REVIEW ARTICLE   **OPEN ACCESS**

EFFECT OF PSYCHOBIOTICS ON ANXIETY SYMPTOMS: A SYSTEMATIC REVIEW

Gabriela F. Brito[1], Eduardo A. da Silva[1], Pedro W.P. Silva[1], Eskálath Morganna S. Ferreira[1], Sérgio R. Stella[2], Vívian N. Gonçalves*[1], Juliana F.M. da Silva[1] and Raphael S. Pimenta[1]

[1]Laboratório de Microbiologia Geral e Aplicada (LMGA), Universidade Federal do Tocantins (UFT) - Campus Palmas, Palmas - Tocantins, Brasil; [2]Instituto Equilibrium. Palmas - Tocantins, Brasil

ARTICLE INFO

*Corresponding author: Vívian N. Gonçalves

ABSTRACT

Anxiety has become increasingly frequent in the entire world population and from the relationship of this pathology with the gut-brain axis, the idea of the present study emerged, with goal of analyzing the possible relationship between anxiety and intestinal microbiota. This is a systematic review, according to the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) guidelines, supported by articles published between January 2016 and June 2021, to present the advance of studies regarding psychobiotics in human beings. The search strategy used was developed by combining MeSH terms (Medical Subject Heading) and using the following descriptors: "("*probiotics and anxiety*" OR *probiotics* OR *anxiety*) AND (*psychobiotics* OR *probiotic*)". A total of 406 articles were identified from Medical Literature Analysis and Retrieval System Online (Medline), Biblioteca Nacional de Medicina (PubMed), Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS) and Scientific Electronic Library Online (SciELO) databases. Seven articles were included in the present study and 85.7% (6) of the studies evaluated, it was possible to observe a link between the use of psychobiotics and the reduction of symptoms of anxiety and stress. However, new studies are fundamental for further investigation of relationship between ingesting psychobiotics and minimization of anxiety symptoms.

INTRODUCTION

Anxiety disorders are complex, can be characterized by excessive fear and anticipation of threats that disrupt daily function and cause underlying pathophysiology influenced by environmental and polygenic factors (Mufford et al 2021). Based on this, researchers like psychologists and psychiatrists separate anxiety into two types: state and trait [Belzung and Griebel, 2001; Goes *et al.,* 2018; Spielberger *et al.,* 1983; Takagi *et al.,* 2018). Basically, the difference between the two types is the duration of the symptoms. State anxiety is an acute response to a potential threat, while trait anxiety is chronic, manifesting itself constantly during the individual's life; it is, therefore, considered a personality trait (Endler and Kocovski, 2001; Spielberger *et al.,* 1983). State anxiety can be defined as a state of alertness to a threat that can be triggered by acute stress. Its primary function is avoiding dangerous situations. On the other hand, trait anxiety is an individual's predisposition to express continuous anxiety (Endler and Kocovski, 2001; Spielberger *et al.,* 1983). It is important to emphasize that both responses represent an advantage when avoiding dangers and threats (Goes *et al.,* 2018; Spielberger *et al.,* 1983; Takagi *et al.,* 2018).

However, when anxiety is sustained and/or provoked by non-threatening stimuli, it becomes dysfunctional and can cause suffering to the individual (Belzung and Griebel 20012; Sylvers *et al.,* 2011). Research has shown that intestinal microbiota can play an extremely important role in brain behavior, through the gut-brain axis, where communication can occur between intestinal microorganisms and the organ, favoring brain development and even social behavior (Dinan *et al.,* 2015). The gut-brain axis provides a bidirectional homeostatic communication pathway that, if dysfunctional, can have important pathophysiological consequences. It is regulated at a neural, hormonal, and immunological level (Breit *et al.,* 2018), through partially modulate the availability of circulating tryptophan, serotonin, kynurenine, and short-chain fatty acids (SCFA), as well as blood-brain barrier (BBB) permeability and activation of peripheral immune cells and brain glial cells (Generoso *et al.,* 2020). With better knowledge about the intestinal microbiota, many studies have correlated the effect of probiotic microorganisms on various psychological conditions, such as: stress, anxiety, irritable bowel syndrome and depression (Chong *et al.,* 2019; Colica *et al.,* 2017; Foster *et al.,* 2017; Gualtieri *et al.,* 2020; Liang *et al.,* 2018; Patterson *et al.,* 2020). These probiotic organisms are defined as live microorganisms that, when administered regularly and in adequate

amounts, provide health and psychological benefits to those who ingest them (FAO/WHO, 2002). This idea led to the formation of a new term called psychobiotics. Currently, there is a growing interest in the impact and benefits of these agents on central nervous system processes, especially regarding stress, mood, anxiety, and cognition (Cheng *et al.,* 2021; Dinan *et al.,* 2015; Misdra and Mohanty 2019). Thus, psychobiotics are probiotics that contain psychotropic properties, that is, they are live bacteria that directly and indirectly produce positive effects on neuronal functions by colonizing the intestinal microbiota and through interaction with commensal intestinal bacteria (Luang-in *et al.,* 2020). If the right amount is ingested, they can positively affect the brain, benefiting people who suffer from chronic stress or anxiety symptoms (Adikari *et al.,* 2019). Some studies, although limited in number, have already described the action of certain species of probiotic bacteria on anxiolytic and antidepressant activities. Thus, with a range of application possibilities, the idea of a new class of psychopharmacology is emerging (Colica *et al.,* 2017; Chong *et al.,* 2019; Eszandarzadeh *et al.,* 2021 Gualtieri *et al.,* 2020; Ma *et al.,* 2021; Patterson *et al.,* 2020). Therefore, in this article, the results of studies from the last five years about the use of psychobiotics and their effect on symptoms of trait anxiety in humans will be discussed.

MATERIALS AND METHODS

The study was developed using the exploratory-descriptive method, according to the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) guidelines (Page *et al.,* 2021). The literature review was performed using the folllowing databases: Medical Literature Analysis and Retrieval System Online (Medline), National Library of Medicine (PubMed), Latin American and Caribbean Health Sciences Literature (LILACS) and Scientific Electronic Library Online (SciELO). Studies of clinical trials performed on humans, published in English, Spanish and Portuguese in the last 5 years (January 2016 to June 2021), that analyzed the effects of administering psychobiotics/probiotics to individuals with symptoms of and/or diagnosed with trait anxiety were considered for the systematic review. The search strategy used was developed by combining MeSH terms (Medical Subject Heading) and using the following descriptors: "("*probiotics and anxiety*" OR *probiotics* OR *anxiety*) AND (*psychobiotics* OR *probiotic*)". Articles with the following eligibility criteria were included in this study: 1) clinical studies; 2) studies in humans; 3) studies that have administered probiotics to people with symptoms of and/or clinically diagnosed with trait anxiety. The following exclusion criteria were also used: 1) animal studies; 2) systematic review studies or narrative review and abstracts; 3) studies that evaluated the effectiveness of probiotics for treating depression and anxiety in pregnant women and/or during postpartum; 4) studies with patients with any other pathology outside the research context, including state anxiety; 5) studies with the administration of other components or medications, except in cases of patients submitted to probiotic supplementation who are diagnosed with anxiety and use medication for such disease.

After the search in the databases, the initial selection of the articles was carried out. This step was performed by three independent reviewers, who read the abstracts in order to observe whether each article met the study's eligibility criteria. When the abstracts were suitable for the research, the articles were read in their entirety, and those that did not meet all the criteria were excluded. In cases of divergence between the three reviewers, it was resolved through consensus. From the selected articles, the data obtained were computed in an Excel spreadsheet with the following information: article title, abstract, year of publication, language, and authors. A descriptive statistical analysis with a narrative approach was performed, where the included studies were detailed, highlighting their respective characteristics. Subsequently, all points of convergence and divergence between studies were analyzed, with their explanations, and the results were grouped to facilitate data synthesis. The presentation of the data was performed in Table 1, with the aim of facilitating the visualization and the inquiry of the

information gathered and developed from interpretive and analytical readings of the selected research material.

RESULTS

The methodology used allowed the identification of 406 articles, of which seven were excluded for duplicity, resulting in 399 articles for the initial screening. When reading the titles and abstracts, 321 articles were excluded for not directly belonging to the research scope, totaling seventy-eight articles selected for full reading in the eligibility assessment step. After that, another seventy-one articles were excluded, resulting in the seven articles that were used to carry out our systematic review, as shown in Figure 1, with the research's methodological steps flowchart.
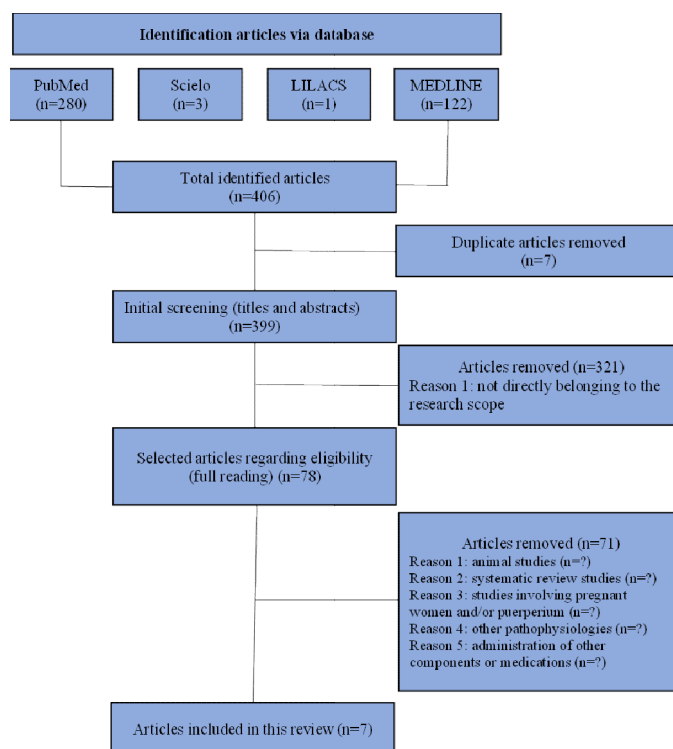

Figure 1. Flowchart of the methodological steps used to select the articles in this study, according to *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) guidelines.[24]

Through the analysis of the 7 selected articles, it was observed that PubMed database was the most relevant, since 85.7% (n=6) of the articles were obtained from it, followed by Medline database, with 14.3% (n=1). These databases were predominant as they contain bibliographic collections dedicated exclusively to the areas of biomedical sciences and aim to preserve scientific information efficiently and free of charge (Falagas *et al.,* 2008). PubMed is one of the most prevalent sources in the health sciences area, as it is one of the most easily accessible systems for satisfactory searches, being convenient for both theories and practical conducts of professionals based on scientific studies (Anders and Evans, 2010). It is known that the association between psychotherapy and pharmacotherapy is the most efficient treatment for anxiety disorders. However, new therapeutic alternatives have been studied. Among them, the link between intestinal microbiota and neurological and psychiatric disorders has been described (Gualtieri *et al.,* 2020; Patterson *et al.,* 2020). Psychobiotics belong to a more recent area of research, which justifies the years chosen to limit the publications used in this study (last 5 years); there were publications in the years 2020 (28.5% n=2), 2019 (28.5% n=2), 2017 (28.5% n=2) and 2021 (14.5% n=1). Publications in English prevailed, with a 100% of the total (n=7), which demonstrates that the articles have significant impact and scope, since publications in this language are the most scientifically disseminated (Lukka and Kasanen, 1996).

Table 1. Characteristics of the studies selected for the systematic review: authors: study design, sample, bacterial species intervention and main findings place where the research was developed, study goal, evaluated sample, type of study and results obtained

Author	Study design	Sample	Bacterial species intervention	Main findings
Colica et al. (2017)[13]	Prospective intervention.	Psychobiotics oral suspension group (POSG) (n=11) Dietary treatment group (DTG) (n=10) Combined treatment group (CTG) (n=9)	*Streptococcus thermophilus* (CNCM I-1630), *Bifidobacterium animalis* subsp. Lactis, *B. bifidum*, *S. thermophiles*, *L. bulgaricus* (CNCM I-1632 and I-1519), *Lactococcus lactis* subsp. *Lactis* (CNCM I-1631), *L. acidophilus*, *L. plantarum*, *L. reuteri*, DSM 17938 (3 g/each 1.5×10^{10} CFU/day).	A significant reduction in anxiety has been demonstrated in individuals with a high degree of anxiety.
Chong et al. (2019)[14]	Double-blind, randomized, placebo-controlled trial.	Probiotic group (n=56); age 31.1±7.8 Placebo group (n=55); age 32.1±11.00	*L. plantarum* DR7 (1×10^9 CFU/day).	The consumption of Lactobacillus plantarum for 12 weeks reduced symptoms of stress and anxiety in adults participating in the study.
Gualtieri et al. (2020)[15]	Double-blind, randomized, placebo-controlled trial.	Probiotic group (n=65); age 43.81±14.88 Placebo group (n=32); age 32.92±11.75	*Streptococcus thermophilus* (CNCM I-1630), *Bifidobacterium animalis* subsp. Lactis, *B. bifidum*, *S. thermophiles*, *L. bulgaricus* (CNCM I-1632 and I-1519), *Lactococcus lactis* subsp. *Lactis* (CNCM I-1631), *L. acidophilus*, *L. plantarum*, *L. reuteri* DSM 17938 (3 g/ each 1.5×10^{10} CFU/day).	Consumption of probiotics alleviates anxiety symptoms. The results suggest genetic association studies for personalized therapy with psychobiotics.
Patterson et al. (2020)[16]	Double-blind, randomized, placebo-controlled trial.	Probiotic group (n=55); 29 F/26 M Placebo group (n=58); 28 F/30 M	*Lacticaseibacillus paracasei* Lpc-37® (1.75×10^{10} CFU/day).	Use of the probiotic *L. paracasei* reduced perceived stress and trait anxiety compared to placebo.
Ma et al. (2021)[22]	Double-blind, randomized, placebo-controlled trial.	Probiotic group (n=43); 33 F/10 M Placebo group (n=36); 28F/8M	Probiotic: *Lactobacillus (L.) plantarum* (2 g; 2×10^{10} CFU/sachet/day).	There was a link between the modulation of the intestinal microbiota induced by probiotics and the relief of anxiety in adults, confirming that the gut-brain axis was involved in mitigating symptoms related to stress and anxiety.
Eskandarzadeh et al. (2019)[23]	Double-blind, randomized, placebo-controlled trial.	GAD probiotic group (n=24); 19 F/5 M; age 34.17±6.14 GAD placebo group (n=24); 20 F/4 M; age 36.67±6.56	*Bifidobacterium longum*, *B. bifidum*, *B. lactis* and *L. acidophilus* (18×10^9 CFU).	The combination of probiotics plus sertraline was superior to sertraline alone in reducing anxiety symptoms after 8 weeks in patients with generalized anxiety disorder.
Romijn et al. (2017)[29]	Double-blind, randomized, placebo-controlled trial.	Probiotic group (n=40); 32 F/8 M; age 35.8±14.0 Placebo group (n=39); 30 F/9 M; age 35.1±14.5	*L. helveticus* R0052 and *B. longum* R0175 ($\geqslant 3 \times 10^9$ CFU/sachet).	No evidence has been found that the probiotic formulation is effective in treating mood swings (such as stress and trait anxiety) or in moderating levels of inflammatory and other biomarkers.

CFU = colony-forming unit; GAD = Generalized anxiety disorder; F = female; M = male.

Amongst the methodological classification of the studies, 85.7% (n=6) were double-blind, randomized, placebo-controlled clinical trials and 14.3% (n=1) were observational studies. Randomized trials are important because this type of study uses an interest group, where a therapy or exposure is applied. This group is then monitored by comparing its results with the results of a control group (De Oliveira and Parente, 2010). It is noteworthy that the included studies differed from each other in the following aspects: strains used in the experiments; assessment of trait anxiety symptoms; duration of the study and age range of the participants involved. The general characterization of the articles included in this systematic review is shown in Table 1. The experiments in the included studies involved a total of 581 participants. The data showed that the age of those involved ranged from 18 to 75 years. Regarding gender, 42% were women, 20% were men and 38% were undisclosed. Probiotics were administered in the form of sachets (71%) and capsules (29%). About 42% of the studies used only one strain in the probiotic formulation and 58% used formulations with at least two strains of bacteria. The duration of the studies ranged between 3 and 12 weeks. Regarding the probiotics used, *Lactobacillus* plantarum (Chong *et al.*, 2019; Colica *et al.*, 2017; Gualtieri *et al.*, 2020; Ma *et al.*, 2021) was the most prevalent, being used in four experiments; followed by *Lactobacillus acidophilus*, *Bifidobacterium lactis* (Colica *et al.*, 2017; Eszandarzadeh *et al.*, 2021; Gualtieri *et al.*, 2020) and *Bifidobacterium longum* (Eszandarzadeh *et al.*, 2021; Ma *et al.*, 2021; Romijn *et al.*, 2017) that were used in 3 studies; *Lactobacillus bulgaricus*, *Lactobacillus reuteri*, *Lactococcus lactis*, *Streptococcus thermophilus* (Colica *et al.*, 2017; Gualtieri *et al.*, 2020) and *Bifidobacterium bifidum* (Eszandarzadeh *et al.*, 2021; Gualtieri *et al.*, 2020) were used in two studies; and *Lactobacillus fermentum*, *Lactobacillus rhamnosus* (Ma *et al.*, 2021) *Lactobacillus helveticus* (Romijn *et al.*, 2017) and *Lacticaseibacillus paracasei* (Patterson *et al.*, 2020) were used in one experiment.

DISCUSSION

It is known that gut-brain axis involves a biochemical signaling pathway between the gastrointestinal tract and the central nervous system (Generoso *et al.*, 2020) and because this psychobiotics have been used to alleviate anxiety symptoms. In the genesis of anxiolytic effects, it is known that bacteria used as probiotics are capable of secreting neurotransmitters and neuromodulators, which are efficient in modulating mental health. For example, the γ-aminobutyric acid (GABA) is produced by *Lactobacillus* and *Bifidobacterium*; norepinephrine is released by *Escherichia*, *Bacillus* and *Saccharomyces* spp.; serotonin is synthesized by species of *Candida*, *Streptococcus*, *Escherichia* and *Enterococcus* spp.; and dopamine is secreted by *Bacillus* (Breit *et al.*, 2018).

The administration of a psychobiotic formulation to anxious subjects who had a score higher than or equal to 18 points, according to the Hamilton anxiety rating scale (HAM-A) was analyzed (Colica *et al.*, 2017) The subjects selected for the study were divided as follows: those who received only the psychobiotic formulation formed the POSG group (*Psychobiotics Oral Suspension Group*), the patients who took the psychobiotic formulation associated with a dietary treatment constituted the CTG group (*Combined Treatment Group*) and those who only followed the dietary treatment formed the DTG group (*Dietary Treatment Group*).

In the groups that received the psychobiotic formulation only (*Psychobiotics Oral Suspension Group* – PSOG) and the formulation associated with dietary treatment (*Combined Treatment Group* – CTG), there was a significant reduction in the HAM-A total score ($\Delta = -5$ points and $\Delta = -9.5$ points, resp.), compared to the results established before the clinical intervention, confirming that psychobiotic supplementation was associated with a reduction of anxiety (Colica *et al.*, 2017). Similarly, in a prospective study (Gualtieri *et al.*, 2020) used HAM-A ($\geq$ 18 points) as a criterion for diagnosing individuals with trait anxiety disorder. However, in this study, the psychobiotic regulation of anxiety symptoms occurred from the perspective of individuals with the IL-1β gene (rs16944), identified through genotyping performed on DNA extracted from salivary samples, which is related to high cytokines levels that can potentially affect mood disorders. Carriers of this gene, according to the HAM-A score, had a substantially higher risk of being anxious compared to non-carriers ($p < 0.01$; OR = 5.90; CI: 1.73 – 20.16), highlighting the interaction between IL-1β polymorphism and mood disorders. The same authors, when evaluating whether the ingestion of probiotics would alleviate anxiety symptoms, noted an improvement in psychometric parameters. In individuals carrying the IL-1β gene who used the psychobiotic formulation (*Psychobiotic Oral Suspension Group* - POSG), there was a 16.2% reduction in anxiety symptoms, compared to the placebo group (*Placebo Control Group* - PCG), where there was a 12.5% reduction. For non-carriers of the genetic polymorphism in the POSG group, a 5.1% reduction in anxious symptoms was detected, whereas in the PCG group no rate of symptomatic reduction was observed. These results show that probiotic intake has an impact on anxiety and on the increased sensitivity of IL-1β carriers to its administration in reducing mood disorders.

The use of psychiatric drugs is a protagonist in the treatment of generalized anxiety disorder (GAD), a subtype of trait anxiety identified as a chronic anxious state with symptoms that last for at least 6 months. Selective Serotonin Reuptake Inhibitors (SSRIs) and Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs) are first-line drugs for treating GAD. In this context, the effects of a probiotic formulation as an aid in the treatment of GAD was studied (Eszandarzadeh *et al.*, 2021). For this, subjects with GAD were randomly assigned to two groups: the treatment group (T) used probiotics associated with 25 mg sertraline (PS), an IRSR, and the placebo group (S) only used 25 mg sertraline. The results obtained from this prospective study after 8 weeks showed that the association of psychobiotics together with sertraline was superior to the use of sertraline alone, and reduced anxiety symptoms in individuals diagnosed with GAD. The score of Hamilton Rating Scale (HAM-A) decreased more in the combination group in comparison with the S group (-11.84 points ± 8.08 *versus* -8, 52 ± 6.85, respectively, p = 0.003). Thus, the use of psychobiotics as adjunctive therapy in the treatment of GAD constituted an important resolution for the main objective of the study, which was to assess changes in anxiety symptoms using the HAM-A scale (Eszandarzadeh *et al.*, 2021). It is noteworthy that the use of psychobiotic formulations with multiple species, which may generate greater chances of a positive result in reducing anxiety symptoms because of the activation of different pathways and the release of a greater number of neuroprotective substances (Colica *et al.*, 2017; Eszandarzadeh *et al.*, 2021; Gualtieri *et al.*, 2020). It is observed that a variety of species leads to greater improvement in the subject's well-being and mental health (Ma *et al.*, 2021). The use of *Lactobacillus plantarum* (DR7) in the relief of

stress and anxiety in adults of the Penang province, in Malaysia, with a prospective, double-blind, randomized, placebo-controlled study was evaluated [14] and it was observed that the administration of DR7 reduced anxiety scores assessed by the *Depression Anxiety Stress Scale* 21, DASS- 21, in all subpopulations: young adults (< 30 years) and normal adults (> 30 years). A greater reduction in total anxiety scores (quantified using the DASS-21) was observed in the two subpopulations compared to the placebo group. Regarding the physiological changes observed, the administration of DR7 reduced the plasma expression of neurotransmitters, such as dopamine β-hydroxylase (DBH) and tryptophan 2,3-dioxygenase (TDO), while increasing the concentrations of tryptophan hydroxylase 2 (TPH2) and serotonin receptor (5-HT6) in all subjects in the experimental group who received the probiotic for 12 weeks. Serotonin plays a key role as a mood regulator. A dysregulation of this neurotransmitter's concentration has been associated with inflammation and psychological disorders such as trait anxiety. Thus, the placebo group showed higher concentrations of DBH and TDO, converging the synthesis of tryptophan to the production of kynurenines, which would disfavor the endogenous pathway for maintaining optimized levels of serotonin, compromising its regulatory function in the central nervous system. Thus, in individuals who used DR7, as they have higher concentrations of TPH2 and 5-HT6 and lower levels of DBH and TDO, the endogenous route of tryptophan was directed towards the production of serotonin, enhancing its role in regulating cognitive health. These results mean that lower stress and anxiety parameters were observed in the experimental group (Chong *et al.*, 2019). Effects of ingesting *Lactobacillus plantarum* P-8 for 12 weeks on the intestinal microbiota of people with anxiety were investigated (Ma *et al.*, 2021), in order to understand the possible biological mechanisms involved in controlling the disorder. The placebo group and the experimental group did not obtain significant differences regarding microbial diversity. However, the prevalence of the species *Bifidobacterium adolescentis*, *Bifidobacterium longum* and *Faecalibacterium prausntizii* after the *L. plantarum* diet alludes to protective benefits regarding neurological diseases and memory. There is also the potential increase of neuroactive pathways, such as the synthesis of methaquinone (vitamin K2), GABA metabolism and SCFAs (Short Chain Fatty Acids), substances that have neuroimmune qualities and antidepressant responses. Another study analyzed the intake of the *Lacticaseibacillus paracasei* Lpc-37 strain for 8 weeks in a sample of 113 participants, distributed between placebo and experimental groups (Patterson *et al.*, 2020). At the end, they observed the effects of probiotics in increasing productivity, controlling fatigue and stress and, consequently, anxiety. The study used salivary cortisol analysis, heart rate (HR), blood pressure (BP), self-report scales, validated inventories, and diary entries as methods of evaluation. The Lpc-37 contributed to the identification of BP as a biomarker of trait anxiety since a reduction in this parameter was observed in the subgroup with low anxiety and in the high anxiety stratum the opposite was seen. Changes in inventories for anxiety were not noticed; however, the was an increase in health perception throughout the study and a decrease in exhaustion/fatigue, which contributed to a reduction of anxiety symptoms in the assessed group. However, the sample stratification into low and high chronic anxiety may have been a limiting factor, since the instruments used did not faithfully represent the diversity within the portion with trait anxiety.

Similarly, no differences between the probiotics and placebo groups were found (Romijn *et al.*, 2017) in the research which seventy-nine participants, with the probiotics group using *L. helveticus* and *B. longum* for 8 weeks. The anxiety rating measures were the Montgomery–Åsberg Depression Rating Scale, the QIDS-SR16 and the DASS-42. It is important to highlight that some confounding factors may have compromised the safe development of the protocol, like the exclusion of individuals using antidepressants and the inclusion of those who participated in psychotherapy. From this perspective, it is worth noting that the contrasting aspects of the studies, such as the intervention period, instruments for assessing trait anxiety and the species of bacteria used (Patterson *et al.*, 2020; Romijn *et al.*, 2017), may compromise the comparative analysis between the results achieved. In addition, sample heterogeneity is

also an important aspect to be observed, as the fact that subjects undergoing psychotherapy for more than 6 months were included in the study (Romijn *et al.,* 2017). Therefore, the analysis of the studies allowed us to observe the positive role of psychobiotics for controlling anxiety disorder, directly or indirectly, as in the modulation of pathways in the gut-brain axis, that is, in the metabolism of substances. In view of this, the realization of new studies that could eliminate the limiting factors displayed by the researchers is fundamental for reaching new conclusions regarding the ingestion of probiotics as a way of mitigating anxiety symptoms.

CONCLUSION

In the studies listed in this systematic review, it was evidenced that psychobiotics have positive results in treating behaviors and symptoms related to trait anxiety. However, some limitations, such as standardization of strains and psychobiotic formulations, study time, number of participants and influence of external factors limit the comparison between the results obtained. Since this is an innovative approach, more studies in humans need to be carried out, as most results so far have come from animal experiments. More clinical studies are needed to elucidate the findings of this review and to clarify the knowledge we have about the positive impact of these biological agents. Furthermore, it was evidenced in the literature that the gut-brain axis has a profound influence on the behavior of individuals with psychological disorders such as anxiety. It is important to emphasize that the use of psychobiotics in people who are diagnosed with some type of anxiety should be considered as an adjunct, since it is a multifactorial pathology; in most cases, there is need for a multidisciplinary follow-up, with psychotherapeutic treatment and the use of medications.

REFERENCES

Adikari AMGCP, Appukutty M, Kuan G 2019. Psychotropic Properties of Probiotics: A Systematic Review. *Int J Public Health Clin Sci.* 6:18-34.

Anders ME, Evans DP (2010). Comparison of PubMed and Google Scholar literature searches. *Respir Care.* 55:578-583.

Belzung C, Griebel G (2001). Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res.* 125:141-149.

Breit S, Kupferberg A, Rogler G, Hasler G 2018. Vagus Nerve as Modulator of the Brain-Gut Axis in Psychiatric and Inflammatory Disorders. *Front Psychiatry.* 9:1-15.

Cheng Y, Liu J, Ling Z 2021. Short-chain fatty acids-producing probiotics: A novel source of psychobiotics. *Crit Rev Food Sci Nutr.* 6:1-31.

Chong HX, Yusoff NAA, Hor YY, Lew LC, Jaafar MH, Choi SB, et al. 2019. *Lactobacillus plantarum* DR7 alleviates stress and anxiety in adults: a randomised, double-blind, placebo-controlled study. Benef Microbes. 10:355-373.

Colica C, Avolio E, Bollero P, de Miranda RC, Ferraro S, Salimei PS, et al. 2017. Evidences of a new psychobiotic formulation on body composition and anxiety. Mediators Inflamm. 2017:1-10.

De Oliveira MAP, Parente RCM 2010. Entendendo ensaios clínicos randomizados. *Braz J Videoendosc Surg.* 3:176-180.

Dinan TG, Stilling RM, Staton C, Cryan JF 2015. Collective unconscious: How gut microbes shape human behavior. *J Psychiatr Res.* 63:1-9.

Endler NS, Kocovski NL 2001. State and trait anxiety revisited. J Anxiety Disord.15:231-245.

Eszandarzadeh S, Effatpanah M, Khosravi-Darani K, Askari R, Hosseini AF, Reisian M, et al. 2021. Efficacy of a multispecies probiotic as adjunctive therapy in generalized anxiety disorder: a double blind, randomized, placebo-controlled trial. Nutr Neurosci. 24:102-108.

Falagas ME, Pitsouni EI, Malietzis GA, Pappas G 2008. Comparison of PubMed, Scopus, Web of Science, and Google Scholar: strengths and weaknesses. Scientific Databases, Pros and cons. FASEB J. 22:338-342.

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization) (2002). Guidelines for the Evaluation of Probiotics in Food. Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food. Available at https://www.who.int/foodsafety/ fs_management/en/probiotic_guidelines.pdf. [Accessed August 04, 2021].

Foster JA, Rinaman L, Cryan JF (2017). Stress & the Gut-Brain Axis: Regulation by the Microbiome. Neurobiol Stress. 7:124-136.

Generoso JS, Giridharan VV, Lee J, Macedo D, Barichello T (2020). The role of the microbiota-gut-brain axis in neuropsychiatric disorders. Braz J Psychiatry. 43:293-305.

Goes TC, Souza TH A, Marchioro M, Teixeira-Silva F (2018). Excitotoxic lesion of the medial prefrontal cortex in Wistar rats: effects on trait and state anxiety. Brain Res Bull. 142:313-319.

Gualtieri P, Marchetti M, Cioccoloni G, De Lorenzo A, Romano L, Cammarano A, et al. (2020). Psychobiotics regulate the anxiety symptoms in carriers of allele A of IL-1β gene: a randomized, placebo-controlled clinical trial. Mediators Inflamm. 2020:1-11.

Liang S, Wu X, Hu X, Wang T, Jin F (2018). Recognizing Depression from the Microbiota–Gut–Brain Axis. Int J Mol Sci. 19:1592.

Luang-in V, Katisart T, Konsue A, Nudmamud-Thanoi S, Narbad A, Saengha W, et al. (2020). Psychobiotic effects of multi-strain probiotics originated from Thai fermented foods in a rat model. Food Sci Ani Resour. 40:1014-1032.

Lukka K, Kasanen E (1996). Is accounting a global or a local discipline? Evidence from major research journals. Account Organ Soc. 21:755-773.

Ma T, Jin H, Kwok LY, Sun Z, Liong MT, Zhang H (2021). Probiotic consumption relieved human stress and anxiety symptoms possibly via modulating the neuroactive potential of the gut microbiota. Neurobiol stress. 14:2352-2895.

Misdra S, Mohanty D (2019). Psychobiotics: A new approach for treating mental illness? Crit Rev Food Sci Nutr. 59:1230-1236.

Mufford MS, van der Meer D, Andreassen OA, Ramesar R, Stein DJ, Dalvie S (2021). A review of systems biology research of anxiety disorders. Braz J Psychiatry. 43:414-423.

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Syst Rev.* 10:1-11.

Patterson E, Griffin SM, Ibarra A, Ellsiepen E, Hellhammer J 2020. *Lacticaseibacillus paracasei* Lpc-37® improves psychological and physiological markers of stress and anxiety in healthy adults: a randomized, double-blind, placebo-controlled and parallel clinical trial (the Sisu study). *Neurobiol Stress.* 13:2352-2895.

Romijn AR, Rucklidge JJ, Kuijer RG, Frampton C 2017. A double-blind, randomized, placebo-controlled trial of *Lactobacillus helveticus* and *Bifidobacterium longum* for the symptoms of depression. *Aust N Z J Psychiatry.* 51:810-821.

Spielberger CD, Gorsuch RL, Lushene RD, Vagg PR, Jacobs GA 1983. Manual for the state-trait anxiety inventory (STAI). Consulting Psychologists Press: Palo Alto.CA, USA.

Sylvers P, Lilienfeld SO, Laprairie JL 2011. Differences between trait fear and trait anxiety: implications for psychopathology. J Clin Psychol. 31:122-137.

Takagi Y, Sakai Y, Abe Y, Nishida S, Harrison BJ, Martínez-Zalacaín I, et al. 2018. A common brain network among state, trait, and pathological anxiety from whole-brain functional connectivity. Neuroimage. 172:506-516.

****