally, salivary CGA levels were significantly lower in the CP2305 group than in the placebo group, while there was no significant change in salivary cortisol levels among the two groups throughout the intervention period.
Chong et al, 2019 [28]	Moderately stressed adults (18-60 years)	111	Double-blinded, randomized, placebo-controlled design study	12 weeks	*Lactobacillus plantarum* DR7 (1×10^9 CFU /day)	Sachet	Anxiety and stress/DASS-42, PSS-10/plasma cortisol, DBH, TDO, TPH2, 5-HT6	PSS-10 and DASS-42 improved. DR7 significantly reduced plasma cortisol levels, DBH, TDO and increased TPH2 and 5-HT6.
Marotta et al, 2019 [29]	38 healthy volunteers (18 – 35 years)	38	A double-blinded, randomized, placebo-controlled study	6 weeks	4×10^9 colony forming unit/active fluorescent unit (CFU/AFU) 2.5 g freeze-dried powder of the probiotic mixture containing *Lactobacillus fermentum* LF16 (DSM 26956), *L. rhamnosus* LR06 (DSM 21981), *L. plantarum* LP01 (LMG P-21021) and *Bifidobacterium longum* BL04 (DSM 23233)	Sachet	Anxiety/STAI	No significant effect was found on STAI scores in the experimental and control groups.
Lew et al, 2019 [30]	Stressed adults (18-60 years)	103	A double-blinded, randomized, placebo-controlled study	12 weeks	2×10^{10} CFU of *Lactobacillus plantarum* P8	Sachet	Anxiety and stress/DASS-42, PSS-10/plasma cortisol	Anxiety and stress, improved insignificantly. There was no change in the level of plasma cortisol.

*** Only results and scales associated to anxiety and psychological stress are reported.**

Discussion

As shown in Table 1, all studies were RCT and blinded (double-blind or triple-blind). The participants in the 12 studies [8,9,12,15–18,20,23,24,27,29] were healthy and in 11 studies [10,11,13,14,19,21,22,25,26,28,30] had moderate to severe symptoms of anxiety. All studies used questionnaires as a scale to assess anxiety symptoms. In 15

studies [8–11,16,17,19–23,25,27,28,30], biomarkers were used in addition to questionnaires.

Analyzing Results of the Questionnaires

Based on the questionnaires from 16 studies [8,10,11,13,14,16,17,20–25,27,28,30], the intervention generally improved anxiety symptoms. Although in cases, the improvement was small. In seven studies [8,16,17,20,23,24,27], participants did not have anxiety symptoms, when they were not exposed to stressors. This suggests that probiotics may decrease stress associated to difficult conditions and specific situations such as students' anxiety about exams. In seven studies [9,12,15,18,19,26,29], the intervention did not affect decreasing anxiety and in five studies [9,12,15,18,29], the participants were not anxious under normal conditions. In other words, the intervention carried out in participants who faced difficult conditions (e.g. exam anxiety) did not affect decreasing anxiety.

Analysis of Changes in Biomarkers

Out of 15 studies that used biomarkers as a scale, no changes were observed in biomarkers in nine studies [9–11,16,17,19,21,25,30]. Of the remaining six studies, cortisol levels (saliva or serum) decreased in three studies [20,23,28]. In one study [22], Hs-CRP levels decreased and in one study [8], serotonin levels increased. In one study [22], the plasma TAC level increased and in another study [27], CGA decreased. In one study [28], TDO and DBH decreased and TPH2 and 5-HT6 increased.

Detailed Analysis and Limitations of the Studies

Studies included in this review had limitations. These limitations included non-assessed changes in gut microbiota in fecal samples [8,10,12–15,19,22,23,25,26,29], small sample sizes [15,17,29], short treatment times [12,15–17,19,20,23], non-assessed biomarkers [12–15,18,24,26,29] and non-assessed the menstrual cycles [20,27], which could affect the cortisol level. One of the important limitations that could affect the results of the studies as a confounding factor was the COVID-19 pandemic. Therefore, this factor should be addressed in post-pandemic studies. This factor could increase anxiety of the participants and invalidate the results of the study. However, this factor could decrease anxiety of the participants in studies. Slykerman et al. [18] study (2022) reported that online tests for students could decrease their anxiety levels and might affect the study result. (During the pandemic, the university attended by students participating in the study was closed, resulting in their teaching and assessments being carried out entirely online.) One important key point in all studies was that the participants did not consume pre or probiotic-rich foods during the treatment as this might act as a confounding factor in the study outcome.

Based on the results from the studies of Nikolova et al. (2023) [13] and Baiao et al. (2023) [19], similar probiotic products were used in the two studies, but the results of these two studies varied. This variation might be because the duration of treatment in the study of Nikolova et al. [13] was eight weeks and four weeks in the study of Baiao et al. [19]. In the study of Nikolova et al. [13], probiotics were used as adjuvant treatments and the participants received probiotic supplements in addition to medication. In the study of Baiao et al. [19], the participants did not receive medication. Another reason for the difference in the results of these two studies could be that severity of the disease of the participants in these two studies varied. Situation of the disease of the participants in the study of Nikolova et al. [13] was severe but participants of the study of Baiao et al. [19] had moderate depression. The limitation of the study by Nikolova et al. [13] was that the therapeutic results could not be generalized to other drug categories except SSRIs.

In a study by Chahwan et al. (2019) [26], probiotic supplementation did not affect decreasing participants' anxiety. In this study similar to the study of Baiao et al. [19], the participants did not receive medication to decrease anxiety and this might be why the intervention was not effective. Based on the results of a study by Eskandarzadeh et al. (2021) [25], probiotic supplementation as an adjunctive treatment decreased anxiety of the patients with GAD. In this study, it was shown that receiving probiotic supplements with sertraline could decrease the symptoms of patients. Results of a study by Akhgarjand et al. in 2022 [14] demonstrated that using probiotic supplements as adjunctive treatments could increase the effectiveness of drugs used in patients with AD. In this study, the GAD-7 scale significantly improved after supplementation in the patients, indicating a decrease in their anxiety. In a study by Sephermanesh et al. in 2021 [22], a decrease in the intensity of anxiety in children with ADHD was observed after receiving a probiotic supplement with Ritalin.

Based on the results of the studies by Walden et al. (2023) [8] and Marotta et al. (2019) [29], similar probiotics were used in the two studies; however, results of these studies varied. This variation might be due to the smaller sample size in Marotta et al. study (2019) [29]. In the study of Marotta et al. [29], the reason that probiotics did not affect decreasing anxiety could be clarified by the fact that healthy people already had low anxiety scores, which could not decrease by the probiotics. In a study by Makela et al. in 2023 [9], this was similarly reported.

Time of the treatment is one of the points that should be addressed. Study of Nishida et al. (2019) [27] included the longest treatment time within these 23 studies. It is recommended to increase the time of treatment in future studies, as this factor may have a positive effect on the study results. The major goal of this review was to identify effective probiotic species for decreasing anxiety. Based on the findings, *L. plantarum* (*Lactiplantibacillus plantarum*)

and *B. longum* were more effective than other probiotics for decreasing anxiety. Therefore, it is suggested to carry out further clinical trials on the efficiency of these probiotics to investigate the mechanism of their effects.

Conclusion

Based on the results from this review, probiotics can be effective as adjunctive treatments with drugs (especially SSRIs) in decreasing anxiety in people with psychiatric disorders (e.g. depression and GAD). However, further clinical trials with larger sample sizes are necessary. Further clinical studies on the role of probiotics as adjunctive treatments with a variety of anxiolytic drugs are needed as well. Additionally, probiotics may be effective in decreasing anxiety in people without psychiatric disorders; however, further clinical trials are needed to verify this hypothesis.

Abbreviations

GAD, Generalized anxiety disorder: GBA, gut-brain axis: GABA, gamma-aminobutyric acid: ADHD, attention deficit hyperactivity disorder: AD, Alzheimer's disease: MDD, major depressive disorder: BAI, Beck anxiety inventory: STAI, state-trait anxiety inventory: CRP, C-reactive protein: HADS, hospital anxiety and depression scale: PSS, perceived stress scale: DASS, depression anxiety stress scales: VAS, visual analogue scale: CAR, cortisol awakening response: BSI, brief symptom inventory: SRI, stress response inventory: POMS, profile of mood states: BDNF, brain-derived neurotrophic factor: HAM-A, Hamilton anxiety rating scale: MRI, magnetic resonance imaging: ACTH, adrenocorticotropic hormone: RCT, randomized controlled trial: CFU, colony forming units: DBH, dopamine β-hydroxylase: TDO, tryptophan 2,3-dioxygenase: TPH2, tryptophan hydroxylase-2: 5-HT6, 5-hydroxytryptamine receptor-6: CGA, chromogranin A: TAC, total antioxidant capacity: SSRI, selective serotonin reuptake inhibitor: CNS, central nervous system.

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