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A systematic review of the effects of probiotics on depression and anxiety: an alternative therapy?

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Abstract *This review aims to understand and analyse the effects of probiotics on depression, anxiety and psychological stress. These disorders are among the leading causes of disability worldwide. Conventional pharmacotherapies usually have a poor response or adverse side effects. In this context, recent studies have demonstrated a dense bi-directional communication named gut-brain axis. Evidences are demonstrating the relationship between disturbance in the enteric microbiome and psychiatric disorders, paving the way for the emergence of alternative therapies. A systematic search for randomized double/triple blind placebo-controlled clinical trials was performed in PubMed, Scopus and Lilacs. The studies selection followed the recommendations of the main items for report systematic reviews and meta-analyses (PRISMA). Nine articles met the criteria and were analysed for effects on depression, anxiety, psychological stress and biomarkers. Seven found positive results in at least one of the items. We concluded that the use of probiotics to alleviate depressive symptoms and anxiety is promising, mainly due to its potential anti-inflammatory effect, but additional and more rigorous double blind randomized clinical trials are necessary to endorse such conclusions.*

Key words *Depression, Anxiety, Probiotics, Microbiota, Gut-brain axis*

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Introduction

Depression and anxiety are complex heterogeneous psychiatric disorders[1] and one of the leading causes of disability worldwide. About 4.4% of the world population, 320 million people, are estimated to suffer from depression, and the anxiety disorder affects more than 260 million[2]. According to a study[2] carried out by the World Health Organization (WHO), Brazil is a world leader in the prevalence of anxiety disorders (9.3% of the population) and ranks fifth in depression rates (about 5.8% of Brazilians, which corresponds to 11.5 million cases).

The prevalence of these mental disorders increases mainly in low- and middle-income countries, even with people experiencing depression and anxiety disorders simultaneously[2,3]. Depression symptoms such as loss of interest in life, hopelessness, altered sleep patterns and appetite, can be long-lasting or recurrent and negatively affect the functions of individuals' daily lives. Excessive/supraphysiological anxiety with intense behavioral changes, generalized concerns, gastrointestinal and respiratory discomfort, and muscle tension[4] also profoundly affect individuals' routines, school or work performance, and quality of life[5]. Both disorders are also associated with increased risk for coronary heart disease, alcohol abuse, and abuse of other harmful substances[6].

In 1991, Smith[7] proposed the macrophage theory of depression, in which he stated that immunological and inflammatory imbalances are the main factors that lead to the onset or maintenance of depression. The author argues that the intestine would play the role of immune activation. According to this theory, the brain-intestine axis has a bidirectional communication between the central nervous system (CNS), the enteric nervous system (ENS), and the endocrine system, connecting the brain's emotional and cognitive centers to the peripheral intestinal functions[8,9]. The advancement of scientific knowledge towards what gathers fields such as psychiatry, nutrition, gastroenterology, and neurology, among others, has robustly established the extensive biochemical communication between the central nervous system and the gastrointestinal tract (GIT)[10].

Human GIT is inhabited by almost 100 trillion microorganisms, collectively known as intestinal microbiota[11]. The interaction of these microorganisms with human metabolism is so deep-rooted, that, according to Gill et al.[11], humans are superorganisms whose metabolism represents an amalgam of microbial and human attributes. Some evidence points to intestinal microbiota's close interaction with the primary neuroendocrine system: the hypothalamic-pituitary-adrenal axis (HPA), which controls various bodily stress-responsive processes. Communication between the intestinal microbiota and the HPA axis is also deeply related to other systems, as mentioned earlier[12]. Thus, just as an appropriate and coordinated physiological response – such as an immune response to stress – is necessary for survival, a dysfunctional response can be harmful to the host, contributing to the development of various CNS disorders[13].

Logan and Katzman[14] were the first to suggest modulation of the intestinal microbiota using probiotics as adjuvants in the treatment of depression. A growing number of clinical and preclinical studies have corroborated this theory after them. Tests on germ-free animals, for example, have shown that bacterial colonization of the intestine is fundamental for the maturation and development of the enteric and central nervous system[8]. The absence of this colonization is associated with changes in the expression of neurotransmitters in both systems and the various motor and sensory dysfunctions of the GIT. The anomalies are restored[8] after the colonization of animals with specific bacterial species.

Likewise, scientific studies have shown that the intestinal microbiota performs essential functions affecting the intestinal wall's integrity, the secretion of cytokines, the suppression of intestinal inflammation, and the restoration of its tight joints' (TJ)[15] structure. Therefore, in line with the macrophage theory and the study by Logan and Katzman[14], enteric microbiome eubiosis can have a beneficial effect on inflammation and adequate communication of the brain-intestine axis. Such findings are in harmony with the observations of Dean and Keshavan[1]. These authors state that, while the exact pathophysiology of depression remains unknown, some inflammatory patterns with HPA axis hyperactivity and reduced neuroplasticity causing neurotransmission disorders[1] are observed. Thus, psychological distress has been shown to increase cytokines production, such as interleukin (IL) -1β -6, and tumor necrosis factor (TNF)-β showing positive feedback between depression and inflammation. In this process, inflammation causes depression and psychological distress, which in turn is proinflammatory[1,16].

While several well-established pharmacological treatments for anxiety and depression are

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observed, many patients experience inadequate response or adverse side effects, such as nausea, agitation, headaches, drowsiness, and sexual dysfunction[5]. In this context, the emergence of alternative therapies such as administering certain probiotics to manipulate the enteric microbiome to improve anxiety and depression psychological symptoms can be an up-and-coming field. Dinan et al.[17] called psychobiotics those living microorganisms that produce mental health benefits when ingested in adequate quantities.

This systematic review aims to elucidate the effects of probiotics on depression, anxiety, and psychological distress in healthy human beings – which can display one or more of these psychological symptoms. To this end, the most recent randomized placebo-controlled clinical trials found in the selected databases will be analyzed.

Methods

Search strategy

A systematic and computerized search was performed, according to the PRISMA[18] guidelines, in the PubMed, Scopus, and LILACS databases. The reference lists of the identified papers were carefully researched for possible additional information. The strategy used consisted of retrieving papers through the use of terms "depressive disorder" OR "depression" OR "anxiety" AND "probiotic" from January 1, 2015, to March 31, 2020, with a filter applied to human trials in English, Spanish, and Portuguese. The search was carried out on April 1, 2020. The results and steps of this process are shown in the flowchart below (Figure 1).

Study selection criteria

The selection included double and triple-blind, randomized, placebo-controlled human studies (mean age over 16 and up to 65 years) that analyzed the effects of probiotic supplement consumption on depression, anxiety, cognition levels, stress, or mood symptoms. There was no restriction on the dosage, strains, or administration modes of the probiotics. Exclusion criteria included animal studies; children (mean age ≤ 16 years) or older adults (mean age > 65 years); pregnant women; unhealthy adults (except those with depression, anxiety, or stress), longitudinal studies, and absence of a scale for measuring anxiety and depression. Book chapters, letters to the editor, and critiques were excluded. Likewise, clinical trials using other substances – pharmacological or not – in conjunction with probiotic strains were removed from the qualitative analysis.

Outcome

The expected primary outcome is clarifying the psychological effects of probiotic administration on depression and anxiety or psychological distress, using at least one psychological scale for symptom assessment. Secondly, in this work, the biochemical processes that may be part of the pathophysiology of depression are examined.

Data extraction and risk of bias

The following data were extracted from each study: authors and year; sample characteristics; the number of individuals; study design; duration of the intervention; strains, number of viable bacteria (expressed in CFU, colony-forming units) and dose; administration mode of probiotics; psychological symptoms and measurement scales; results and main findings. The title and summary of each research result were screened by two reviewers who independently applied the same inclusion and exclusion criteria and had no dissent.

Results

As shown in Figure 1, 119 papers were initially found. The first screening to excluded duplicates, leaving 107 works. Sixteen of these were submitted to full-text analysis, and only nine papers met the selection criteria established in the methods.

Thus, this review includes the analysis of nine studies[19-27], whose characteristics are described in Table 1. Eight of those selected[19-26] address randomized, double-blind, placebo-controlled clinical trials. In this set, one is a cross-sectional study[22], and the other was carried out three times during three consecutive years[26]. Only one study consists of a randomized, triple-blind, placebo-controlled trial[27] before and after the intervention. The studies' intervention time ranged from four to twelve weeks, and the total number of randomized individuals was 698.

Two studies[19,21] evaluated stressed patients, one study[23] evaluated adults with moderate mood swings, and another, depressive adults[24]. The other studies[20,22,25-27] evaluated healthy pa-

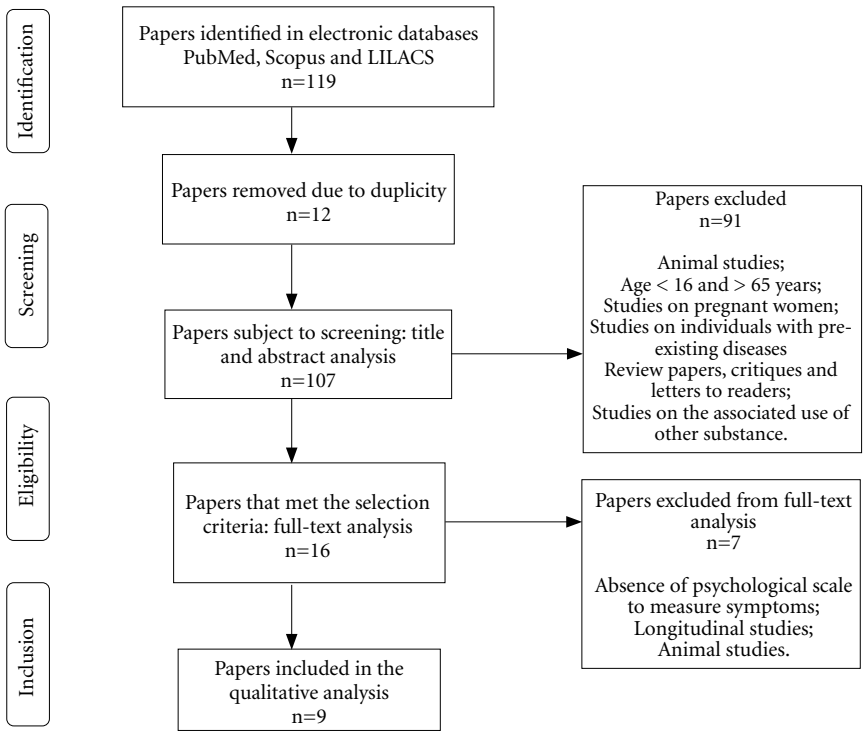

Figure 1. Flowchart of the systematic literature search and selection process according to the PRISMA recommendation.

Source: Author´s elaboration.

tients without psychological or psychiatric disorders symptoms. In one of them[25], the sample consisted of petrochemical workers, and the other, only male individuals[22].

All selected studies used questionnaires and scales to assess depression or anxiety symptoms in patients. All clinical trials consisted of the administration of commensal bacterial species, which may be with the use of only one strain[19,21,22,26] or a mix of probiotic strains. The genera of the microorganisms administered are as follows: *Lactobacilli* (L.), *Bifidobacteria* (B.), *Streptococcus* (S.), or *Lactococcus*. The most used strain was the *Lactobacilli*. Two studies[24,25] do not specify the strains of the bacterial species used. Tran et al.[20] only mention the number of strains administered for each sample group without stating the microorganisms used.

Effects on depression

Six studies[19,21,23-25,27] evaluated the effects of daily probiotic administration on depression, with interventions ranging from four to twelve weeks. Only one of them examined just depression symptoms[24]. All the others also analyzed other psychological changes, such as anxiety and psychological distress. The authors used questionnaires with scientifically validated scales to qualify and quantify changes in psychological symptoms.

Three[24,25,27] of the six studies reported improved depressive symptoms, and the other three[19,21,23] concluded that there was no change in symptoms or change was insignificant. However, among the studies that found no significant changes, Lew et al.[21] concluded that *L. Plantarum P8* has positive effects on other psychological symptoms – which will be addressed later – evaluated by the DASS-42. Similarly, Chong et al.[19] found that, while not showing beneficial effects on depression-related items in the DASS-42, *L. Plantarum DR7* administration results in reduced stress and anxiety symptoms and the improvement of several cognitive functions and memory, reduced levels of plasma cortisol and

Table 1. Characteristics of selected clinical trials.

Authors, year	Sample Characteristics	Number of individuals	Study design	Duration of intervention	Bacteria species and dose (CFU)	Administration mode	Psychological symptom / scale / biomarker	Outcome/main findings
Chong et al., 2019[19]	Moderately stressed adults (18 to 60 years)	111	Double-blind, randomized, placebo-controlled	12 weeks	1x 10^9 UFC of L. *plantarum* DR7	Sachet	Anxiety and depression/ DASS-42 Stress/PSS-10 and DASS-42 Memory and cognition/ CBB Plasma biomarkers	Improved stress levels (DASS-42) and anxiety. No effect on depression. Improved memory and cognition and in biomarkers.
Tran et a.l, 2019[20]	Healthy adults, mean age 20.59 years (18-31 years)	86	Double-blind, randomized, placebo-controlled	4 weeks	A. 50 x 109 UFC of 18 strains; B. 50 x 109 UFC of 10 strains; C. 15 x 109 UFC of 18 strains; E. 10 x 109 UFC of 10 strains.	Tablet	Anxiety / BAI, ACQ-R State of mind / PANAS Negative mood regulation / NMR Preoccupation / PSWQ	Improved anxiety, negative affectivity, mood regulation.
Lew et al., 2018[21]	Stressed adults, mean age of 31.7 years (18-60 years)	103	Double-blind, randomized, placebo-controlled	12 weeks	2x 10^{10} UFC of L. *plantarum* P8	Sachet	Anxiety and depression/ DASS-42 Stress/PSS-10 and DASS-42 Plasma biomarkers	Improved stress levels (DASS-42). Insignificant effect on depression and marginal effect on anxiety. Improved biomarkers.
Kelly et al., 2017[22]	Male healthy adults (20-33 years)	29	Cross-sectional, double-blind, randomized, placebo-controlled	4 weeks	1 x 10^9 UFC of L. *rhamnosus* JB-1	Capsule	Stress / BDI, SCL-90 BAI, SAI, TAI, PSS-10, PSQI, SECPT Cognitive impairment / CANTAB Plasma biomarkers	Unchanged
Romijn et. al 2017[23]	Adults with moderate mood swings (> 16 years)	79	Double-blind, randomized, placebo-controlled	8 weeks	3 x 10^9 UFC of L. *helveticus* R0052 e B. *longun* R0175	Sachet	Depression / QIDS-SR16, DASS-42, MADRS, iCGI Anxiety / DASS-42 Plasma biomarkers	Unchanged
Akkasheh et al., 2016[24]	Adults diagnosed with depression (20-55 years)	40	Double-blind, randomized, placebo-controlled	8 weeks	*2x 109 UFC of each strain: L. acidophilus, L. casei and B. bifidum*	Capsule	Depression / BDI Plasma biomarkers	Improved depression scale (BDI) and plasma biomarkers

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Table 1. Characteristics of selected clinical trials.

Authors, year	Sample Characteristics	Number of individuals	Study design	Duration of intervention	Bacteria species and dose (CFU)	Administration mode	Psychological symptom / scale / biomarker	Outcome/main findings
Mohammadi et al., 2016[25]	Healthy adult petrochemical workers	70	Double-blind, randomized, placebo-controlled	6 weeks	Probiotic yogurt: 1x 107 UFC/ml of L. acidophilus LA5 and B. lactis BB12 Probiotic capsule: L. casei 3 × 103 L. acidophilus 3 × 107, L. Rhamnosus 7 × 109, L. bulgaricus 5 × 108, B. breve 2 × 1010, B. longum 1 × 109, S. thermophilus 3 × 108 UFC/g	Yogurt and capsule	Depression, anxiety and stress / DASS and GHQ Plasma biomarkers	Improved mental health. No effect on the HPA axis.
Takada et al., 2016[26]	Healthy adults (< 30 years)	140	Three double-blind, randomized, placebo-controlled trials, in a parallel group	8 weeks	1x109 UFC of L. casei Shirota YIT 9029	Milk	Stress and anxiety/STAI, HHQ, Saliva biomarker	Improved physical symptoms related to stress and anxiety and improved biomarker.
Steenbergen et al., 2015[27]	Healthy adults, mean age 19.7 years of the placebo group and 20.2 years of the probiotic group	40	Randomized triple-blind placebo-controlled pre and post intervention	4 weeks	2.5 x 109 UFC/g de B. bifidum W23, B. lactis W52, L. acidophilus W37, L. brevis W63, L. casei W56, L. salivarius W24, and Lactococcus lactis W19 e W58.	Sachet	Depression/LEIDS-r, BDI -II Anxiety / BAI	Improved depression and no effect on anxiety.

Captions: ACQR - Anxiety Control Questionnaire Revised; BAI- Beck Anxiety Inventory; BDI - Beck Depression Inventory/BDI - II; CANTAB - Cambridge Neuropsychological Test Automated Battery; CBB -Cogstate Brief Battery; DASS-42 - Depression and Anxiety Stress Scale; GHQ - General Health Questionnaire; HHQ - Health History Questionnaire; iCGI - Improved Clinical Global Impression; LEIDS-r - Leiden Index of Depression Sensitivity-revised; MADRS - Montgomery-Åsberg Depression Scale; NMR - Negative Mood Regulation; PANAS - Positive and Negative Affect Schedule; PSQI - Pittsburgh Sleep Quality Index; PSWQ - Penn State Worry Questionnaire; PSS -10 - Perceived Stress Scale - 10 questions; QIDS-SR16 - Quick Inventory of Depressive Symptomatology- Self Report 16 items; SAI - State Anxiety Inventory; SCL-90 - Symptom Checklist-90; SECPT - Socially Evaluated Cold Pressor Test; STAI - State-Trait Anxiety Inventory; TAI - Test Anxiety Inventory.

Source: Author´s elaboration.

proinflammatory cytokines. In turn, while they did not find any significant difference in any measure of the psychological outcome between the groups that ingested the sachet containing *L. helveticus R0052* and *B. longum R0175* and those with placebo, Romijn et al.[23] concluded that vitamin D baseline level moderated the positive effect of the treatment, which is found in several scales used.

Regarding the positive results for the improved depressive symptoms, Akkasheh[24] and his colleagues observed that depressed patients who received probiotic supplements – containing *L. casei*, *L. acidophilus*, and *B. bifidum* – significantly decreased the total score of the BDI compared to placebo after eight weeks of intervention, indicating a general improvement in symptoms, including mood. Additionally, these subjects showed a significant development in some plasma biomarkers, which will be analyzed later. Mohammadi et al.[25] analyzed the effects of two probiotic interventions: (1) Probiotic yogurt containing *L. acidophilus LA5* and *B. lactis BB12* and (2) probiotic capsule containing *L. casei*, *L. acidophilus*, *L. rhamnosus*, *L. bulgaricus*, *B. breve*, *B. longum*, *S. thermophilus*, in healthy petrochemical workers. A significant improvement in mental health assessed by GHQ and DASS scores was observed after six weeks of treatment, which was not detected in the group that received conventional yogurt. The authors did not break down the scores by psychological symptom. Finally, Steenbergen et al. found that, after four weeks of multi-species probiotic intervention (*B. bifidum W23*, *B. lactis W52*, *L. acidophilus W37*, *L. Brevis W63*, *L. casei W56*, *L. salivarius W2*, and *Lactococcus lactis W19* and *W58*) in healthy individuals, the participants showed a significantly reduced cognitive reactivity to depression – assessed by LEIDS-r, mainly by the reduced rumination and aggressive thoughts.

Effects on anxiety and psychological distress

Eight[19-23,25-27] of the nine studies included in this review assessed stress or anxiety, and five reported improved symptoms after intervention with a probiotic. Among these, while not finding an evolution on the psychological scale, one[26] described positive effects on physical symptoms and one stress biomarker. Among the three who did not observe a positive impact, in their trial with 29 healthy male adults, Kelly et al.[22] found no beneficial effect of using *L. rhamnosus JB-1* on the anxiety, stress, mood, or sleep quality measures, either in the subjective measures of stress or in the response of the HPA axis to the SECPT. In the same vein, Romjin et al.[23] also found no significant difference between the probiotic and placebo groups in any psychological outcome measure after eight weeks. Steenbergen et al.[27] found no significant effects of the intervention on anxiety symptoms but attributed it to the fact that the BAI scale addresses self-reported anxiety, and the selected individuals have minimal or mild scores at the onset of treatment.

In the recent study carried out by Chong et al.[19], the authors concluded that the consumption of DR7 reduced the stress and anxiety symptoms – assessed by the DASS-42 questionnaire – by up to eight weeks in stressed adults compared to the placebo group. These authors divided the individuals studied into two groups (age below and above 30 years) for those who received the probiotic and the placebo. Stress was assessed using the PSS-10 questionnaire and the DASS-42. According to this research, the administration of DR7 has a more significant benefit for young adults (<30 years), with a higher reduction in the total stress scores of DASS-42 after the eighth week compared to the placebo group. The administration of DR7 also reduced anxiety scores in all populations studied – assessed by the same scale. Also, DR7 improved cognitive and memory functions in adults older than 30 years. In other words, according to the authors, although the probiotic has benefited both the young population and older individuals regarding anxiety symptoms, its use favors only the young adult population in assessing stress.

In a recent clinical trial, Tran et al.[20] divided their sample of healthy adults into five groups. Group A received probiotic tablets with a high number of CFU and species of microorganisms; B received a high number of CFU and a low number of species; C was the control group, which ingested placebo; D received a low number of CFU and a high number of species and, finally, E, received a low number of both (see Table 1). The study's findings suggest that probiotics improved panic, neurophysiological anxiety, negative affect, preoccupation, and negative mood regulation. Also, they found that the amount of CFU is more effective than the number of species when counting the number of significant improvements. Finally, they note that the participants who reported greater suffering were also the ones who showed the best development.

Lew et al.[21] observed that stressed individu-

als who consumed *L. Plantarum P8* had reduced scores of stress and anxiety after 12 weeks compared to placebo after the fourth week – assessed by DASS-42. P8 effectiveness in reducing stress, compared to placebo, was predominantly attributed to the reduction of irritability, irritation, increased calm, and tolerance. As for anxiety, P8 proved to be efficient, mainly in reducing shortness of breath, abnormal heartbeats unrelated to physical activities, and decreasing fear of the unknown. Secondly, the intervention had positive effects on women's speed of social and emotional cognition, while men showed no differences compared to placebo.

For three consecutive years, Takada[26] and his team analyzed *L. casei Shirota*'s effects on the psychological and physiological responses in healthy medical students. The authors concluded that the level of psychological stress rose – according to STAI – before taking a national exam. With the exam approaching, both the placebo and probiotic groups had increased scores for stress. However, those who took *L.c. Shirota YIT 9029* significantly reduced the increase in salivary cortisol and experienced a significant decrease in physical symptoms, including abdominal complaints. The *L.c. Shirota*-induced suppression of salivary cortisol secretion may have been associated with the relief of physical symptoms associated with stress.[26]

Finally, as already described in the previous section, Mohammadi et al.[25] assessed the effects of using probiotics for anxiety, stress, and depression according to DASS-42 and GHQ and found a significant improvement in the overall score.

Effects on inflammatory, hormonal, and neurohormonal biomarkers

Besides assessing psychological symptoms, six trials[19,21-25] incorporated the test of plasma biomarkers and one salivary cortisol test[26]. Three[22,23,25] of these seven studies found no impact of the probiotic intervention on the evaluated markers.

Chong et al.[19] analyzed plasma samples for cortisol, IL-1β, -4 and -10, TNF-α, and interferon (IFN) -γ concentrations. The researchers found that the plasma cortisol level was reduced among individuals who received DR7 compared to placebo, with a decrease in proinflammatory cytokines – IFN-γ and TNF-α – and an increase in anti-inflammatory drugs, such as IL-10. When the results were analyzed by age group, they found that DR7 had more prevalent effects in increasing the IL-10 anti-inflammatory cytokines and in decreasing the proinflammatory IFN-γ in young adults, while reducing only the proinflammatory IFN-γ, TNF-α, and IL-1β cytokines in older adults, without any effect on anti-inflammatory cytokines. From the viewpoint of neurohormonal signaling, the administration of DR7 improved the serotonin pathway, through reduced expressions of dopamine β-hydroxylase (DBH), tyrosine hydroxylase (TH), indoleamine 2,3-dioxygenase (IDO) and tryptophan 2, 3-dioxygenase (TDO), and increased values of tryptophan hydroxylase (TPH) -2 and 5-hydroxytryptamine (HT)-6. In parallel, the dopamine pathway was regulated with the stabilization of TH and DBH levels. Therefore, Chong et al.[19] observed that probiotic treatment could have beneficial effects through mechanisms that involve positive regulation of serotonin pathways and stabilization of dopamine pathways along the brain-intestine axis. Finally, the use of DR7 did not affect the expressions of glutamic acid decarboxylase (GAD)-65, gamma-aminobutyric acid α-5 (GABA5), brain-derived neurotrophic factor (BDNF), and cAMP response elements (CRE).

Lew et al.[21] found no significant difference in plasma cortisol levels. Instead, they observed a significant reduction in IFN-γ in subjects who received the probiotic and an increase in the placebo group. TNF-α values increased in both groups. However, those administered with P8 showed a smaller increase than those who ingested placebo, especially in women. Both groups did not produce changes in plasma levels of IL-10, IL-1β, and IL-4 during the study.

Romijn et al.[23] observed that 75% of their sample had at least one inflammation marker analyzed (C-reactive protein-CRP, IL-1β, IL-6, or TNF-α). After treatment, the researchers found no significant differences in the biomarkers' levels between those who consumed the probiotic and the placebo group. However, among those randomized to the probiotic group, individuals with higher vitamin D levels at baseline had more positive effects on mood than those with lower levels, indicating, according to the authors, a relevant role of vitamin D in moderating the effects of the intervention.

Kelly et al.[22] found no changes with the use of *L. rhamnosus JB-1* in the concentrations of IL-10, IL-1β, IL-6, or IL-8. Thus, no significant anti-inflammatory effects were observed, assessed by baseline and stimulated cytokine levels.

Significant decreases were observed in serum insulin levels, HOMA-IR (model for assessing insulin resistance homeostasis), and lower

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CRP concentrations after supplementation with probiotics in the evaluation of plasma samples performed by Akkasheh et al.[24], compared with placebo. Also, probiotic intake resulted in a significant increase in plasma glutathione (GSH) values compared to placebo. However, the authors found no relevant changes in fasting blood glucose levels, HOMA-β (pancreatic β cells), QUICKI (quantitative insulin sensitivity index), lipid profiles, and total antioxidant capacity (CAT) after supplementation.

Mohammadi et al.[25] revealed that supplementation with probiotics in petrochemical workers for six weeks did not affect HPA axis biomarkers such as kynurenine, tryptophan, neuropeptide Y, and cortisol via the adrenocorticotropic hormone (ACTH).

Finally, Takada[26] and his colleagues tested a salivary cortisol sample and concluded that its stress-induced increase was significantly suppressed in the group that consumed milk with probiotics compared to the group that ingested a placebo.

Discussion

This review aimed to understand the effects of probiotics in some mental disorders, especially depression, anxiety, and psychological distress. When analyzed together, the clinical trials' findings that evaluated the effects on depression do not allow a definitive conclusion regarding the effectiveness of one or more probiotic strains. Three studies[19,21,23] with 293 randomized subjects showed that probiotics' consumption did not benefit depression symptoms. Another three carried out with a total of 150 adults, observed beneficial effects. However, Mohammadi et al.[25] did not specify which parameters of the DASS scale significantly affected the treatment with microorganisms, limiting the possibility of knowing the intervention's real scope.

Analyzed together, the psychological indicators of anxiety and stress showed better development than those of depression. Studies that propose more extended intervention strategies or even in an adjuvant way to pharmacological treatments may be necessary due to the more profound and severe psychological depression conditions (including deteriorated mental states, such as dysphoria, hopelessness, life devaluation, and self-depreciation). Despite advances in research on the disease, depression remains a challenging treatment disorder. Among other reasons, this is due to the countless side effects of drugs currently available and because they generally induce remission of major depressive disorder in about one-third of patients only.[28] Therefore, it is essential to identify new therapies.

However, the varying use of probiotic strains, in the dosage, the duration of the intervention, and the sample characteristic itself, shows significant limitations to reach a consensus regarding the beneficial effects for the disorders analyzed. Furthermore, the studies did not preliminarily examine the conditions of eubiosis/enteric dysbiosis of randomized individuals, which can strongly impact the results found.

From the viewpoint of biomarkers, studies[29] have shown that depression is associated with activation of the inflammatory response, with increased positive acute-phase proteins, including CRP, and proinflammatory cytokines such as IL-1, IL-2, IL-6, and IFN-γ. Similar serum marker patterns have been observed in states of anxiety and are known to occur due to stress[17].

In this study, four[19,21,24,26] trials observed changes in biomarkers after intervention with probiotics. Works by Chong et al.[19] and Lew et al.[21] were the only ones examining the impact on the TNF-α, IFN-γ, IL-1β, -4, and -10 indices. Both groups found a significant reduction in IFN-β and TNF-α, although Lew et al.[21] found a relative decrease in the last marker. As for the other cytokines, Chong et al.[19] concluded that DR7 significantly reduced its levels. Lew et al.[21] did not see the effect of P8 on these biomarkers. Akkasheh et al.[24] observed a significant decrease in insulin, HOMA-IR, CRP levels, and an increase in GSH, while Takada et al.[26] observed the suppression of increased salivary cortisol levels.

These results are essential evidence of a positive impact of specific microorganisms on the inflammatory profile of adults. As previously mentioned, evidence[19,21,30] shows that depressed, stressed, or anxious patients have an increased profile of proinflammatory cytokines and positive acute-phase proteins. Also, Dinan et al.[17] observed that studies with rodents indicate that stress alters the intestinal barrier's function, allowing the access lipopolysaccharides (LPS) and other molecules to the bloodstream, stimulating the Toll-Like Receptor 4 (TLR4) and other TLRs, resulting in the production of inflammatory cytokines. Therefore, the intestine relationships, the inflammatory/immunological profile, and the neuroendocrine responses are intrinsic.

The analyzed literature possibly points to two dominant hypotheses addressing probable action

mechanisms by which probiotics affect mental health. These theories involve the regulation of inflammatory markers and the neurotransmission of serotonin. However, as the immune system, the central nervous system, and the enteric nervous system are so intricately connected, we could affirm that the two action mechanisms produce the effects induced by probiotics[5]. In line with this observation, O'Brien et al.[29] affirm that HPA axis function disorders have long been recognized as a significant depression trait, especially in patients with melancholic characteristics. The authors argue that various new antidepressants targeting receptors on that axis are being developed, and it seems appropriate to explore immune sites. Proinflammatory cytokine antagonists such as IL-1 or IL-6 would reduce the HPA unit and, in so doing, could alleviate depressive symptoms. An alternative strategy would be to search for new compounds that increase the production of anti-inflammatory cytokines. As a result of the present review, DR7 showed effects in this direction, although they were not enough to influence the DASS-42 scores.

According to Dinan et al.[17], as a class of probiotics, psychobiotics can produce and distribute neuroactive substances, such as GABA and serotonin, which act on the brain-intestine axis. Much research on psychobiotics is based on rodent models, which use stress inductions and behavioral tests to assess motivation, anxiety, and depression[31]. According to Sakar et al., the psychophysiological effects of psychobiotics fall into the following three categories: (i) psychological effects on emotional and cognitive processes; (ii) systemic effects on the HPA axis, the stress response (glucocorticoid), and inflammation – characterized by enormous concentrations of proinflammatory cytokines and (iii) neural effects on neurotransmitters and proteins such as GABA, glutamate, and BDNF[31]. Accordingly, with these categories, this review found that DR7[19], P8[21], and the mixture of *L. casei*, *L. acidophilus*, and *B. bifidum*[24] acted positively in at least two of these action pathways. The study by Chong et al.[19] showed that DR7 focused on the three pathways proposed by Sakar et al.[31], Lew et al.[21], and Akkasheh et al.[24] evidenced benefits in the inflammatory profile and emotional processes.

As for neural effects, the role of the intestinal microbiota in the regulation of serotonin biosynthesis from intestinal enterochromaffin cells has recently been established in the literature[32]. Approximately 90% of serotonin is known to be derived from these cells and 5% of neurons in the ENS[33], and that the brain's serotonin pathways are involved in the regulation of cognition and mood[34,35]. Therefore, dysfunctionalities in these pathways may contribute to comorbidities of gastrointestinal and mood disorders. Simultaneously, the benefits of using probiotics for intestinal integrity and healthy microbiota[36-38] – fundamental for the serotonin biosynthesis – are known. In this sense, although the studies included in this review do not bring definitive conclusions to the question that gave rise to this study, the fact that seven (of the nine) studies have observed some positive impact from the use of probiotics is encouraging.

However, although the evidence mentioned above and the results of some trials included in this review expose a very promising and thriving ground for advancing research on depression and other disorders such as anxiety and psychological stress, clinical studies to support new therapies using probiotics are critical. Undoubtedly, the great diversity of microbial species that inhabits the gastrointestinal tract and some species' ability to synthesize neuroactive molecules make the digestive tract a place with great pharmacokinetic possibilities and with a potential capacity to impact mental health.

Conclusion

This systematic review revealed limited but promising preliminary research on the effects of probiotics on depression and anxiety symptoms in humans. It also exposed gaps and inconsistencies in the studies published to date. Some limitations observed in the trials analyzed in this study prevented a consensus on probiotics' beneficial effects for each of the disorders analyzed.

More randomized, double-blind, placebo-controlled clinical trials are needed to overcome the main limitations and determine the effective relief of symptoms, and the optimal duration of treatment, dosage, and probiotic strain to achieve positive effects on depression and anxiety. Even so, we can conclude that the evidence presented in this review shows that the use of probiotics affects the inflammatory profile of patients and the regulation of the serotonin's pathway. These findings are consistent with the promising potential use of probiotics in depression and anxiety, given changes in the HPA axis and the serum levels of inflammatory biomarkers in depressed patients. When analyzed togeth-

er, seven of the nine studies have shown some benefit from the administration of probiotics concerning mental health. This conclusion corroborates with a recent meta-analysis[39], which showed the promising use of probiotics in relieving depressive symptoms, emphasized the need for more evidence with larger samples and more rigorous randomized controlled trials.

Collaborations

MS Minayo worked on the conception, methodological design, research, data analysis, writing and final revision of the text. I Miranda worked on the conception and analysis of the data. RS Telhado worked on the final revision of the manuscript.

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