

## **Probiotics supplementation in prophylaxis and treatment of depressive and anxiety disorders – review of current research**

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### **Summary**

In recent years increased interest in the connection of intestinal microflora and the state of human health resulted in a great deal of research on the influence of intestinal microflora in particular on mental health, including works on affective functions of the central nervous system (CNS). Previous studies on animals have revealed the existence of a bi-directional communication system between the gastrointestinal microbiota and the central nervous system, the so-called 'gut-brain axis' which modulates functioning of CNS through immunological, endocrine and neuronal mechanisms. Clinical studies have shown a connection between supplementation of probiotics containing specific species and strains of bacteria and the regulation of the body's response to stress and with the exacerbation of depressive and anxiety symptoms in humans. Studies have also demonstrated differences in the composition of the intestinal microflora of patients with a diagnosis of major depressive disorder in comparison with the healthy population. The aim of this article is to present the current state of knowledge on the relationship between composition of intestinal microflora and affective functions of CNS and on effects of supplementation of probiotics on depressive and anxiety symptoms in humans. Previous studies on the use of probiotics in the prophylaxis and treatment of depressive and anxiety disorders included too small groups of persons, especially in groups of patients diagnosed with depression, to be able to clearly determine the effectiveness of probiotics in prevention and, in particular, treatment of these disorders in humans.

**Key words:** depression, probiotics, microbiota

### **Introduction**

In recent years, the interest in the influence of microbial gut bacteria on development and proper functioning of the human body has increased. Some published works prove that there is a certain dependence between the composition of intestinal microflora and the human mental state. Animal studies have shown that there is an effect of interven-

tion with probiotics containing specific bacterial strains on the results of behavioral tests; they have allowed us to formulate hypotheses concerning the mechanisms of the influence of intestinal microflora on the central nervous system. The studies also describe experiments on the relationship of administered probiotics with the regulation of body response to stress and exacerbation of depressive and anxiety symptoms in humans. Moreover, they show differences in the intestinal microflora composition of patients diagnosed with major depressive disorder, compared to the healthy population.

### **Aim**

The aim of this article is to present the current state of knowledge on the relationship between composition of intestinal microflora and affective functions of the CNS and on the effects of supplementation of probiotics on depressive and anxiety symptoms in humans.

### **Method**

While preparing the article, the PUBMED database was searched (2004–2018) using the following key words: depression, probiotics, anxiety disorders, and intestinal microbiota. Articles in English and Polish were taken into account.

### **Intestinal microbiota**

The term ‘microbiota’, ‘microflora’ refers to microorganisms – bacteria, fungi, viruses – occurring in a specified environment (e.g., the human body). Definition of ‘microbiome’ applies to a set of genes of microflora organisms [1].

In the process of evolution, the microbe of the digestive tract has become a commensal organism in its relation to humans (commensalism is the type of relationship between species in which one of the organisms benefits it without harming the other). At the time of birth, the neonate’s digestive tract is sterile [2]. Immediately after birth, bacterial colonization occurs; proper colonization of the intestine affects the development of immune tolerance [3, 4], body response to stress [5], angiogenesis [6]. Between the 5<sup>th</sup> and the 7<sup>th</sup> year of life full intestinal microbial stabilization occurs; at the time its composition is similar to microflora of an adult [7, 8]. A number of factors affect the variety of the bacterial flora in adults, including: health condition, age, diet, antibiotic use [9].

It is estimated that the number of microbial cells in the human body is ten times higher than that of our own somatic cells – the microbiota consists of about  $10^{14}$  cells [10]. The microbiota is composed of more than a thousand species of bacteria, fungi, archaea, and viruses [11]. The most numerous type of bacteria are Firmicutes (64%), Bacteroidetes (23%), Proteobacteria (8%) and Acinetobacter (3%) [12]. The composition of the flora varies depending on the section of the gastrointestinal tract [13]. Intestinal microbiota performs a variety of functions in the body, i.e., it synthesizes B and K vitamins [14], affects the absorption of magnesium, calcium and iron ions,

conversion of fatty acids, stimulates the development of the immune system [15] and performs protective functions – commensal bacteria compete with pathogenic bacteria for nutrients and receptors on the surface of the intestine. Intestinal bacteria affect the intestinal epithelial tightness by influencing the gene expression of some structural proteins in the so-called tight junctions between intestinal epithelial cells [16].

Increasing awareness of the impact of intestinal microflora on human health resulted in starting the so-called *Human Microbiome Project* in 2008, conducted by the National Institute of Health of the United States, aimed at gaining comprehension of the composition of human microbiota and its relationship to various disease states [11]. Studies on the effects of microbiota influence on tumors, obesity, diabetes and irritable bowel syndrome have been carried out [10]. Research on animal models has revealed that intestinal microbiota may also be associated with the functioning of the central nervous system. It has been stated that children diagnosed with autism have displayed a significant increase of the *Clostridium* bacteria in comparison to healthy group [17].

One of the research topics addressed in this area is the meaning of the so-called gut-brain axis in mood disorders. The gut-brain axis is a complex, two-way system of dependence between intestinal microflora and brain operation. Fluctuations of intestinal microflora may cause changes in mood and behavior, while psychological stress can cause disturbances of the normal condition of the intestinal microflora [18].

### **Mechanisms of the action of the gut-brain axis in preclinical animal studies**

Studies on animal models provided much knowledge on mechanisms of the influence of intestinal bacteria on the central nervous system and the regulation of mood. Inter alia, it has been proved that the increase of the permeability of the intestinal barrier, leading to the inflammatory reaction is associated with the occurrence of depressive symptoms [19]. It has been shown that probiotics have a positive influence on the integrity of the intestinal barrier, reduce the body's response to stress, increase the level of serotonin and the expression of GABA ( $\gamma$ -aminobutyric acid) receptors in the CNS. Intestinal bacteria found in probiotics have also the ability to produce neurotransmitters.

In 2004, Sudo et al. [5] conducted a study on the model of GF (germ free) mice grown in completely sterile conditions, devoid of any intestinal bacterial flora and SPF (specific pathogen free) mice, with normal microbiota devoid of specific pathogens. Both groups of mice were subjected to stressors, which resulted in a higher increase in corticotropin (ACTH) and corticosterone in GF mice than in SPF mice, indicating the functioning mechanism of bacteria through activity modulation of the hypothalamic-pituitary-adrenal axis (HPA). In addition, this study showed that the HPA axis response normalized after colonizing GF mice with bacteria from SPF mice if GF mice were colonized at the early stage of development. Colonization at a later stage of development did not lead to normalization of stress response.

The experiment also proved that GF mice showed lower levels of brain-derived neurotrophic factor (BDNF) in the cortex and hippocampus than SPF mice. BDNF is a protein belonging to the family of nerve growth factors, affecting the development of

serotonergic, dopaminergic, noradrenergic and cholinergic neurons. The relationship between BDNF concentrations in serum and exacerbation of depressive symptoms has been demonstrated (concentration of BDNF measured in plasma corresponds to the concentration in the CNS) [20].

A similar research methodology based on the GF mice model was presented in 2011 by Heijtz et al. [21]; however, no stress factors were used in the experiment. In behavioral tests, it has been proved that GF mice showed less control over their behavior than SPF mice, as manifested by increased motor activity in GF individuals. It was shown again that the colonization of these individuals with the bacteria of SPF mice early in life causes normalization of behavior. This is the consequence of the different expression of genes responsible for inter-synaptic transmission in the brain on which motor functions and anxiety behavior is dependent. The study also showed lower gene expression for BDNF in areas of the brain responsible for feeling anxiety, in particular the hippocampus, amygdala, cingulate gyrus, in GF mice.

The relationship between the development of depression, immune response and bowel function is currently explained by the so-called leaky gut syndrome. Studies have shown that tight junctions deteriorate under stress. Tight junctions are tight connections between intestinal epithelial cells. Loss of integrity between epithelial cells leads to the translocation of intestinal bacteria through the intestinal barrier to the circulatory system [22]. Zareie et al. [23] conducted a study in which they showed that in mice subjected to chronic stress, increased uptake of intestinal bacteria was observed in the intestinal lymph nodes; this indicates a certain weakening of the intestinal barrier. Penetration of bacteria outside the intestinal lumen leads to inflammation in the body. The relationship between elevated markers of inflammation and lowered mood has been observed in animal models for many years [24]. In the same study, it was demonstrated that the probiotic containing *Lactobacillus rhamnosus* R0011 and *Lactobacillus helveticus* R0052 prevented the adhesion of pathogenic bacteria to the intestinal epithelium and their translocation to the mice blood vessels. Probiotics compete with pathogenic bacteria for receptors on the surface of the intestinal epithelium; this may explain the influence exerted by probiotics.

The impact of probiotic bacteria on serotonin concentration in the central nervous system was studied by Desbonnet et al. [25]. In the study the levels of tryptophan, the precursor of serotonin, and kynurenine acid, a product of metabolic transformation of tryptophan with a neuroprotective effect [26] in mice treated with a probiotic containing *Bifidobacterium infantis* 35624 were assessed in relation to individuals from the control group. In the group of mice receiving the probiotic, higher concentrations of both factors were noted. The examined mice were also tested for cytokines: IFN-gamma, TNF-alpha and interleukin-6, whose levels were lower in mice receiving *B. infantis*. Cytokines lower the amount of tryptophan in the serum, which may affect the occurrence of depressive symptoms. The study shows that the probiotic *Bifidobacteria infantis* reduces the level of proinflammatory cytokines, which has a positive effect on the redirection of tryptophan to the metabolic pathway of conversion to serotonin (kynurenine pathway). A year later, the same research group presented the results of studies on the maternal separation model where infant mice were separated from

mothers in the early-childhood period, confirming the observations described above. Separation from mothers causes profound and long-lasting changes in the development of the central nervous system, including the regulation of the body's response to stress. It is a well-established model for studying mental disorders induced with stress (including depression) [22]. In the test experiment, mice were given a probiotic or citalopram. Probiotic therapy resulted in lowering interleukin-6 level, elevation of norepinephrine levels in the brainstem and improvement in behavioral tests. Similar effects were obtained in the group of mice receiving citalopram.

Intestinal bacteria influence the central nervous system via the vagus nerve. Study performed by Bravo et al. in 2011 [27] showed that the administration of *Lactobacillus rhamnosus* (JB-1) has changed the expression of GABA receptors in the central nervous system and reduced the body's response to stress manifested in the growth of corticosterone level. Administration of probiotics also improved performance in behavioral tests – less anxiety and depressive behaviors have been observed in behavior of tested mice. In mice that underwent vagotomy, the same tests were performed without observing any effects of the kind described above.

Later research has shown that intestinal bacteria produce substances such as noradrenaline, serotonin, melatonin, GABA, histamine, acetylcholine in a sufficiently large amount to affect the host's physiology [28].

Understanding the mechanisms described above that explain the relationship between the regulation of the body's response to stress, pathogenesis of depression and the functioning of the bacterial intestinal flora, as well as the effectiveness of interventions with probiotic bacteria in animal experiments, underlay further research in the field of application of probiotics in treatment of depressive and anxiety disorders in humans.

### **Clinical trials**

The relationship between intestinal and mental disorders was observed, inter alia, on the basis of the frequent co-occurrence of irritable bowel syndrome (IBS) and depressive/anxiety disorders in humans. Studies have shown that even as much as 70 to 90% of patients treated for IBS suffered from mood disorders [29]. Most likely, it is the inflammation in the body that plays a common role in the pathogenesis of IBS and depression.

In a clinical study on a group of 28 patients with major depressive disorder and a 23-person control group, Maes et al. [19] confirmed the relationship between elevated levels of inflammatory cytokines and symptoms of depression, so far observed in the animal study described above. The study showed that patients diagnosed with depressive disorders had significantly elevated levels of IgM and IgA immunoglobulins against lipopolysaccharides of the Gram-negative enterobacteria, that occur in the normal intestinal microflora, compared to the control group; this may suggest an involvement of leaky gut syndrome and intestinal bacteria translocation to the circulatory system in the pathogenesis of depression. The hypothesis that changes in the intestinal microbiota may be an etiological factor of mood disorders was put forward at the beginning of the 20<sup>th</sup> century. In 1910, Dr. George Porter Phillips made an

attempt to treat adults with 'melancholia' using a gelatin with live lactic acid bacteria; he observed a reduction of depressive symptoms [30].

Two studies were designed to search for correlation between the condition of the intestinal microflora and symptoms of depression. Jiang et al. [31] compared fecal samples of 46 patients with the diagnosis of major depressive disorders to the samples of 30 healthy individuals. The study displayed a statistically significant increased proportion of Bacteroidetes, Proteobacteria and Actinobacteria bacteria and a reduced proportion of Firmicutes in the intestinal microbiota of patients diagnosed with depression. There was also a greater diversity of species in microbiota of patients diagnosed with depression. The study also compared the indicators of inflammation (IL-6, TNF- $\alpha$ , IL-1 $\beta$ ) and BDNF levels in the control group and the ill patients. No differences were found in the indicators of inflammation, while BDNF level was lower in patients with depression compared to the healthy subjects.

Naseribafrouei et al. [32] compared the microbiota of 37 patients diagnosed with depression with the microbiota of 18 healthy individuals. There was no significant difference in the diversity of bacterial species in the healthy controls and the ill patients with a slight tendency to a larger number of bacterial species in patients who suffered from depression. In depressed patients, however, the proportion of Bacteroidetes in the composition of microbiota was higher than in healthy ones.

Next to the animal studies, which resulted in a better understanding of the functioning of the gut-brain axis, a few studies in human population were carried out to date; they were designed to supply specific probiotics to the participants and assess their mood changes and/or the severity of anxiety using scales such as: General Health Questionnaire (GHQ), Depression Anxiety and Stress Scale (DASS), Leiden Index of Depression Sensitivity-Revised (LEIDS-r), State-Trait Anxiety Inventory (STAI), Development Behavior Checklist (DBC), Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Hopkins Symptom Checklist (HSCL-90), Hospital Anxiety and Depression Scale (HADS), Perceived Stress Scale (PSS), Coping Checklist (CCL), Profile of Mood State (POMS). Some of the studies evaluated the markers of inflammation and BDNF in the serum or the level of cortisol as an assessment of the body's response to stress. Two studies assessed brain activity in the functional magnetic resonance imaging before and after probiotic supplementation.

The majority of studies described below were conducted on healthy volunteers, one of them concerned patients with chronic fatigue syndrome, two of them involved patients diagnosed with depression, in one of them patients were additionally diagnosed with irritable bowel syndrome.

In a double-blind study conducted by Benton et al. [33], 124 healthy volunteers consumed milk enriched with *Lactobacillus casei*, a Shirota strain, for 20 days. Volunteers were divided into three subgroups depending on the initial mood. Results – evaluated using the POMS (Profile of Mood States) – showed statistically significant improvement in the mood of the group of people who initially assessed their mood as the most lowered.

In the study conducted by Messaoudi et al. [34], 55 healthy volunteers were given a mixture of probiotic bacteria containing *Lactobacillus helveticus* R0052 and

*Bifidobacterium longum* R0175 or placebo for 30 days (double-blind study). Before and after the trial participants were assessed using the Hopkins Symptom Checklist (HSCL-90) and Hospital Anxiety and Depression Scale (HADS), Perceived Stress Scale (PSS), and Coping Checklist (CCL), the level of cortisol in the urine was examined in addition. Probiotic supplementation reduced the severity of anxiety, depression, somatization, anger–hostility indicator in the Hopkins Symptom Checklist (HSCL-90), and improved the results in the Hospital Anxiety and Depression Scale (HADS); there was also a decrease of urinary cortisol level in the group that received the probiotic. Re-examination of 25 patients from the same group of volunteers with the lowest level of stress assessed by the urinary cortisol level at the beginning of the study revealed a sustained improvement in HSCL-90 and HADS scores [35].

Chung et al. [36] in 2014 conducted a study aimed at the assessment of cognitive functions and mood after 12-week administration of a probiotic containing *Lactobacillus helveticus* IDCC3801 bacteria to 35 healthy people at the age of 60–75. The patients' mood was evaluated using the Perceived Stress Scale (PSS) and the Geriatric Depression Scale-Short Form (GDS-SF). In this study, no significant improvement in the mood was found after the intervention. The study also assessed the level of BDNF; there was no increase in BDNF after the intervention.

In 2012, Shinkai et al. [37] conducted a study involving 300 healthy volunteers at the age of 65 or older. Participants of the study were divided into three groups: group taking high dose of *Lactobacillus pentosus* strain b240, group taking low dose of the same probiotic and the last group was given placebo. The study lasted for 20 weeks. Patients were evaluated for mood and the level of stress before and after the intervention with the POMS and a questionnaire to assess the quality of life (SF-36v2 Health Survey, containing items concerning anxiety and mood as elements affecting the quality of life of the subjects). The results of the experiment showed a statistically significant improvement in well-being and subjective perception of subjects' health in the group receiving probiotics compared to the placebo group.

In a double-blind study by Östlund-Lagerström et al. [38], 290 healthy subjects aged 65 and over, received *Lactobacillus reuteri* DSM 17938 or placebo for 12 weeks. Depression and anxiety symptoms were assessed using the HADS, the severity of patients' stress was assessed using the Perceived stress scale (PSS) and the EQ-5D-5L scale (EQ-5D index and EQ-VAS) was used for subjective assessment of the quality of life of the subjects. After probiotic intervention, there was no improvement in severity of depressive and anxiety symptoms in patients whose baseline questionnaires revealed presence of such symptoms. There was also no reduction in stress or difference in the general well-being of patients receiving the probiotic compared to the placebo group after 12 weeks of study.

Steenbergen et al. [39] examined a group of 40 healthy students who received a probiotic containing *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, and *Lactococcus lactis* W19, and W58 or placebo for 28 days. Participants were assessed before and after the intervention using the following scales: the Beck Anxiety Inventory (BAI), Beck Depression Inventory II (BDI-II) and

Leiden index of Depression Sensitivity Scale (LEIDS-r). No differences were found in the first two questionnaires, nevertheless the initial score was very low in the healthy volunteers, which could be the reason of no change observed after the intervention. The probiotic supplementation influenced the reduction of a cognitive response to lowered mood, especially in the form of ruminations and aggressive thoughts measured on the Leiden scale.

In a study by Mohammadi et al. [40], 70 patients – petrochemical workers, received a probiotic containing *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Bifidobacterium breve*, *Bifidobacterium longum*, and *Streptococcus thermophilus* (strains of probiotic bacteria were not specified) or placebo for 6 weeks. Participants mood was assessed at the beginning and at the end of the study using the General Health Questionnaire (GHQ) and Depression Anxiety and Stress Scale (DASS). The level of cortisol in the serum of participants was also evaluated at the beginning and at the end of the intervention. The study showed a statistically significant improvement in mood of the group receiving the probiotic, but showed no difference in the activity of the HPA axis measured by evaluating the serum cortisol level.

Romijn et al. [41] supplied a probiotic containing *Lactobacillus helveticus* R0052, *Bifidobacterium longum* R017579 (strain I-1722 in the French National Collection of Cultures of Microorganisms [CNCM], Institut Pasteur, Paris, France) to 79 participants with depressive symptoms on self-report mood measures for 8 weeks. The study evaluated the impact of the probiotics on mood and anxiety using the Montgomery-Åsberg Depression Rating Scale (MADRS), Improved Clinical Global Impressions (iCGI), Global Assessment of Functioning (GAF), DASS-42, and QIDS-SR16. Levels of proinflammatory cytokines IL-1 $\beta$ , IL-6, TNF- $\alpha$  in serum were also evaluated. In this study, no significant differences were found prior to administration of the probiotic and after the end of the trial in both psychometric results and the indicators of inflammation in the blood.

Akkasheh et al. [42] conducted a study involving 40 patients diagnosed with major depressive disorder. Patients received *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium bifidum* probiotics or placebo (double-blind trial) for 8 weeks. The Beck Depression Inventory (BDI) was used for the evaluation at the beginning of the study and after its completion. The results showed that consumption of probiotic significantly reduced BDI scores. In addition, the metabolism of insulin, lipidogram, hs-CRP, and oxidative stress biomarkers were compared at the beginning and at the end of the study. The group receiving probiotics obtained improvement in the metabolism of insulin, probiotics also reduced oxidative stress in patients with major depressive disorder.

Rao et al. [43] examined 35 patients with chronic fatigue syndrome who received the *Lactobacillus casei* strain Shirota (LcS) probiotic for two months (double-blind trial). Patients were evaluated for mood and anxiety before and after the intervention with the BDI and BAI scales. The results showed a significant improvement in the BAI, with no improvement in the BDI. In this study, fecal samples were also collected to assess aerobic, anaerobic bacteria, *Lactobacillus* spp, and *Bifidobacteria* spp.

As expected, the increase in the number of *Lactobacillus* was observed in the group receiving probiotic. Unexpectedly, in this group an increase in *Bifidobacteria* number was observed; this is a desirable phenomenon, because *Bifidobacteria* belong to the healthy intestinal bacterial microflora and its number is reduced in patients who suffer from chronic fatigue syndrome.

In 2013, a study on the impact of probiotic intervention on the activity of the areas of the brain responsible for emotional responses, assessed with the fMRI was published [44]. In this study, for 4 weeks, 12 healthy women consumed milk products with the addition of a set of probiotics containing *Bifidobacterium animalis* subsp *lactis* (CNCM strain number I-2494), *Streptococcus thermophilus* (CNCM strain number I-1630), *Lactobacillus bulgaricus* (CNCM strain number I-1632 and I-1519), and *Lactococcus lactis* subsp *lactis* (CNCM strain number I-1631), 11 women consumed unfermented milk, 13 women did not take any products. All of the participants were examined with the fMRI method before and after the intervention to observe the brain's response during the task of recognizing emotions on faces. The results showed that the consumption of probiotics reduced the activation in the insular cortex, somatosensory cortex and other neuronal areas responsible for affective functions in response to the task of recognizing emotions.

A pilot study conducted in 2017 by Pinto-Sanchez et al. [45] assessed the effectiveness of probiotics in patients diagnosed with IBS and mild to moderate depressive and anxiety symptoms. 44 patients were treated with *Bifidobacterium longum* NCC3001 or placebo for 6 weeks. The study showed an improvement in depressive symptoms after probiotic supplementation, no improvement in the severity of anxiety symptoms assessed using the HADS and STAI, and an improvement in the subjective quality of life of IBS patients. CNS imaging in the fMRI study performed before and after the intervention showed a reduction in limbic reactivity in response to negative emotions in patients receiving probiotic compared to those taking placebo.

## Discussion

Two meta-analyses of studies on the influence of probiotics on depressive symptoms have been published so far. The meta-analysis of five randomized, double-blind, placebo-controlled clinical trials published in 2016 found the effects of probiotics on mood to be statistically significant in both depressed and healthy individuals [46]. The other meta-analysis of the research on the impact of probiotic interventions on patients' mood published in 2018 in relation to the meta-analysis of 2016 included five additional randomized clinical trials on healthy volunteers and two studies conducted in individuals with depressive symptoms [47]. The results of this meta-analysis showed that probiotic supplementation significantly improved the mood of individuals with mild to moderate depressive symptoms, but had statistically insignificant effects in healthy individuals. The results are therefore inconclusive.

In the five studies described above, healthy volunteers showed an improvement in psychometric tests after probiotic supplementation; however, in three studies no statistically significant difference in the results was observed, while in one of the studies

the improvement occurred only in the group of patients with the lowest initial mood. Two studies assessed the level of cortisol as an indicator of the body's response to stress; only in one study a decrease in the level of cortisol after finishing the probiotic supplementation was observed. One attempt to intervene with probiotics was conducted on a group of patients diagnosed with depression, obtaining an improvement in the BDI. Another one was carried out on a group of patients with mild or moderate depressive and anxiety symptoms and IBS diagnosis, obtaining improvement in depressive symptoms after the probiotic supplementation, without improvement in the severity of anxiety symptoms.

The results of the evaluation of the composition of the intestinal microbiota in the healthy volunteers and patients with depression are also ambiguous; testing of fecal samples showed some differences, but in one of the studies they were not statistically significant. In both studies the diversity of bacterial flora was greater in patients with depression than in the healthy individuals, and the percentage of Bacteroidetes bacteria, as well as the number of bacteria *Alistipes* – one of the genus in the Bacteroidetes cluster – was higher than in the healthy group. The results of the study by Rao et al. [43], which showed an improvement in the composition of the intestinal flora after the probiotic supplementation of patients with chronic fatigue syndrome, are promising.

Based on the presented studies, the influence of probiotics supplementation on the symptoms of depression is difficult to determine because most of the studies have been conducted only on healthy volunteers. Different scales were used to assess mood changes of the subjects; they assess non-specific parameters such as lowered mood, irritability, severity of anxiety and stress. Factors such as sleep disorders were not taken into account.

Several studies have used MRI techniques in addition to psychometric tests to assess the effects of probiotic interventions in humans. Studies by Tillish et al. [44] and Pinto-Sanchez et al. [45], in which fMRI examination was performed before and after the intervention, showed that probiotic reduced brain reactivity associated with affective functions compared to placebo. A study in which 54 healthy volunteers received a mixture of probiotic *Lactobacillus casei* W56, *Lactobacillus acidophilus* W22, *Lactobacillus paracasei* W20, *Bifidobacterium lactis* W51, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19, *Bifidobacterium lactis* W52, *Lactobacillus plantarum* W62, and *Bifidobacterium bifidum* W23 or placebo for 4 weeks, was published In 2018. The study aimed at investigating the influence of a multi-strain probiotic administration on whole-brain functional and structural changes in healthy volunteers. After supplementation of probiotics, the changes of brain activation in areas associated with emotions, attention control and behavior were observed in the fMRI study, but no structural changes in the brain were observed [48].

Studies described above did not consider diet, which has a significant impact on the intestinal microflora, although it was assumed that participants will not significantly change their diet during the study. Differences in the microbiota depending on sex and age were not taken into account either. The studies conducted so far concerned the adult population. Three of the above-mentioned studies concerned the population of people at the age of 60 or over. Studies on elderly people gave inconclusive results,

therefore it is not possible to assess how the effectiveness of probiotics supplementation is determined by the age of the patients. There have been no studies on the impact of probiotic supplementation on depressive and anxiety disorders among children and adolescents. Another limitation of the studies was the duration of the trials – while antidepressants are tested for a minimum six week period [49], in some of the studies the supplementation of probiotics lasted for a shorter time. The examined groups were relatively small.

Based on the clinical trials carried out so far, the problem is the determination of the proper probiotic dose for the possible prophylaxis of affective disorders. In addition, there are signals from control institutions that many probiotic supplements available in Poland contain lower number of viable probiotic bacteria than declared by the producer still in the shelf-life period. It is therefore necessary to conduct studies of probiotics in order to assess the content of live bacteria during the shelf-life period. It is therefore necessary to carry out detailed studies of probiotics in order to assess the content of live bacteria during their shelf-life.

Different species and strains of bacteria were used in the above-mentioned studies, mainly rod-shaped bacteria from the genera *Lactobacillus* and *Bifidobacterium*. In many studies, a combination of several strains of bacteria was used, which does not allow to determine exactly which strains specifically affected the mood of the subjects. In an answer to a clinical question whether probiotic supplementation is effective in the treatment of symptoms of depression and anxiety, published in *The Family Physicians Inquiries Network* (FPIN) in 2018, authors cited a study in which the combination of three probiotic species slightly improved symptoms in patients diagnosed with depression [42], while in another study it was shown that *Lactobacillus casei* does not affect depressive symptoms in patients with chronic fatigue syndrome, but it reduced the anxiety symptoms [43, 50]. The supply of *Lactobacillus* increases the expression of GABA receptors in mice in areas of the brain associated with emotions [27]. Studies in mice showed that bacterial infections of the gastrointestinal tract and long-term inflammation were associated with the activation of brain areas related to anxiety and the occurrence of anxiety behaviors [51].

Further research on the potential use of probiotics as prophylaxis of anxiety disorders appears to be important due to high prevalence of anxiety disorders causing the necessity of using anxiolytic drugs, with a number of side effects, while probiotics are safe, even with long-term use [52]. This attribute of probiotics preparations may be of great importance for pregnant women and breast-feeding mothers, for whom the possibility of using anxiolytic drugs is limited. In 2018, a systematic review of human and animal studies regarding the relationship between stress, bacterial microflora of pregnant subjects, anxiety and depressive disorders in perinatal period was published. Stress affects changes in bacterial microflora both in reproductive tract and intestine of pregnant individuals. Fecal samples of animals subjected to stressors during pregnancy showed changes in relation to fecal samples of non-stressed animals, additionally differences in placental microbiota were found, however, not statistically significant. Changes in microbiota which occur under the influence of stress, can have potentially great impact on the mental state of pregnant women due to inflammation they cause

[53]. In a study by Slykerman et al. [54] published in 2017, pregnant women consuming the probiotic had statistically significant reduction in the severity of depressive and anxiety symptoms and lower rate of postpartum depression compared to subjects taking placebo.

### Recapitulation

Previous clinical trials on effectiveness of probiotics in prevention and treatment of depressive and anxiety disorders included too small groups of persons to be able to unequivocally assess the effectiveness of probiotics in this application. The object of concern in the published studies was not so much treatment of depression, but improvement of patients' mood; the reason is that they were carried out on healthy volunteers.

It is possible that taking probiotics could prevent disturbances in the composition of intestinal microbiota as a result of chronic stress; by reducing inflammation and increasing serotonin biosynthesis, the use of probiotics could be part of prevention of depression.

An important advantage of probiotic supplementation is the fact that, as current studies show, no serious side-effects have been observed after ingestion of the products in question.

It is necessary to conduct further research, especially in groups of patients diagnosed with depression, children and adolescents, taking into account a possibly larger number of respondents and longer duration of tests to assess the use of probiotics as an element of prophylaxis and perhaps, in the future, of treatment of depressive and anxiety disorders.

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