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Exploring the gut-brain axis: probiotic intervention as an additional treatment in depression.

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A mis padres, por ser mi gran suerte. Por confiar en mí, apoyarme y estar a mi lado durante todo este proceso, y siempre.

A mi hermano, por seguir creciendo de la mano y disfrutar esta profesión que compartimos.

Y por supuesto, a todas las personas que me han acompañado estos cuatro años. Desde la universidad, desde casa y desde la pista. Todos formáis parte de esta etapa.

En memoria a mi abuelo Claudio. Gracias por ser mi mayor referente.

Resumen

El eje intestino-cerebro es una comunicación bidireccional entre los centros emocionales y cognitivos del cerebro y las funciones intestinales periféricas. La posibilidad de los probióticos como tratamiento adicional a los antidepresivos ha ido cogiendo fama a lo largo de los últimos años, gracias a las múltiples ventajas que ofrecen a nuestro organismo. Esto, junto al impacto y el crecimiento de la depresión en la sociedad han sido las razones de esta revisión, cuyo objetivo era estudiar la relación entre el eje intestino-cerebro y el Trastorno Depresivo Mayor, y cómo los probióticos pueden afectar a esta conexión. A partir de un análisis exhaustivo de los ensayos clínicos más relevantes en este campo de investigación, los resultados obtenidos han sido, por un lado, que la administración de probióticos mejora los síntomas depresivos y, en segundo lugar, que existe una microbiota alterada y específica en pacientes depresivos que están bajo tratamiento probiótico, destacando las cepas de Ruminococcus como las más relevantes. Esta revisión apoya el uso de probióticos como terapia complementaria para pacientes con depresión, pero aun habiendo obtenido estos resultados ha enfatizado que esta área de investigación está creciendo de forma desestructurada y destaca por no tener suficientes ensayos clínicos, por lo que más investigación en este campo es necesaria, para así obtener conclusiones más concretas y generalizables.

Abstract

The gut-brain axis is a two-way communication between the emotional and cognitive centres of the brain and peripheral gut functions. The possibility of probiotics as an additional treatment to antidepressants has become increasingly popular over the last years, thanks to the wide range of benefits probiotics offer to our body. This, together with the impact and growth of depression in society have been the reasons behind this review, which aimed to study the relationship between the gut-brain axis and Major Depressive Disorder, as well as how probiotics may affect this connection. Based on a thorough analysis of the most relevant clinical trials in this field of research, the results obtained are, firstly, that probiotic administration improves depressive symptoms and, secondly, that there is an altered and specific microbiota in depressive patients who are under probiotic treatment, highlighting Ruminococcus strains as the most relevant. This review supports the use of probiotics as an adjunctive therapy for patients with depression, but even with these results, it is emphasized that this area of research is growing in an unstructured way and is notable for its lack of clinical trials, therefore more research is needed in this field to provide more accurate and generalizable conclusions.

Keywords: depression, gut-brain axis, probiotics, psychobiotics

Abbreviations

BDI = Beck's Depression Inventory

DSSS = Depression and Somatic symptoms Scale

EPDS = Edinburgh Postnatal Depression Scale

HAM-D = Hamilton Depression Rating Sale (HAM-D or HRSD or HDRS)

HAMD-17 = 17-item reduced version of Hamilton Depression Rating Sale

ASV = Amplicon Sequence Variant

STAI = State-Trait Anxiety Inventory

DASS = Depression Anxiety Stress Scale

DSM-V = Diagnostic and Statistical Manual of Mental Disorders, 5ª Ed.

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Introduction - Exploring the gut-brain axis: probiotic intervention as an additional treatment in depression.

Over the past decade, considerable emphasis has been placed on the examination of the gut-brain axis concerning various social behaviours, particularly those associated with major depression. The significance of this subject has become so profound that it permeates our everyday lives, influencing the literature we engage with and the profiles we encounter on social media platforms. However, it is crucial to ascertain the extent to which the renowned gut-brain connection impacts individuals suffering from depression. What manner of relationship does it exist between the two? Might the gut-brain axis serve as a potential target for novel antidepressant treatments? Could probiotics offer a means of enhancing specific behaviors? Furthermore, is there an altered microbiota implicated in cases of major depression?

The gut-brain axis (GBA) is a bidirectional communication between the central nervous system (CNS) and the enteric nervous system (ENS), linking the emotional and cognitive centres of the brain with peripheral gut functions (Carabotti et al., 2015). GBA is crucial to maintain gut homeostasis and brain function (Watanabe et al., 2022). The gut microbiota plays a key role in emotional processing, regulating behaviour and higher cognitive funcitons (Bagga et al., 2019; Roman et al., 2018; Rode et al., 2022; Watanabe et al., 2022). It has also an important function in brain processes such as stress responsiveness, anxiety-related behaviours, pain perception and social cognition (Sanchez et al., 2017; Bagga et al., 2019; Roman et al., 2018). As well as an important task in modulating biochemistry, brain plasticity and the activation of the immune system (Bagga et al., 2019; Roman et al., 2018). The other way around, the brain influences mobility, appetite sensations, secretions and intestinal permeability (Sanchez et al., 2017).

The interaction between the gut and the brain is made up of a complex network of endocrine, immunological and neural mediators, which has been highlighted as a critical target for the manipulation of brain health, neurodegenerative diseases and physical and psychological health (Chahwan et al., 2019; Kim et al., 2021; Steenbergen et al., 2015). This two-way communication occurs through three different routes: the neural pathway, where we find the vagus nerve, known as the main route of information transmission in this axis, and the enteric nervous system; the endocrine pathway, through the release of hormones, as cortisol;

and the immune pathway, by the action of cytokines (Roman et al., 2018; Watanabe et al., 2022).

Moreover, the study of the GBA has related the gut microbiota to neurodegenerative pathologies including Alzheimer's disease and to mood disorders such as depression and anxiety (Kim et al., 2021). What's more, Wallace et al. (2020) have found that the microbiome of patients with depression varies in terms of its composition and diversity (Wallace et al., 2020).

The following illustration (Figure 1) schematises how the central nervous system communicates via afferent and efferent autonomic pathways (SNA) with different intestinal targets, that is, the ENS, muscle layers and intestinal mucosa, modulating motility, immunity, permeability and mucus secretion. The enteric microbiota maintains bidirectional communication with these gut targets, modulating gastrointestinal functions and in turn being itself modulated by gut-brain interactions (Carabotti et al., 2015).

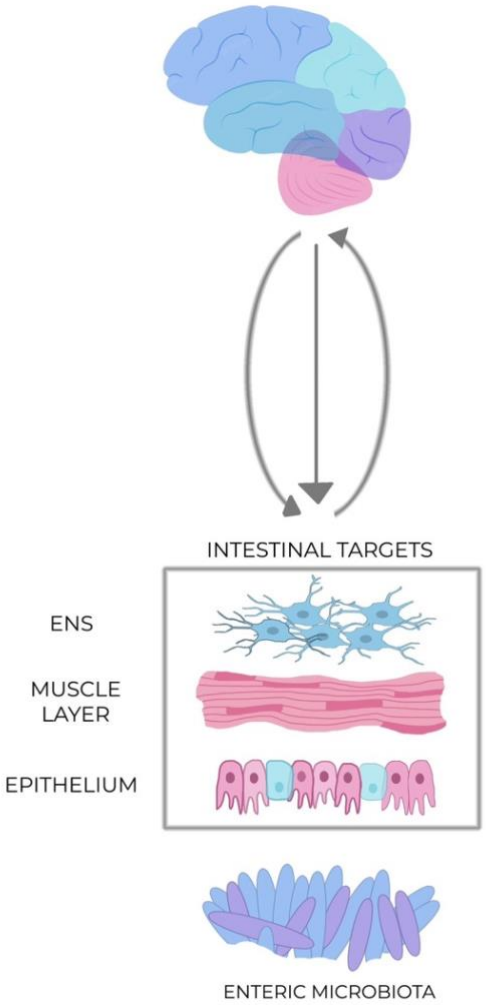
As indicated above, GBA is essential for the maintenance of homeostasis and brain function. Therefore, an imbalance in the communication between the gut microbiota and the CNS could contribute to neuropsychiatric disorders - such as depression (Reininghaus et al., 2020; Herselman et al., 2022). Many other health problems are associated with microbial dysbiosis i.e., neuropsychiatric disorders, such as autism spectrum disorder, depression and anxiety; and at the same time mood in healthy adults is affected by alterations in the pattern of gut microbial composition (Slykerman et al., 2017). Dysbiosis is bounded with high levels of pro-inflammatory cytokines, increased oxidative stress, altered gastrointestinal (GI) function, and decreased micronutrient and omega-3 fatty acid status (Slykerman et al., 2017).

Along these same lines, stress is a clear example of a mechanism that can produce changes in homeostasis. The release of stress hormones have an impact on the gut ecosystem, modulating gastrointestinal blood flow, secretion, permeability and motility, as well as activating the immune system; chronic exposure may predispose dysregulation in the GBA, leading to immune imbalances and the development of stress-related disorders (Roman et al., 2018; Peter et al., 2018). Stress and diet, acting together, are believed to modulate gut health and the composition of the gut microbiota (Herselman et al., 2022).

Stress and other environmental factors, such as emotion, can activate the hypothalamic pituitary adrenal (HPA) axis, which leads to the release of cortisol through a complex interaction with the limbic system, formed by the amygdala, the hippocampus and the hypothalamus (Carabotti et al., 2015). It is clear that stress is a significant factor in the gut-brain axis.

Figure 1

Gut-brain Axis



Note. Gut brain-axis schematic representation. Adapted from "The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems" (p.204), by Carabotti et al., 2015, *Annals of Gastroenterology, 28* (2).

According to Schneider et al. (2023), recent studies have found that alterations in the gut microbiota are associated with a decrease in anti-inflammatory butyrate-producing bacteria and an increase in pro-inflammatory bacteria in patients with depression; moreover, the prevalence of butyrate-producing Faecalibacterium and Coprococcus bacteria has been linked to a better life quality (Schneider et al., 2023).

In addition, preclinical research has found that changes in the composition of the gastrointestinal microbiota can affect immunity within the central nervous system and blood-brain barrier resistance (Mysonhimer et al., 2023), and further studies suggest that imbalance in the levels of monoamine neurotransmitters, such as serotonin and norepinephrine, is a critical factor in the pathophysiology of depression and anxiety disorders (Barandouzi et al., 2022). As a matter of fact, preclinical studies have shown in mice that faecal transplantation, on the one hand, can modify the anxiety phenotype, and on the other hand, can induce depression-like behaviours from MDD patients' stool and therefore that microbiota and brain chemistry changes are accompanied (Schaub et al., 2022; Slykerman et al., 2017; Reiter et al., 2020)

Barandouzi et al. (2022) also state that there are similarities in the faecal microbiome of people with irritable bowel syndrome (IBS) and people with depression, denoting a high abundance of proteobacteria and low abundance of bifidobacteria (Barandouzi et al., 2022). Indeed, research demonstrates that 90% of serotonin is synthesized in the gut, so both the CNS and the ENS have the capacity to produce neurotransmitters, supporting the previous statements (Barandouzi et al., 2022). Feng et al. (2022) present clinical evidence on the implication of gut microbiota dysbiosis interacting with bile acid metabolism alterations in the psychological disorders of Crohn's disease (Feng et al., 2022), providing information and illustrating this association.

Major Depressive Disorder

Major Depressive Disorder (MDD) is a chronic and life-threatening disorder that, according to the World Health Organization (WHO), affects more than 33 million people in the European Union and is one of the leading causes of disability worldwide (Karakula-Juchnowicz et al., 2019; Chinna-Meyyappan & Milev, 2020; Reiter et al., 2020). In European

countries, also reported by the WHO, the cost of mood disorders amounts to about 170 billion euros yearly; even so, more than 50% of patients with Major Depressive Disorder do not receive treatment (Karakula-Juchnowicz et al., 2019). MDD affects both personally and socio-economically, those suffering from it experience a persistent low mood, and a lack of pleasure experience, along with a wide range of cognitive, physical and behavioural symptoms that prevent them from carrying on with their daily lives as usual, such as energy shortage, anhedonia, sleep disorders, and appetite changes (Reiter et al., 2020; Wallace et al., 2020). In addition to all these symptoms, several studies have documented a decline in cognitive performance regarding memory and attention (Schneider et al., 2023). DSM-V states that the main feature of a major depressive episode is at least a two-week period of time when there is low mood or loss of interest and/or pleasure in nearly every activity (APA, 2014)

Traditionally it was thought that MDD was a brain disease, but it is now recognised as a systemic disease of the whole body, caused by multiple factors, including genetic, neurological, inflammatory, personality, cognitive and environmental (Chahwan et al., 2019; Karakula-Juchnowicz et al., 2019).

Inflammation is an important pathophysiological pathway when discussing affective disorders; indeed, inflammation and depression are interrelated, and there is growing evidence that reducing peripheral inflammation is an encouraging approach for the treatment of depression (Karakula-Juchnowicz et al., 2019; Reininghaus et al., 2020).

The cytokine and neuroinflammation hypothesis appears in Chinna-Meyyappan & Milev (2020), which suggests that an increase in proinflammatory cytokines (interleukin 6 [IL-6] and tumour necrosis factor-alpha [TNF-α]) and a reduction in anti-inflammatory cytokines (interleukin 10 [IL-10] and transforming growth factor beta [TGF-β]) contributes to a proinflammatory condition in patients with depression, suppresses negative feedback of the HPA axis, enhances blood-brain barrier permeability, decreases 5-HT synthesis, impairs glutamatergic systems, and influence neuroglial cells (Chinna-Meyyappan & Milev, 2020).

Moreover, several studies show how altered levels of proinflammatory cytokines are found in other psychiatric illnesses, such as schizophrenia and bipolar disorder, supporting an association between depression and chronic inflammation, as well as oxidative stress (Chahwan et al., 2019; Reiter et al., 2020)

Brain structure and microbiota changes

It is known that the prefrontal cortex, hippocampus and amygdala play a vital role in the regulation of emotion, anxiety, stress responses, self-control, motivation and cognitive reactions. Chinna-Meyyappan & Milev (2020) also tries to clarify the pathophysiology of MDD naming the monoaminergic neurotransmitter deficiency hypothesis, which states that the monoaminergic neurotransmitters serotonin (5-HT), norepinephrine (NE), and dopamine (DA) remain at insufficient levels ultimately resulting in depressive symptoms; this hypothesis supports the argument that in depressed patients, the prefrontal cortex and hippocampus functions are damaged, while amygdala activity is increased (Chinna-Meyyappan & Milev, 2020).

Notwithstanding the increase in research, there is still not a thorough understanding of the changes in the intestinal microbiota. Currently, research indicates, that both pharmacological treatment and psychological therapies are effective in decreasing depressive symptoms and that approximately 60-70% of patients respond to treatment; nevertheless, it is estimated that one-third of patients do not respond to treatment, simultaneously, further research claims that concerning the initiation of antidepressant treatment up to two-thirds of these depressed patients do not respond adequately, and even up to 30% of treatment-resistant patients experience remaining symptoms once they have received their optimised treatments; on top of this, we must include the large number of people who do not seek treatment at all, due to the associated stigma (Chahwan et al., 2019; Reiter et al., 2020; Schaub et al., 2022). Making it clear, therefore, that additional or complementary treatment strategies for depression are needed.

It is recognised that the gut microbiota is an important factor in human health and disease, its influence goes beyond the gut and has been related to diseases such as obesity, type 2 diabetes, Crohn's disease and depression; suggesting that microbiota manipulation could be a viable therapeutic option due to its strong influence on mental health (Chahwan et al., 2019).

Over the last few years, a growing number of studies have investigated the possibility of using probiotics as a therapeutic intervention. For instance, Rudzki et al. (2019) observed an improvement in cognitive functions in the depressed patients' group receiving the probiotic

Lactobacillus Plantarum 299v in comparison to the placebo group (Rudzki et al., 2019). On the other hand, Yamanbaeva et al., (2023) suggest in their findings that probiotics affect brain structure and function in the front-limbic network and that these effects are partly related to decreased depressive symptoms (Yamanbaeva et al., 2023).

Even so, conclusions from clinical trials investigating probiotic treatment have shown mixed results, while some human trials concluded that evidence supporting the beneficial effects of probiotics on mood might not be as consistent as some recent literature reports (Slykerman et al., 2017), other preclinical studies, in both animals and humans, have shown behavioural and mood improvements under probiotic treatment (Chahwan et al., 2019).

Probiotics

Probiotics were first suggested as a potential additional therapy for major depression in 2005 (Slykerman et al., 2017). According to the Food and Agriculture Organization of the United Nations (FAO) and WHO, probiotics are strains of live micro-organisms that, when administered in adequate amounts, benefit the health of the host (Reiter et al., 2020). Many studies refer to probiotics as "psychobiotics" when they may provide mental health benefits (Chahwan et al., 2019; Heidarzadeh-Rad et al., 2020; Lu et al., 2021; Rode et al., 2022; Roman et al., 2018).

Probiotic consumption appears to exert a wide range of benefits, influencing inflammatory pathophysiology, enhancing immune response, reducing faecal enzymes involved in cancer initiation, improving intestinal barrier function, influencing gut and distal organ physiology, affecting gut microbial dynamics and homeostasis, among others (Chahwan et al., 2019; Kim et al., 2021; Roman et al., 2018; Wallace et al., 2020).

The strains belonging to Lactobacillus helveticus, Lactiplantibacillus plantarum and Bifidobacterium longum, according to studies in both animals and humans, have shown potential to improve memory, social interactions, emotion, attention and psychological symptoms, this last one by reducing anxiety and depression (Rode et al., 2022). Indeed, a large number of Lactobacillus and Bifidobacterium strains have been investigated in relation to mental health, and these genera appear to have the most beneficial effects (Slykerman et al., 2017).

It is also well-known that several Lactobacillus strains have the capacity to produce neurotransmitters, notably gamma-aminobutyric acid (GABA), and to increase the activity of certain enzymes that are implicated in the metabolism of tryptophan, a precursor of serotonin (Sanchez et al., 2017).

Relevant evidence to understand how probiotic treatment could affect patients with depression since GABA and serotonin are recognized for their positive impact on depression and anxiety. In addition, probiotics can also improve several cognitive functions, as Schneider et al. (2023) highlight, verbal episodic memory is enhanced in healthy individuals and in patients with MDD, Alzheimer's disease and other disorders after administration of probiotics. (Schneider et al., 2023).

Objectives

Given this theoretical framework and the wide variety of studies concerning the effects of probiotics, and more specifically in relation to major depressive disorder, it is necessary to describe and synthesize this topic. Therefore, the general aim of this systematic review is:

- To study the relationship between the gut-brain axis and Major Depressive Disorder, and how probiotics may affect it.

More specifically, the following specific objectives derive from this general objective:

I. To determine which parameters are altered due to the effects of probiotics in patients with Major Depressive Disorder.
II. To examine if probiotic administration leads to an improvement in depressive symptoms.
III. To observe if there is a correlation between altered microbiota and Major Depressive Disorder.

Methodology

To achieve the objectives proposed in this review and as a strategy for identifying the relevant bibliography, a search equation was used in PubMed and Web of Science databases.

First, a synonym search was performed in the database EBSCO (APA Thesaurus of Psychological Index Terms), to avoid documentary noise and obtain the most accurate results in line with the topic. Based on the selection of the optimum vocabulary, the final research equation was as follows: (Gut-brain axis) AND (Major Depression OR depression) AND (clinical trial).

To obtain results, Boolean operators were used, both "AND", a system for retrieving documents that contain all the terms, and "OR", which allows retrieval of at least one of the terms. In addition, a series of filters have been applied in the search, such as the range of years between 2010-2023, and the type of article so that reviews and systematic reviews can be excluded; thereby creating inclusion criteria that allowed us to obtain the most relevant articles for the review.

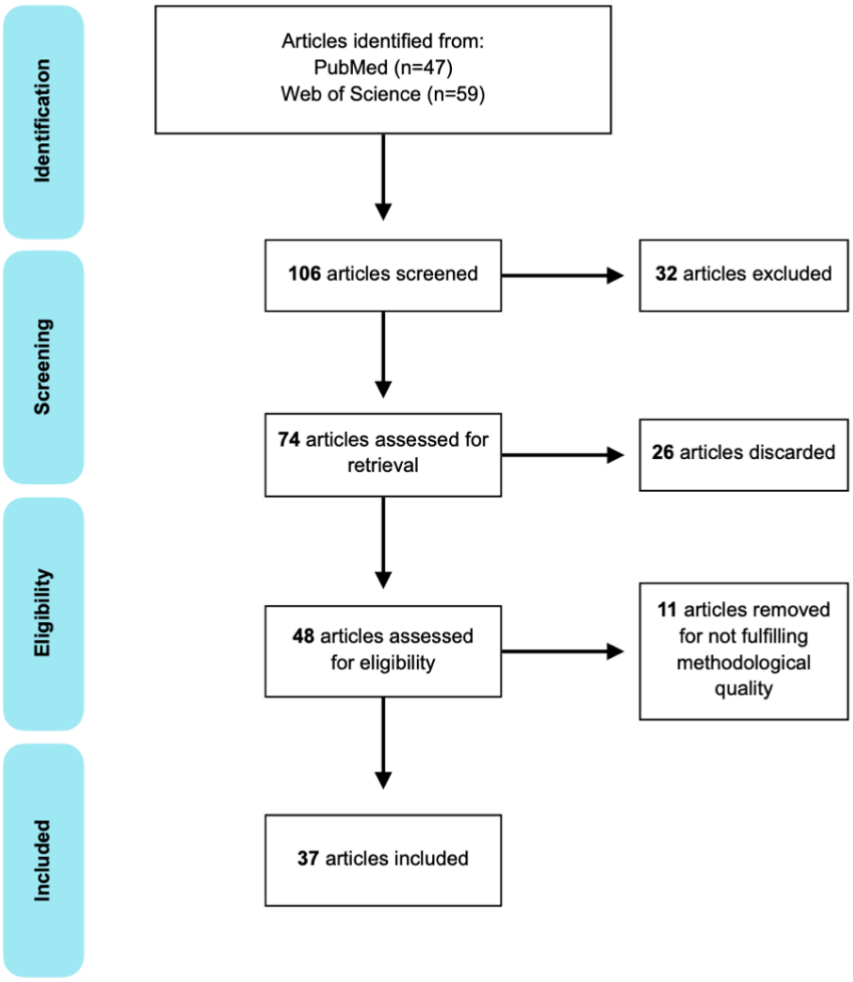
The equation returned a total of 106 articles, 47 articles in PubMed and 59 articles in WoS, of which 32 were discarded for being reviews or systematic reviews after the filter was applied. Therefore 74 articles were selected. After reading the abstracts of each of the articles, 26 were discarded, in this case also considering whether there was access or not to the full text, leaving a total of 48 articles. This search yielded articles that connected the brain-gut axis with MMD, as set out in the objectives of the review.

Subsequently, after reviewing all the selected articles, 11 more were discarded for not fulfilling the methodological. This left a total of 37 articles that were included in the bibliography of this review. Additionally, DSM-V reference was included to provide complementary and necessary information to the review.

As shown in Figure 2, the following flow chart summarizes what has been mentioned above, in order to simplify the process of bibliography selection.

Figure 2

Flow Chart



Results

Prior to explaining the results in detail, note that 12 clinical trials have been considered from all the studies. The clinical trials have been divided into two different headings, according to the respective results that each of the studies supported or, instead, argued against.

1. Probiotic therapeutic intervention improves depression symptoms.

Across 37 studies with unique effects due to probiotics, a total of 9 clinical trials have been selected to highlight the first result of this review, the improvement in depressive symptoms that can be observed because of probiotics interventions in patients undergoing treatment for depression.

Among all these studies, only two related their results to an improvement in the BDI score, demonstrating that the intake of probiotic supplements in patients with MDD can improve their depressive symptoms. Akkasheh et al. 2016, firstly, after 8 weeks of intervention supplemented with the following probiotic mixture: Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium bifidum, observed that patients in the probiotic group had an improvement in the BDI score, in addition, the probiotic group improved insulin function and decreased oxidative stress (Akkasheh et al., 2016). In the same line, Kazemi et al. 2019 reported that probiotic supplementation resulted in a greater decrease in BDI score compared to the placebo group and, also showed that probiotic supplementation decreases the serum kynurenine/tryptophan ratio compared to placebo (Kazemi et al., 2019)

Regarding HAMD-17 and DSSS scales. An open trial with a small sample size that studied Lactobacillus plantarum PS128 (PS128) within an eight-week intervention, found that HAMD-17, DSSS, and subscales of DSSS were significantly improved, whereas the markers of inflammation and gut permeability did not change remarkably (Chen et al., 2021)

Also, but using a more specific and concrete scale, the EPDS, Slykerman et al. (2017) evaluated postpartum depression and anxiety symptoms by administrating the Lactobacillus rhamnosus HN001 (HN001) during pregnancy and postpartum, women who received HN001 had significantly lower postpartum depression and anxiety scores, therefore the study demonstrated that probiotic supplementation with HN001 significantly reduces the prevalence of postnatal depression and postpartum anxiety symptoms during pregnancy and after pregnancy (Slykerman et al., 2017)

The kynurenine/tryptophan ratio is relevant as tryptophan is a precursor of 5-HT (serotonin), which contributes to feelings of well-being. In relation to this, Rudzki et al. 2019, has studied the psychobiotic and immunomodulatory effects of the probiotic bacterium Lactobacillus Planetarium 299v in patients with MDD treated with selective serotonin reuptake inhibitors (SRRI) and found an improvement in cognitive functions and a significant decrease

in the concentration of kynurenine, both in the probiotic group (Rudzki et al., 2019). The lower the ratio of kynurenine/tryptophan, the more likely it is to synthesize 5-HT, therefore Rudzki et al. (2019) by indicating that probiotic supplementation decreases this ratio, suggest that there is an improvement in depressive symptoms when administrating probiotics (Rudzki et al., 2019). Kazemi et al. (2019), obtained similar results supporting probiotic supplementation as a possible way to decrease the kynurenine/tryptophan ratio, and thereby depressive symptoms (Kazemi et al.,2019).

BDNF refers to Brain-Derived Neurotrophic Factor, a protein associated with the physiological processes of plasticity and development of the nervous system. Only one article has been able to demonstrate how probiotics can improve depressive symptoms, based on an increase in this protein, which is suggested to be essential for the response to antidepressants. Heidarzadeh-Rad et al. 2020, a double-blind, randomised, controlled trial of patients with low to moderate depression, found that BDNF levels after probiotic intervention increased significantly compared to the placebo group; and that after 8 weeks of supplementation, changes in serum BDNF levels and BDI score were significantly correlated (Heidarzadeh-Rad et al., 2020). In contrast to this study, Schneider et al. 2023, claim to have found no significant difference between groups over time in BDNF levels (Schneider et al., 2023)

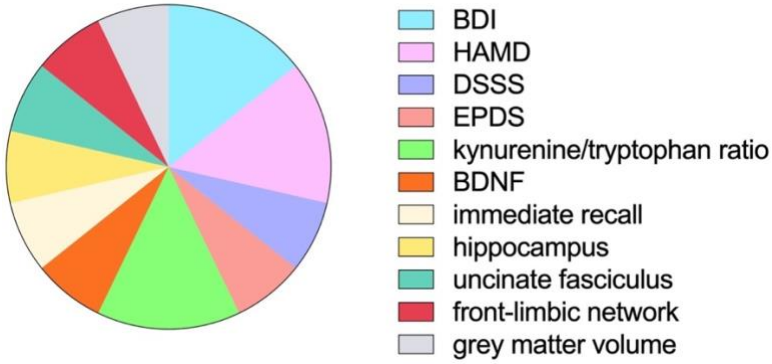
Although this last study does not indicate positive results with BDNF, it does indicate positive results in brain activity. Schneider et al. 2023, which carried out a 4-week intervention of high-dose probiotic supplementation in patients with depression, showed after the intervention that probiotic supplementation significantly improved immediate recall, and in addition, at the neural level they found significant differences in activation changes over time in the hippocampus, reflecting the beneficial effect of probiotics in mental disorders connected with depression (Schneider et al., 2023). Accompanying this study but in a different line, there are two more articles that observed alterations in the brain structure. On the first hand, using a multimodal neuroimaging approach, Watanabe et al. 2022, explored probiotic supplementation for four weeks in depressed patients. In this study a significant negative correlation was demonstrated between changes in fractional anisotropy (FA) and mean diffusivity (MD) in both the left and right uncinate fasciculus (UF) within the probiotic group (Watanabe et al., 2022). These results were obtained along with changes in resting-state functional connectivity (rsFC) between specific front-limbic seed regions and, a cluster in the precuneus and another in the left superior parietal lobe, over time between the groups (Watanabe et al., 2022). These effects

suggest that probiotics influence the brain structure and function of the cortico-limbic network, which is associated with a decrease, and consequently an improvement, in depressive symptoms. And, on the other hand, the results of Schaub et al. 2022 suggest that additional probiotic treatment improved depressive symptoms as a decrease in HAM-D scores over time was observed in the probiotic-supplemented group compared to the placebo group. Simultaneously, an increase in grey matter volume in the calcarine sulcus extending to the lingual gyrus, and a significant decrease activation of the putamen during emotion processing were also observed, both brain changes in the probiotic group, indicating the relevance of these areas in depression and the effect that probiotics may have on them (Schaub et al., 2022).

The graph (Figure 3) illustrates schematically all the different parameters affected after probiotic administration in depressive patients, so that briefly it can be clearly identified, which has been discussed in the previous section. It can be observed in a larger size the parameters that have been named in more studies, such as BDI, HAMD and the kynurenine/tryptophan ratio. And to a minor extent, the parameters that have been mentioned in fewer studies, these being: DSSS, EPDS, BDNF, immediate recall, hippocampus, uncinate fascicules, front-limbic network and grey-matter volume. It is therefore a graph to identify the parameters that have been reported and which have been the most discussed, according to the number of articles that have mentioned them.

Figure 3

Data Studied



2. Specific target of bacterial population for MDD patients under probiotic treatment.

Four studies evaluated the change in microbial composition in depressed participants and attempted to correlate MDD with a specific altered microbiota. Two of them reported Ruminococcus strains as relevant bacteria, while the other two reported other strains as relevant when talking about benefits caused by probiotic intervention.

Reininghaus et al. 2020 highlighted after their study, significantly altered beta diversity and a significant increase of the Ruminococcus gauvreauii among depressed individuals who received probiotics; in addition, after one month of treatment they also found an increase of Coprococcus 3; as well as a significant improvement of psychiatric symptoms in both groups, probiotic and placebo groups (Reininghaus et al., 2020). Alongside these results, Chahwan et al. 2019, revealed a significant positive correlation between an Operational Taxonomic Unit (OTU), classified as Ruminococcus gnavus, and the DASS depression score (Chahwan et al., 2019). This OTU was present in 72% of depressed participants compared to only 25% of non-depressed participants, and was present in relative abundance in the BDI severe depression range; although there were no significant differences in gut microbiota between pre-and post-intervention subjects in either the probiotic or placebo groups, they highlighted that the gut microbiota of all participants was dominated by two strains, Bacteroidetes and Firmicutes (Chahwan et al., 2019).

The graph below (Figure 4) maps the bacterial strains that, according to the clinical trials, have negatively correlated with depression scales, while also increasing their population after probiotic administration. Most of the studies have reported results related to Ruminococcus, which is therefore the population of bacteria of most interest to be administered to seek benefits in depressed patients' symptoms.

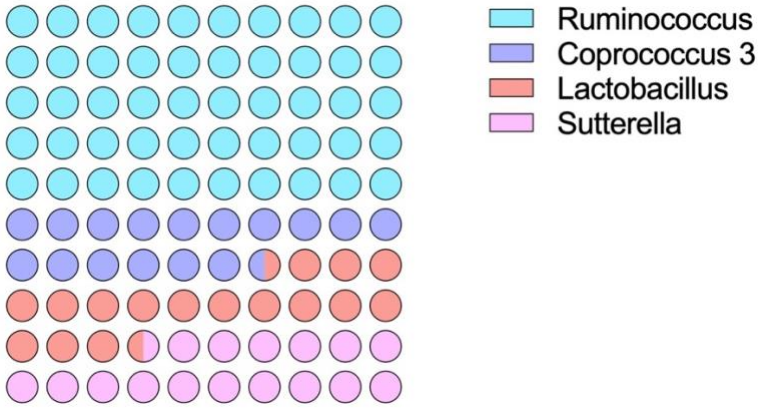
Against the previous two articles, Schaub et al. 2022, discussed in the first result of this review, observed after their intervention that subjects who received the probiotic supplement increased the abundance of the Lactobacillus strain, and interestingly that the Lachnospiraceae ASV increased slightly but not significantly; the increase in Lactobacillus genus was negatively associated with HAM-D and BDI; it is also observed that Ruminococcaceae and Lachnospiraceae ASV showed an increase over time in the placebo group, but there was no significant change between groups; indeed, the group Ruminococcaceae ASV was associated

with decreased HAM-D and Lachnospiraceae ASV was negatively associated with STAI (Schaub et al., 2022).

In relation to PS128, Chen et al. 2021, investigated what effects PS128 could have on psychophysiology in patients with MDD, after 8 weeks of intervention they observed that markers of inflammation, gut permeability and gut microbiota composition did not change significantly, however, some genera were correlated with changes of symptoms and biomarkers like Bifidobacterium and Lactobacillus, who correlated positively with DSSS, and Sutterella who correlated negatively with DSSS; what is more, Coprococcus and Lactobacillus, significantly correlated with both biomarkers and depressive symptoms and, genus Akkermansia was strongly correlated with the level of IL-6 (Chen et al., 2021).

Figure 4

Bacterial Strains



Discussion and conclusions

The overall aim of the present review is to study the relationship between the gut-brain axis and Major Depressive Disorder, and how probiotics may affect it. After analyzing the results obtained in the articles selected, the following conclusions are proposed to address the specific objectives established at the beginning of this paper, which, if we recall, were as follows: to determine which parameters are altered due to the effects of probiotics in patients

with Major Depressive Disorder; to examine if probiotic administration leads to an improvement in depressive symptoms; and to observe if there is a correlation between altered microbiota and Major Depressive Disorder.

Probiotics have been shown to influence Major Depressive Disorder, playing an important role through different approaches, as will be discussed later, leading to an improvement in depressive symptoms. When reference is made regarding the improvement of depressive symptoms, it is referred to the improvement of the main and additional symptoms as defined by the DSM-V. MDD is a complex pathology in which main symptoms are depressed mood, anhedonia, and addittional physiological alterations in sleeping, weight, fatigue and altered psichomotor activity (APA, 2014). Additionally, cognitive symptoms like impaired ability to think or concentrate, indecisiveness, recurrent thoughts of death or suicidal ideation could also appear.

Therefore, the improvement of depressive symptoms has been observed in several different changes after taking probiotics. Firstly, studies show improvements in different scales such as the HAMD-17, DSSS, BDI and EPDS, following probiotic intervention in patients with Major Depressive Disorder (Akkasheh et al., 2016; Chen et al., 2021; Kazemi et al., 2019; Slykerman et al., 2017). These scales are different measuring instruments used to diagnose depression, and the severity of depressive symptomatology. They consist of questionnaires with different items that assess mood severity, guilt, suicidal ideation, insomnia, agitation, anxiety, weight loss and somatic changes.

Secondly, serotonin is a neurotransmitter known as the "happy hormone" and is necessary for the nerve cells and the brain to function. Studies suggest that increased levels of serotonin can reduce stress and depression, as a deficit in this hormone leads to low mood or irritability. As regards serotonin in this review, it was observed that the administration of probiotics reduces the ratio of kynurenine/tryptophan, promoting better synthesis of 5-HT (Kazemi et al., 2019; Rudzki et al., 2019). A better synthesis may imply a decrease in depression, based on what has been explained previously.

Another change that has been reported which allows us to state that taking probiotics improves depressive symptoms, is the increase in BDNF protein levels, as it was significantly correlated with the BDI score (Heidarzadeh-Rad et al., 2020). BDNF is involved in important

neurophysiological processes in the central and peripheral nervous systems and is involved in enhancing neuronal survival, neuroplasticity and neuroprotection (Heidarzadeh-Rad et al., 2020). These BDNF functions are the ones that are being studied and tried to be related to depression, since a better performance of these processes could lead to improvements in mechanisms related to depression and, per se, to depressive syndromes.

Concerning the brain, we can observe changes in its activity and structure. On the one hand, probiotic intake has been observed to significantly improve immediate recall, and a significant difference in activation changes in the hippocampus (Schneider et al., 2023). On the other hand, it has been observed that probiotics influence the brain structure and function of the front-limbic network, increase the volume of grey matter in the calcarine sulcus and alter the activation of the putamen, while also being related to the decrease of HAM-D scores (Schaub et al., 2022; Watanabe et al., 2022). Through these alterations in the activity and structure of the brain, changes in depressive symptoms can be detected since the areas that are affected have been related and studied over the years with depression and the different symptoms suffered by depressed subjects.

All these studies support the idea that probiotics improve depressive symptoms. And at the same time, these studies claim that some parameters are altered due to probiotics treatment in Major Depressive Disorder patients. Therefore, we can state that the administration of probiotics as a complementary therapy to antidepressants can be of great relevance for patients suffering from depression.

As a second result, a specific target of bacterial population has been identified for MDD patients under probiotic treatment, more specifically Ruminococcus and Lactobacillus strains, which show benefits and are present in higher concentrations after probiotic intake. In other words, probiotics have an effect on the abundance of specific strains of bacteria. Consequently, in relation to the third objective, it is true that a specific correlation has not been observed between the altered microbiota and Major Depressive Disorder, but a connection between both of them has been noted, as the improvement in symptoms and the administration of probiotics has modified the microbiota and has increased the abundance of certain strains in the patients we have studied, therefore it can be considered that this relationship does exist. What is needed is more research to specify the correlation between the microbiota and depression.

In relation to the Ruminococcus strain, the following two studies support the above outcome. The first one highlights an improvement of psychiatric symptoms that have been observed along with an alteration of beta diversity and a significant increase of Ruminococcus gauvreauii and Coprococcus 3 among depressed individuals who received probiotics (Reininghaus et al. 2020). Moreover, the other study correlated Ruminococcus gnavus and DASS depression score positively and observed this same strain present in relative abundance in the severely depressed BDI range (Chahwan et al., 2019).

Lactobacillus has also been studied for its benefits in patients with Major Depressive Disorder, but there is a more controversial outcome regarding this strain, as on the one hand, the increase of this genus was negatively associated with HAM-D and BDI (Schaub et al. 2022). Meaning that this increase improves depressive symptoms. On the other hand, together with Bifidobacterium, it has been positively correlated with DSSS (Chen et al., 2021). Meaning the opposite, i.e., the decrease improves depressive symptoms. Therefore, the amelioration of antidepressant symptoms could be related to the increase or decrease in the abundance of Lactobacillus; an ambivalent statement that requires further study in order to establish a relation between this specific strain and Major Depressive Disorder.

What's more, it is necessary to point out that in most of the studies mentioned in the first section of the results, Lactobacillus strains are used, and the favourable results are obtained (Akkasheh et al., 2016; Chen et al., 2021; Rudzki et al., 2019; Slykerman et al., 2017). Further studies are necessary in relation to this strain to determine conclusive findings with respect to its application. Knowing the concentration of this bacterial variety and its effects on patients suffering from depression would be a major breakthrough, in other words, a great advance for the improvement of this disorder and for the betterment of the patients' health situations. Although the latter study mentioned has inconsistent results for Lactobacillus strain, it does show that Sutterella was negatively correlated with DSSS and that the genus Akkermansia and IL-6 level were strongly correlated (Chen et al., 2021). Increasing the range of possible strains for antidepressant enhancement.

If taking probiotics improves depressive symptoms and in turn alters beta diversity while increasing certain strains, as discussed above, it can be stated that specific strains show benefits in MDD patients. Further research focused on the strains that have been described in this study would be worthwhile, not only to see if there is a deficit of these populations in

people with depression but also to verify which strains are the most beneficial as a treatment adjunct to antidepressants.

Both of our reported findings are consistent with those of previous meta-analysis, which claimed a significant probiotic effect on depression (Liu et al., 2019; Sikorska et al., 2023). In this way, this review is up-to-date with the current scenario of probiotics as an additional therapy for depression.

After reaching the preceding results, it can be confirmed that the specific objectives established at the beginning of this review have been fulfilled. Firstly, the effects of probiotics on Major Depressive Disorder have been determined, and the improvement in depressive symptoms has been confirmed. In addition, a correlation between Major Depressive Disorder and specific altered microbiota has been observed. Our main finding is a greater amelioration of depressive symptoms as a result of probiotic administration.

It is worth noting within this discussion that there is a branch in this area of research that focuses primarily on probiotics' effects on healthy subjects. In contrast to the previous outcomes, the following studies support the benefits on stress due to probiotic intervention in healthy subjects and report in their results associations of these benefits with changes in cortisol levels, among other key factors. Beginning with, Messaoudi et al. (2011), provided evidence that the consumption of probiotics, in their study L. helveticus R0052 and B. longum R0175 in combination, mitigated psychological distress in three tests without displaying any adverse event; what's more, the probiotic treatment group reported being more focused on the problem-solving dimension and their cortisol values decreased over time, both facts compared to the control group (Messaoudi et al., 2011).

Following, a pilot study in medical students investigating the effects of the probiotic Lactobacillus casei strain Shirota (LcS) on psychological, physiological and physical responses to stress, failed to detect any significant change in HADS anxiety, STAI-state anxiety, HADS depression, SDS and PSQI scores in any of the groups; although it did show a significant reduction in physical symptoms and a significant increase in salivary cortisol levels one day before the test in the placebo group only, as well as in plasma L-tryptophan (Trp) levels in the same group; these results suggest that LcS daily intake may suppress the stress-induced

modulation of Trp metabolism and can improve serotonin biosynthesis together with Trp metabolism being normalised during post-examination time (Kato-Kataoka et al., 2016).

Results from Watanabe et al. (2022) associated the intake of Lactiplantibacillus plantarum SNK12 (SNK) with an improvement in mood in response to a mental stress load, and suggest that the intake of 50 mg/day SNK could relieve stress, more specifically, negative feelings, anxiety, tension, embarrassment, confusion, anger and hostility; given the significant differences obtained in Total Mood Disturbance scores and salivary cortisol, compared to the placebo group (Watanabe et al., 2022). Similarly, Kim et al. (2021), observed that intervention with probiotics (Bifidobacterium bifidum BGN4 and Bifidobacterium longum BORI) for 12 weeks had an effect on stress scores and improved cognitive function in the probiotic group, while serum BDNF levels increased significantly, this study in healthy older adults provided evidence related to serum BDNF suggesting that the reduction of Eubacterium and Clostridiales strains in the gut, due to probiotic supplementation, is related to increased BDNF and consequently to the improvement of brain functions (Kim et al., 2021).

Contrasting these results, Östlund-Lagerström et al. (2016), when evaluating the effect of the probiotic strain Lactobacillus reuteri DSM17938 on digestive health, well-being and psychological distress in older adults, did not observe any significant improvement effect on well-being, stress or anxiety (Östlund-Lagerström et al., 2016). Along the same lines, Kelly et al. (2017) studied the impact of L. rhamnosus in healthy male participants, and after eight weeks of trial, found that there was no evidence that probiotic treatment was significantly different from the placebo group in modifying stress-related measures, HPA responses, inflammation or cognitive performance; therefore states that the probiotic treatment did not attenuate the stress response and had no clear anti-inflammatory effect (Kelly et al., 2017).

Romijn et al. (2017) is another study that did not find evidence that supports the probiotic formulation's efficacy in treating low mood, and in this case, in moderating the levels of inflammatory and other biomarkers (Romijn et al., 2017). And finally, Rode et al. (2022) found a slight but non-significant effect on sleep patterns after the probiotic intervention, but in relation to stress, this study did not observe that the level of cortisol in saliva was affected by the probiotic intervention either (Rode et al., 2022).

In conclusion, probiotic intervention improves stress in healthy subjects is a very ambivalent result, which reinforces a need for further research into the role of probiotics. Based on the previous discussion, much remains to be studied regarding the role of probiotics in healthy and depressed subjects. This research field has been mentioned because of the role of stress as a factor in triggering depression. Being able to understand stress and also to relate its mechanism to probiotics could be a significant step forward in this research area, and, more importantly, towards the potential role of probiotics in our society.

Limitations, future directions and final conclusions

The GBA research area in psychology is very small, the clinical trials that have been done over the years study different approaches, so most of them confirm that they are "the first ones" to do this kind of investigation. This makes it quite difficult to state specific and reliable results, as each trial focuses on one aspect; in addition to the fact that the samples in most of the studies are very small and therefore not easily generalizable.

Meanwhile, this is an area in high demand, so the number of reviews is overwhelming. There is very limited real research, and it is growing in an unstructured way. Further studies with larger populations are needed to better understand the functioning of GBA in patients with depression, so that solutions could be found to improve antidepressant treatment. As mentioned above, it should also be highlighted that in future studies a focus on certain strains, such as Ruminococcus, would be of great interest to see if these patients have a deficit and therefore an increase in this group of bacteria would lead to an improvement in their symptoms.

To summarize the current situation, more research is required, as has been repeated throughout this review. It is necessary to carry out long-term investigations with larger populations, to measure the effects at different points in time. In addition, studies should focus on the microbiota changes, on the strains that are present during the administration of probiotics, and after this intervention. Obviously, these clinical trials should look at the correlation of all possible results with depressive symptoms, as well as the correlation with the depression scales. Also, if alterations in BDNF and tryptophan are studied in future research, it would be very useful.

To come to an end, this review supports the use of probiotics as a complementary therapy for depressed patients. As there is a lack of good-quality clinical trials in this research field, future studies investigating the impact and efficacy of specific bacterial strains and technical interventions and doses in larger populations are needed. Furthermore, probiotic therapy could be a promising option as an adjunctive treatment, as it appears to be safe and there are no studies contrary to its use. No firm conclusions can be drawn regarding the efficacy of probiotics in healthy populations due to the variability of results in the available studies and the paucity of these studies. However, reliable conclusions can be reached about probiotic intervention in depressive patients, as shown throughout this review, there is evidence of improvement in depressive symptoms after probiotic administration, as well as a possible correlation between this symptom improvement and specific strains, such as Ruminococcus. It would be extremely useful to establish this association in a healthy population or in a population with subclinical symptoms of depression because this would become a key element in depressive disorders prevention. Therefore, further studies, long-term prospective studies, in both healthy populations and depressed populations are necessary.

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