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Exploring the therapeutic potential of the gut microbiome in treating anxiety and depression

Xu Y[1]

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Abstract
The gut microbiome is the collection of microorganisms located in the human gastrointestinal system. Their interaction with food and host cells can profoundly impact human health. Recent studies have shown that the gut microbiome can alter the activity of the human brain. The brain diseases, or anxiety and depression in this review, are influenced by multiple factors, including epigenetics, the Gut-Brain Axis (GBA), inflammation, and the microorganism metabolome; among others. The gut microbiome can affect the Central Nervous System (CNS) through the vagus nerve, release multiple molecules that affect physiological responses such as systemic and neuro-inflammation, secrete neurotransmitters, and also shape the neural pathways. Furthermore, the biological functions of the gut microbiome; including neruromodulation; are influenced by the compositional ratio of different bacterial species. Some clinical trials have confirmed the effectiveness of certain probiotic compositions in treating mood-related diseases. This review discusses in detail how the different brain-related pathways operate, the impact of the gut microbiome on brain activity and behavior, the gut microbiota interactions, the potential for microbiome-based treatments for anxiety and depression, and their limitations.

Keywords
Gut-brain axis, Gut microbiome, Microbiota, Anxiety, Depression, Inflammation, Central nervous system, Fecal microbiota transplantation, Germ free animal models, Telomere

[1]Corresponding author: Yixin Xu, The High School affiliated to Renmin University of China, 37 Haidian Street, Beijing 100080, China. monicayixin@gmail.com

Introduction

Anxiety and depression have become the most pervasive mental disorders worldwide. The increase in the yearly prevalence rates of anxiety disorders as a group is >10% in the USA and in the UK (1). In general, the symptoms of depression are evident: pain, fatigue, and sleep disturbances (2), while anxiety is usually observed as the patient perceiving threat, restlessness, moodiness, tension, and physical symptoms including dry mouth and sweating (3). The above symptoms are disruptive to patients' social lives and affect people of all ages worldwide. Conventional treatments may require drugs or other medications, including serotonin-selective reuptake inhibitors (SSRIs) and serotonin-noradrenaline reuptake inhibitors (SNRI) (4). However, standard SSRI/SNRI monotherapy is not responsive for most patients or becomes less so over time, and dosage increases or the addition of other drugs frequently adds unpleasant side effects that include headache, insomnia, and somnolence, among others (4, 5). Furthermore, patients frequently become tolerant of these drugs, resulting in a progressive loss of effectiveness (4). Thus, there is a need for more effective, tolerance-resistant, and minimal adverse-effect-producing therapies for these mental health disorders.

In recent years, researchers have obtained a better understanding of the gut microbiome and recognized its potential in treating disease. The gastrointestinal microbiome, or gut microbiome, is present in the gut of all mammals in the form of diverse species of fungi, bacteria, archaea, protozoa, viruses, and even prions (6). The absolute and proportional composition of genera and/or organisms populating the human gut microbiome has been found to be generally indicative of an individual's overall health. Enough evidence has accumulated to suggest a correlation between the gut microbiome and mental health as well. Multiple signaling pathways exist by which the immune system, nervous system, and circulatory system can connect the human brain and the gut in a bidirectional manner. The study of this bidirectional Gut-Brain Axis (GBA) is an ongoing active area of investigation because the possibility of treating mental health disorders by modulating the composition of the microbiome offers a tantalizingly effective and safe treatment modality. One of the first attempts to alter microbiota composition involved transplanting the microbiota from disease-free individuals into diseased patients; termed Fecal Microbiota Transplantation (FMT). The anecdotal high success rate of FMT has spawned many next-generation improvements that involve purified or sterile consortia of bacteria or spores respectively from the microbiota of healthy donors. Many of these ‘living drugs’ are in clinical trials.

Overview of mental health disorders: anxiety and depression

To understand how the gut microbiome may help in treating anxiety and depression, it is important to understand the detailed mechanisms of the diseases. In general, environmental adversity may be the biggest contributor to depression and anxiety. Studies have shown that childhood trauma, including physical/emotional abuse, physical/emotional neglect, loss/separation experiences, or sexual trauma, increases the risk of mental disorders over a person’s lifespan (1). Furthermore, in animal models, researchers found

hyperreactivity of the hypothalamic-pituitary-adrenal axis as well as damage to the blood-brain barrier integrity after early-life adversities (1). Prenatal stressors and negative life events in adulthood can also lead to depressive and anxiety disorders.

Anxiety and depression also have a genetic component. However, unlike monogenic diseases, they do not follow a Mendelian mode of inheritance. Schiele et. al. found that there was a complex-genetic inheritance pattern in anxiety disorders based on segregation analysis, including interactions of multiple susceptibility genes and their interactions with environmental factors (1).

Anxiety

Anxiety is classified into several types, including Generalized Anxiety Disorder (GAD), Social Anxiety Disorder (SAD), post-traumatic stress disorder (PTSD), and panic disorder. The activation of an anxiety disorder involves a wide range of biological processes within the human body. For instance, stress-induced inflammation contributes to anxiety disorders. Studies show that stress can induce high levels of proinflammatory cytokines, such as interleukin (IL)-6, which cause the activation of systemic immune responses (7). Moreover, the activation of an immune response is correlated with the increase in anxious behavior in murine models (8). Nevertheless, the true mechanisms of anxiety disorders are still not fully understood, but there are plausible hypotheses, especially for GAD. One hypothesis is that there is an anxiety circuit in the brain that consists of the prefrontal cortex and amygdala. The circuit includes two pathways: the fast path and the slow path, both relying on the amygdala to activate anxiety-like behavior (9). Another hypothesis involves Papez's circuit, a neural circuit proposed by J.W. Papez. This hypothesis asserts that in addition to the amygdala, other structures of the limbic system are also involved in anxiety activation with the prefrontal cortex. The circuit originates in the hippocampus; passes through the mammillary body, the anterior nucleus of the thalamus, and the cingulate gyrus; and, finally, returns to the hippocampus (9).

Depression

Depression consists of 15 subtypes classified under different criteria (10). The most common types are minor and major depressive disorders (MDD). An early hypothesis, the monoamine hypothesis, proposed that MDD is caused by neuro lesions of central noradrenergic and serotonergic systems, thus targeting them can potentially treat the disease (11). This hypothesis is the basis for SSRI intervention. It is known that monoamine neurotransmitters can be produced by gut microbiota, hence repopulating the gut using appropriate bacteria may confer a similar effect as SSRI administration.

Another compelling hypothesis is termed the "pathogen-host hypothesis". First, depression risk alleles promote greater immune activation that would have been an important survival trait for human ancestors, giving them a reproductive advantage. This immune activation then promotes the release of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α (7, 12). Then, similar to anxiety, the increase of proinflammatory cytokines also activates an immune response and thus leads to depression, in a cause-consequence cyclical pattern (7). Consistent

with this hypothesis, randomized trials have shown that anti-inflammatory agents can reduce depressive disorders, yet concomitantly increase susceptibility to infections. According to researchers, western diets with high-calorie foods can activate inflammation, while Mediterranean dietary patterns which contain more fruits and vegetables are less likely to have this effect. Thus, the Mediterranean diet can serve the same effect as anti-inflammatory agents, and intriguingly, populations adopting such a diet are less likely to suffer from depression (13), presumably because the gut microbiome that this diet selects for, or can induce immune activation without causing inflammation.

The metabolism of tryptophan (TRP) *via* the tryptophan–kynurenine (KYN) pathway plays a major role within the GBA. The release of proinflammatory cytokines induces the synthesis of kynurenine. Kynurenine; an amino acid; is a product of tryptophan metabolism. In one pathway, it is converted into kynurenic acid; whereas in another pathway, kynurenine is converted to 3-hydroxykynurenine and eventually to quinolinic acid. These two acids can both act as agonists at the N-methyl-d-aspartate glutamate receptors, which in turn activate the programmed cell death (apoptosis) of the astrocytes. Since astrocytes are neurons responsible for supporting and separating other nerve cells, their apoptosis causes neurodegeneration as it results in the exposure of neurons to the exotoxic kynurenine metabolites which cause chronic depression (14). A variety of probiotics have been shown to lower the KYN/TRP ratio while alleviating depressive symptoms.

The most widely accepted cause of depression is the decrease of 5-hydroxytryptamine (5-HT), a neurotransmitter in the brain, whose level is correlated with mood. SSRIs are used to mitigate this reduction. They inhibit 5-HT re-uptake into the raphe nuclei neurons, thereby increasing the level of 5-HT in the brain (15). Gut bacteria not only manufacture > 90% of the body's supply of serotonin (5-HT); any alterations in their normal composition ratios can modulate serotonin signaling through the GBA as well.

The gut-microbiome may therefore interact with nervous circuits *via* multiple pathways, cause the release of host-derived molecules like IL-6 and cholecystokinin (CCK) as well as modulate the levels of neurotransmitters, and alter host gene expression patterns as they relate to immune activation and inflammation.

The Gut-Brain Axis, Its Microbial Diversity, and Its Relation to Anxiety and Depression

The connection between the gastrointestinal (GI) tract and CNS was first investigated in 2006 by neuroscientist Jane Foster who found that mice with different gut microbiota have altered behavior related to anxiety. The gut microbiome seemed to be influencing the brain and behavior in both murine and human models (16). Though a few other scientists were also interested in the field, it was largely neglected in the neuroscience field until 2014. Today, the interest in exploring the connection has resurged, with a significant number of publications centering on GBA’s development. The US National Institutes of Health has invested millions of dollars to investigate how the human brain is connected with the GI tract (16). The term GBA was then coined to describe the connection formally, referring to

the network of bidirectional connections between the GI tract and the CNS that involves multiple biological systems, as shown in Figure 1 (17).

The gut microbiome

The gut microbiome is predominantly composed of organisms from two phyla of bacteria: Firmicutes and Bacteroides. Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia are also present but are less common. (18). In recent years, a wide range of research has shown that patients with mental disorders like anxiety and depression have a different gut microbiome content and composition compared with healthy individuals. In particular, Bacteroidetes, Protobacteria, and Actinobacteria were increased, and Firmicutes were decreased, in the gut of MDD patients. On the other hand, patients suffering from GAD showed a much lower prevalence of five genera: Faecalibacterium, Eubacterium rectale, Lachnospira, Butyricicoccus, and Sutterella when compared to normal individuals (19). In the rest of this section, the physiological role of microbial diversity in mental health disorders will be discussed in detail. After understanding the potential difference in the gut microbiome community between healthy persons and patients with anxiety or depression, FMT or its recent iterations may be utilized to treat these diseases by changing the composition of the gut microbiome.

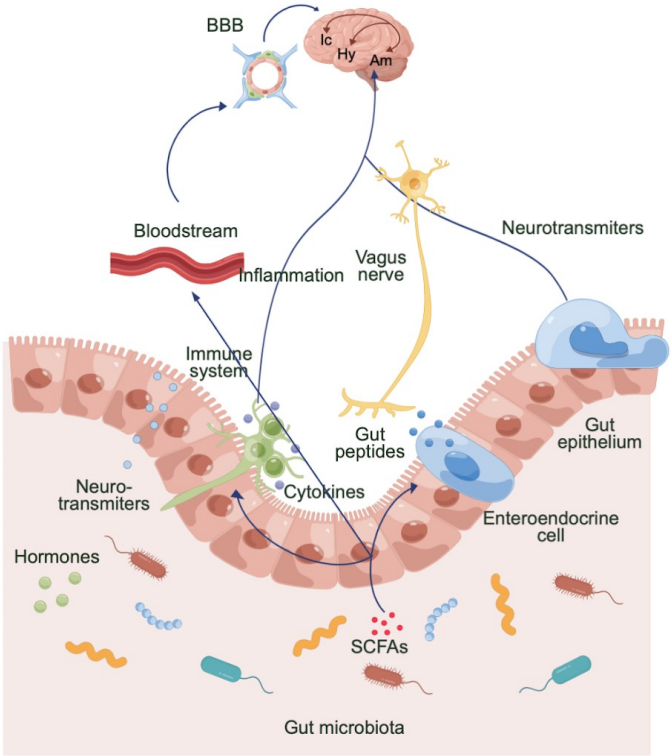

Figure 1. The Gut-Brain Axis, sourced from Figdraw. ID: ORWTObe66b

The gut microbiome and epigenetics

The gut microbiome also influences gene expression. First, the gut microbiome can influence the expression of host epigenetic modifying enzymes to affect gene expression. For example, scientists used intestinal epithelial cell-specific histone deacetylase 3 knockout mice ($HDAC3^{\Delta IEC}$) to illustrate this phenomenon. In the conventionalized groups (not removing any types of gut microbiota), these mice showed dysregulated host gene expression and disrupted homeostasis. Specifically, the dysregulated genes were related to the inflammatory bowel disease (IBD) progression. Germ-free $HDAC3^{\Delta IEC}$ murine models do not have such host gene expression dysregulation, from which it can be inferred that the microbiome mediates the effects (20). Gut microbiomes also alter gene expression directly. When comparing germ-free mice and conventionalized mice, 116 out of 303 xenobiotic processing genes (genes that regulate the expression of specific drug-processing enzymes) were expressed differently (21). Multiple studies demonstrated that the human microbiome affects epigenetic modifications, including DNA methylation, histone acetylation, and many other genetic processes (20). In addition, pathogenic bacteria generate host transcripts. Bacteria like *Listeria monocytogenes*, *Salmonella Typhimurium,* and *Helicobacter pylori* can stimulate host microRNAs which have significant effects on host metabolism and can potentially affect diseases including anxiety and depressive disorders (20).

The physiology of the Gut-Brain Axis

The enteric nervous system (ENS) is a part of the human autonomic nervous system. It is connected with the CNS *via* the vagus nerve so that the brain can be affected by the gut environment (22). Also, the hypothalamic–pituitary–adrenal (HPA) axis plays a vital role in the gut-brain axis. Chronic stress can cause dysregulation of the HPA axis and stress cortisol responses, which suppress the release of anti-inflammatory agents and therefore may be depressogenic (22).

There are multiple molecules produced by gut cells and gut microbiota that can cross the blood-brain barrier into the CNS. These molecules, acting as neuronal factors, include proteins, peptides, and fatty acids. One specific type of fatty acid that generates behavioral changes and disease is categorized as a short-chain fatty acid (SCFA). SCFAs are produced by intestinal microorganisms through the fermentation of dietary fiber. SCFAs can ingress into multiple cells, including colonic cells and hepatocytes. In the latter, these SCFAs are used for the biosynthesis of glucose, cholesterol, and fatty acids (17). SCFAs not absorbed by cells can enter the circulatory system and directly exert hormonal effects *via* interactions with protein-coupled receptors and histone deacetylases of brain tissues. They can also modulate hormones, the immune system, as well as the peripheral nervous system, and thus affect the CNS indirectly (23).

A wide range of gut peptides also have similar effects. For example, peptides including CCK, glucagon-like peptide (GLP-1), peptide YY (PYY) (24), and ghrelin can act on vagal afferent neurons (25). Studies have shown that CCK can affect anxiety. Brawman-Mintzer et. al. used the CCK2 agonist Pentagastrin, an inhibitor of the CCK receptor, to induce panic attacks in GAD and control patients, inducing

panic symptoms in 71% of GAD patients and 14% of non-GAD patients. This finding suggested that CCK may be involved in the development of panic symptoms in patients with GAD. Therefore, CCK is a candidate factor that induces GAD (9, 26). CCK acts through the G protein-coupled receptors CCK; found on vagal afferent neurons, and CCK2, which are mainly distributed in the brain, to suppress appetite and increase anxiety-like behavior (25). GLP-1 receptors are located in the brain, intestinal epithelial cells, and vagal neurons. GLP-1 can alter glucose metabolism (27), send interoceptive information to the hypothalamus and other forebrain areas (28), and stimulate the solitary tract cells which serve as the entrance for impulses into the brain (24). Peptide YY, a member of the Neuropeptide Y Family, can inhibit gastric emptying *via* receptors on the GI tract and through vagus nerve signaling. Its circulatory level is positively correlated to exercise and stress (24). Ghrelin is synthesized in the brain. Association has been found between low plasma Ghrelin levels and anxiety and depression (24). The physical connection between the human gut and brain comprises the vagus nerve that extends from the brainstem to innervate the gut and the ENS. Research has shown that gut microbiota metabolism can affect signaling through such connections (24). For instance, one randomized controlled trial involving glucose-tolerant volunteers showed that their GLP-1 level increased by 76% after ingesting *Lactobacillus reuteri* for four weeks compared to the placebo (29). The gut bacteria also influenced the host environment through the synthesis of neurotransmitters including γ-aminobutyric acid (GAGB), serotonin, dopamine, noradrenaline, melatonin, histamine, and acetylcholine (30), the modulation of brain-derived neurotrophic factor and oxytocin (30), and the shaping of neuronal pathways such as hippocampal neurogenesis (30, 31).

Specific types of microbes influence the secretion of different kinds of gut peptides and other molecules, including PPY, CCK, Ghrelin, GLP-1, GAGB, and SCFA (17, 24). These molecules, released by the enteroendocrine cells (ECCs) and other gut cells, can affect the CNS. In murine models, nutrient-induced *Escherichia coli* growth generates proteins and increases plasma PYY, which then stimulates the CNS (32). The bacteria *Bifidobacterium bifidum* positively regulated intestinal PYY in mice, but not in humans (24, 33), whereas only gram-negative bacteria, or Proteobacteria, increased PYY levels in humans (34). The peptide CCK is released postprandially to reduce food intake, especially for people suffering from obesity (24). Experiments in mice demonstrated that when germ-free mice were infected by *Giardia*, their colon CCK level was upregulated (35). Blood ghrelin levels were negatively correlated with the gut bacteria such as the commensal *Bifidobacterium* and *Lactobacillus* strains (36), whereas reduction of the phyla Firmicutes and Bacteroidetes promoted the production of GLP-1 (37). *Lactobacillus rhamnosus*, by producing GABA and regulating its receptors, increased vagal mesenteric nerve firing and reduced stress responses in mice (17). SCFA-producing bacteria in the gut activated the microglia and lead to neuroinflammation in mice (17). Augmentation of SSRI treatment with the probiotic bacteria *Lactobacillus Plantarum* 299v improved cognitive performance and decreased KYN concentration in MDD patients (38). The placebo in the trial was the probiotic administered by itself. The study demonstrated

that probiotics can be judiciously combined with existing small molecule treatments for depression such that the maintenance dose of the latter may be reduced for prophylactic treatment. Generally, meta-analyses support the effectiveness of probiotic supplementation in reducing depressive symptoms in MDD patients in a statistically significant manner; with the probiotic composition (multi-strain formulation), the quantity of ingested psychobiotics, and the duration of the study being the parameters determining the success of treatment. Nevertheless, probiotics have not yet made it to the mainstream of mood-disorder treatments, presumably because of ambivalent neuronal effects on depression, anxiety, stress, and schizophrenia, the short duration of clinical studies using heterogeneous populations, and ambiguous outcome measures. Larger, more rigorous, better-designed clinical studies on selected populations are needed to provide definitive evidence.

The Gut-Brain Axis and the inflammatory response

The immune system is involved in GBA modulation. The release of proinflammatory cytokines, local infection, dysbiosis, or intestinal inflammation cause alterations in signaling through the GBA. Proinflammatory cytokines are small proteins that can activate multiple immune responses. Normally, proinflammatory cytokines lead to an inflammatory response which can then disrupt epithelial tight junctions, compromising both the gut-vascular barrier (GVB); which then causes a "leaky gut"; as well as the blood-brain barrier integrity. It allows signaling molecules and even pathogens to reach the brain parenchyma through the disruption of the CNS barrier (29). For instance, much like their function in the gastrointestinal system, SCFAs can also affect the brain *via* the immune pathway. Systemic inflammation disrupts the intestinal barrier and increases the translocation of the microbiome and its products. Thus, inflammation increases the interactions between SCFAs and the immune cells, thereby influencing differentiation, recruitment, and activation based on the nature of those interactions (23).

Telomere shortening, known to be related to cell aging, has been observed in chronic inflammatory conditions. Inflammation is a risk factor for the development of mental health disorders such as anxiety and depression. It was observed that (compensatory) telomerase activity was increased in MDD patients compared with controls (39). Telomerase activation has been proposed to be a mechanism by which psychopharmacological drugs exercise their beneficial effects (40). It may be hypothesized that telomere shortening may also be modulated to some extent by the gut microbiome. Administration of Nicotinamide Mononucleotide (NMN) to human volunteers reduced the fecal bacterial diversity with an increased abundance of *Helicobacter, Mucispirillum*, and *Faecalibacterium,* and concomitantly increased the telomere length in peripheral blood mononuclear cells (PBMC) (41). Nevertheless, there is not enough evidence to associate telomere shortening to gut microbial composition (42).

Immune cells including neutrophils, dendritic cells, macrophages and monocytes, and T cells can activate inflammation. Signals from the CNS can induce a massive relocation of immune cells, specifically, intestinal IgA+

plasma cells into the brain and spinal cord to decrease or increase neuroinflammation. This is directly related to mental disorders including anxiety and depression (43). In addition, CNS inflammation is also shown to alter the intestinal immune cells as well as the gut microbiota by an as yet unknown mechanism (43).

The cholinergic anti-inflammatory pathway (CAP) describes the connection between the immune system and the nervous system (44). CAP modulates inflammatory responses that conduct signals through vagal afferent to vagal efferent nerves, leading to the release of acetylcholine; which in turn; binds to the alpha7 nicotinic acetylcholine receptor. It inhibits inflammatory mediators and regulates the function of immune cells (45). This inflammatory reflex made it possible for neurons to respond to, and to modulate immune responses.

The gut microbiome is capable of modulating the autoimmune system, intervening in the GBA, and thus demonstrating that the gut residual microbes may be effective in altering brain-related disorders. Warner et. al. designed a model termed Experimental autoimmune encephalomyelitis (EAE) to study the effect of the microbiome on the CNS. EAE was a T cell-mediated model for demyelinating diseases of the CNS, induced by eliciting an immune response to injected myelin-based proteins. Using this model, Warner et. al. described the gut microbiota's role in autoimmune CNS systems for the first time. Systemic T cells migrate to the CNS, causing proinflammatory Th1/Th17 responses and demyelination. However, germ-free (GF) mice are resistant to EAE. GF mice are protected by altered T cell responses, diminishing the gut dendritic cells' ability to stimulate local and systemic Th1/Th17 cells to produce proinflammatory cytokines and further inflammation. Such models demonstrate the various ways in which the gut microbiota can affect the CNS by mediating innate dendritic cell responses locally and altering T cell responses in the CNS (46).

As mentioned earlier, the vagus nervous system and the gut microbiome have a close bidirectional relationship, where afferent fibers and part of the vagus nerve, can be stimulated by microbial components and metabolites. As a corollary, efferent fibers can influence gut inflammation and gut permeability (46). In a healthy gut, the immune cells are separated from the gut microbes which include many gram-negative bacteria. Nevertheless, when intestinal permeability changes during inflammation, bacteria including *Enterobacteriaceae* can transit through the GVB and cause systemic immune activation due to the presence of the bacterial proinflammatory molecule, Lipopolysaccharide (LPS). Such interactions may further result in hyperimmune response-mediated depression (47).

The Gut-Brain Axis and other vascular systems

The circulatory system and endocrine system also play major roles in the mechanics of the gut-brain connection. Specifically, ECCs release molecules such as GLP1 and PYY hormones (43) into the bloodstream. The production and release of these molecules can be induced by SCFAs (43). Different types of ECCs can produce different peptides (e.g. X/A-like cells secrete ghrelin) (24) which can then affect brain function and can be altered by the

gut microbes. The translocation of hormones, peptides, and cytokines among different organs of the human body requires the bloodstream, or, the circulatory system. For instance, gut peptides are transported in the blood after release, whereby they arrive at the sensory neurons to stimulate impulses (24).

The microbiome and the metabolome

The metabolome refers to the collective products of complex biochemical reactions in the body. The gut metabolome composition and specific metabolome products may depend on the composition of the microbiome. This microbiome-metabolome association may be exploited to alter the composition of psychobiotics so that depression-alleviating or inducing metabolites are increased or decreased respectively. As an example, decreased blood levels of the metabolite LaurylCarnitine; a product of fatty acid oxidation was found to be associated with depressive disorders (48). Similarly, the levels of metabolites such as SCFAs', acylcarnitine, and KYN were significantly associated with depressive disorders (49). Metabolites involved in the tricarboxylic acid cycle (TCA) were significantly altered in patients with MDD (50). The levels of these metabolites could be altered by modulating the gut microbiota constituting the order *Clostridiales*, and the phyla *Proteobacteria* and *Bacteroidetes*, thus demonstrating the interplay between gut microbiota and the blood metabolome in the pathogenesis of MDD.

Gut microbiota interactions and psychobiotics

With the knowledge of GBA and the multiple pathways through which the gut microbiome can influence anxiety and depression, researchers are attempting to incorporate probiotics supplements, or psychobiotics, to treat these mood diseases. Usually, a single microbiome species does not profoundly affect host mental health. Instead, different microbes function synergistically (30). Thus, it is important to discuss the relationships between microbiota and the effectiveness of psychobiotics.

Gut bacteria interact through bacterial quorum sensing (QS), a kind of cell-cell signaling that alters the organism's behavior after reaching a particular density (51). The QS molecules secreted by bacteria can also regulate mood. As an example, segmented filamentous bacteria (SFB) deficient mice were found to be resistant to the induction of depressive-like behavior. SFB produces the quorum sensing molecule autoinducer-2 (AI-2) and promoted the production of serum amyloid proteins-1 and 2 (SAP1 and SAP2) by the host; which in turn, increased host hippocampal production of IL17 (52). Patients with MDD exhibited increased fecal IL17, SAP1, and SAP2 levels. Studies also show that some QS peptides can penetrate the blood-brain barrier, an essential pathway to the brain (53).

The forkhead box protein O1 (Foxo1) is a transcription factor, whose depletion, absence, or mutation in the Intestinal Epithelial Cells (IEC) causes an increase in gut mucinase-producing bacteria and a decrease in SFCA-producing bacteria (54). The resultant reduction in the mucus layer alters the organization of intestinal tight junction proteins, causing the "leaky gut" and eventually increasing inflammation. Foxo1 dysfunction hence affects anxiety and depression *via* the inflammation-mediated modulation of the GBA

(55). This inflammatory–depressive phenotype can be rescued by continuous supplementation of SCFA-producing bacteria, which promote mucus production and secretion.

As reviewed in the previous sections, some species of bacteria affect the host secretion of GAGB, PYY, etc. When combing these species, there also can be synergistic effects (24). Researchers have performed clinical trials of specific dosages of probiotics in treating anxiety and depression. The microbiota used in the studies were dosed in colony-forming units (CFU) (30). The duration of trials lasted from 6 weeks to 13 weeks, and most of them showed positive results as reported on the Beck Depression Inventory (BDI) scores (30). Most of the treatments used combinations of *Lactobacillus* and *Bifidobacterium* species. For instance, Morkl et. al. used 2×10^9 CFU/g *Lactobacillus helveticus* R0052 and 2×10^9 CFU/g *Bifidobacterium longum* R0175 to treat MDD patients (30). Eight weeks later, the patients treated with the probiotic supplements showed improvements in BDI scores when compared with placebo-dosed patients. The study evolved a total of 110 participants, which was considered large enough to concluded that the treatment was effective (30, 56). Other similar treatments combined *Lactobacillus acidophilus* (2×10^9 CFU/g), *Lactobacillus casei* (2×10^9 CFU/g), and *Bifidobacterium bifidum* (2×10^9 CFU/g) or 3 different *Bifidobacterium* and 5 different *Lactobacillus* species (a total of 1×10^{10} CFU/day) (30). Some also used single species as probiotic supplements (30). More importantly, it was found that the beneficial effect lasted for 4 weeks. The patients' BDI scores started to regress gradually 4 weeks after the treatment, yet remained significantly better than their scores before starting treatment (30). On the other hand, the clinical data was not compelling or significant when measured for anxiety. Morkl et. al. treated GAD patients with 18×10^9 CFU/day *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium lactis,* and *Lactobacillus acidophilus* (30). The patients showed an improvement in State anxiety; but not in trait anxiety, after 8 weeks (30).

Fecal microbiota transplantation

FMT involves the transplant of a fecal solution aliquot from a healthy donor into the intestinal tract of a recipient. This comprises three main stages: donor selection, material preparation, and administration. FMT has been used since 1958 to treat *Clostridium difficile* infections. FMT is a promising method and gained attention because of the low rate of adverse events. Even in long-term follow-up studies, FMT is generally reported to be free of adverse events (6).

FMT has been used in *Clostridium difficile* infections and to treat irritable bowel syndrome. The clinical data for the use of FMT in these diseases showed promising results (6). Since gut microbiota or their metabolome products such as SCFAs can modulate intestinal permeability, FMT has the potential to treat metabolic diseases and mental disorders such as anxiety and depression (6).

Furthermore, scientists have discovered probiotics that can relieve anxiety disorders by modulating immunologic factors and regulating GABA receptors. It has been demonstrated in animal models that specific strains of *B. longum*, *B. infantis*, *L. helveticus*, or *L. rhamnosus* alone or in combination normalized

behavioral phenotypes of anxiety animals (57). While literature about FMT in treating depression and anxiety disorders is sparse, its potential in treating psychiatric disorders is recognized by researchers (58).

Limitations

Questions about the GBA remain. First, there remains the controversy of whether depression causes the gut microbiota to change, or whether depression is a consequence of dysbiosis. It is known that depression can modulate gut microbiota (59). In animal models, it was observed that depression and stress precede gut microbiota alterations (60). It was also observed that depression precedes inflammation and other immune responses that cause gut microbiome shifts (60). Another hypothesis, on the contrary, asserts that gut microbes regulate brain functions through the vagal afferent fibers and the modulation of neurotransmitters (60). Clinical trials and reviews indicated that changing the gut microbiota can influence CNS function *via* multiple pathways (59, 60). Some studies have concluded that these pathways are bidirectional (59). Thus, the conundrum of a cause-consequence of the gut microbiome–depression cycle remains to be resolved. Regardless, microbiome composition should still influence the symptoms of depression and anxiety. Also, as presented in the review, some of the models and pathways of GBA need to be confirmed and there is still a long way to go before making probiotics a mainstay treatment for mental diseases.

Furthermore, despite the multiple advantages of probiotics, this treatment for mood disorders, including anxiety and depression, has not yet come into its own. In fact, there is not a single probiotic drug to treat mood diseases that are approved by the US Food and Drug Administration (FDA) (56). Only a few probiotics can be used to supplement the FDA-approved antidepressants, such as *Lactobacillus Plantarum* 299v discussed previously. Probiotics are different from other drugs: their active constituents are alive when administered, so their genetic stability, the potential for pathogenicity or toxigenicity, and immunological effects must be carefully considered before being approved (56). Since the GBA appears to be essential for probiotics to exert their CNS actions, it is important to determine if, and in what physiological or diseased conditions, this signaling pathway and/or its branches may be affected or compromised. It may be speculated that metabolic disorders such as obesity, diabetes, and hypertension; among others may cause a dysfunctional or deregulated GBA, which, in turn, will likely decrease, or even abrogate, the efficacy of psychobiotic treatment.

A significant shortcoming of researching the gut microbiome is that – using the current gold-standard method for bacterial genomic sequencing; the 16S RNA gene - results for bacterial identification are only reasonably accurate to the order or phyla level. Current methods cannot identify the vast number of species of residual bacteria in the gut. The results reported hence only correspond to the *known* microbiome. This effectively means that clinical study results of administering probiotics to MDD patients may have as much to do with causation or correlation as with pure chance (observational bias or the streetlight effect); although the latter can be significantly reduced by administering species subsets from responder and non-responder patients to Germ-

Free (GF) mice. Unless methods to identify gut bacteria down to the species level emerge, it may not be possible to know if specific sub-species even exist, that significantly alleviate anxiety and depression symptoms *vis-a-vis* the probiotic compositions used currently (61).

85% of gut-colonized microbes in mice are not found in humans, hence it is extremely difficult – if not impossible – to extrapolate microbiota engineering results from animal models to humans. The situation is further compounded by the fact that the microbiome in no two individuals is exactly the same. Therefore, unless *ex vivo* GF organ models are developed and species-level identification and quantification methods are discovered, the probability of near-term clinical success of psychobiotics may be realized; but is likely to be uncertain and unpredictable.

Conclusion

In conclusion, the various types of anxiety and depressive disorders correlate with biomarkers comprising of select immunomodulators, neurotransmitters, metabolic products, the inflammasome, gene transcription factors or epigenetic modulators. These biomarkers; in turn, are capable of being modulated by the gut microbiome composition, their quorum sensing protein products or their metabolic products (metabolome) and epitopes in the cell wall or membrane. Altering the gut microbiome hence has a strong potential to provide a therapeutic benefit in depressive disorders. The GBA; which includes an interplay between signaling pathways of the nervous, immune, circulatory, and endocrine systems; is responsible for connecting the human gut and the brain and provides a conduit for the transmission of chemical signals from the microbiome to the CNS. The gut microbiome can alter the activity of the GBA directly or indirectly. As a result, it is plausible that particular genera or species of gut bacteria can be used to treat anxiety and depression.

Ongoing clinical trials of such probiotics (psychobiotics) have had mixed success due to heterogeneity of patient population, short duration of the trials, and ambiguous or subjective outcome measures. The overall evidence however, does point to an observable and clinically significant benefit to treat depression and anxiety. Purified and select bacterial species from healthy individuals; the next iteration of FMT; are on the cusp of gaining FDA approval for treating intestinal bacterial infections and Irritable bowel syndrome. It seems a matter of time before psychobiotics emerge as approved drugs to treat mental health disorders, either as stand-alone treatments or in conjunction with neurotransmitter modulators. A deeper level of understanding of the gut microbiome at the species level, and the detailed mechanisms by which select species or genera modulate mood *via* the GBA opens the possibility of ushering in a new era in the field of psychology and mental health.

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Abbreviations

Gut-Brain Axis (GBA); Fecal Microbiota Transplantation (FMT); Serotonin Selective Reuptake Inhibitors (SSRIs); Serotonin-Noradrenaline Reuptake Inhibitors (SNRI); Central Nervous System (CNS); Enteric Nervous System (ENS); Generalized Anxiety Disorder (GAD); Social Anxiety Disorder (SAD); Post-Traumatic Stress Disorder (PTSD); Interleukin (IL); 5-HydroxyTryptamine (5-HT); Cholecystokinin (CCK); Gastrointestinal Tract (GI); Short-Chain Fatty Acids (SCFAs); Glucagon-Like Peptide (GLP-1); Peptide YY (PYY); Gut-Vascular Barrier (GVB); Cholinergic Anti-Inflammatory Pathway (CAP); γ-AminoButyric Acid (GAGB); Enteroendocrine Cells (ECCs); Experimental Autoimmune Encephalomyelitis (EAE); Lipopolysaccharide (LPS); Intestinal epithelial cell-specific Histone Deacetylase 3 knockout mice ($HDAC3^{\Delta IEC}$).

References

1. Schiele, M. A., & Domschke, K. (2018). Epigenetics at the crossroads between genes, environment, and resilience in anxiety disorders. Genes, brain, and behavior, 17(3), e12423.

2. Rakel R. E. (1999). Depression. Primary care, 26 (2), 211–224.

3. Tyrer, P., & Baldwin, D. (2006). Generalized anxiety disorder. Lancet (London, England), 368 (9553), 2156–2166.

4. Murrough, J. W., Yaqubi, S., Sayed, S., & Charney, D. S. (2015). Emerging drugs for the treatment of anxiety. Expert opinion on emerging drugs, 20 (3), 393–406.

5. Altaf, R., Gonzalez, I., Rubino, K., & Nemec, E. C., 2nd (2021). Folate as adjunct therapy to SSRI/SNRI for major depressive disorder: Systematic review & meta-analysis. Complementary therapies in medicine, 61, 102770.

6. Smits, L. P., Bouter, K. E., de Vos, W. M., Borody, T. J., & Nieuwdorp, M. (2013). Therapeutic potential of fecal microbiota transplantation. Gastroenterology, 145 (5), 946–953.

7. Peirce, J. M., & Alviña, K. (2019). The role of inflammation and the gut microbiome in depression and anxiety. Journal of neuroscience research, 97 (10), 1223–1241.

8. Sominsky, L., Fuller, E. A., Bondarenko, E., Ong, L. K., Averell, L., Nalivaiko, E., et.al. (2013). Functional programming of the autonomic nervous system by early life immune exposure: implications for anxiety. PloS one, 8 (3), e57700.

9. Zuxiao, B., Yufeng, Z., Li, L., & Baojun, C. (2011). Research progress on the pathogenesis of generalized anxiety disorder. The 2011 Academic Annual Meeting of the Psychiatry Branch of Zhejiang Medical Association and the Fourth Annual Meeting of Psychiatrists Branch of Zhejiang Medical Doctor Association.

10. Harald, B., & Gordon, P. (2012). Meta-review of depressive subtyping models. Journal of affective disorders, 139 (2), 126–140.

11. Hirschfeld R. M. (2000). History and evolution of the monoamine hypothesis of depression. The Journal of clinical psychiatry, 61 Suppl 6, 4–6.

12. Kim, Y. K., Na, K. S., Myint, A. M., & Leonard, B. E. (2016). The role of pro-inflammatory cytokines in neuroinflammation, neurogenesis, and the neuroendocrine system in major depression. Progress in neuro-psychopharmacology & biological psychiatry, 64, 277–284.

13. Firth, J., Gangwisch, J. E., Borisini, A., Wootton, R. E., & Mayer, E. A. (2020). Food and mood: how do diet and nutrition affect mental well-being?. BMJ (Clinical research ed.), 369, m2382.

14. Leonard B. E. (2018). Inflammation and depression: a causal or coincidental link to the pathophysiology?. Acta neuropsychiatrica, 30 (1), 1–16.

15. Yohn, C.N., Gergues, M.M. & Samuels, B.A. (2017). The role of 5-HT receptors in depression. Mol Brain 10, 28.

16. Willyard C. (2021). How gut microbes could drive brain disorders. Nature, 590 (7844), 22–25.

17. Morais, L. H., Schreiber, H. L., 4th, & Mazmanian, S. K. (2021). The gut microbiota-brain axis in behaviour and brain disorders. Nature reviews. Microbiology, 19 (4), 241–255.

18. Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., et.al. (2005). Diversity of the human intestinal microbial flora. Science (New York, N.Y.), 308 (5728), 1635–1638.

19. Jiang, H. Y., Zhang, X., Yu, Z. H., Zhang, Z., Deng, M., Zhao, J. H., et.al. (2018). Altered gut microbiota profile in patients with generalized anxiety disorder. Journal of Psychiatric Research, 104, 130–136.

20. Nichols, R. G., & Davenport, E. R. (2021). The relationship between the gut microbiome and host gene expression: a review. Human genetics, 140 (5), 747–760.

21. Fu, Z. D., Selwyn, F. P., Cui, J. Y., & Klaassen, C. D. (2017). RNA-Seq Profiling of Intestinal Expression of Xenobiotic Processing Genes in Germ-Free Mice. Drug metabolism and disposition: the biological fate of chemicals, 45 (12), 1225–1238.

22. Forssten, S. D., Ouwehand, A. C., Griffin, S. M., & Patterson, E. (2022). One Giant Leap from Mouse to Man: The Microbiota-Gut-Brain Axis in Mood Disorders and Translational Challenges Moving towards Human Clinical Trials. Nutrients, 14 (3), 568.

23. Dalile, B., Van Oudenhove, L., Vervliet, B., & Verbeke, K. (2019). The role of short-chain fatty acids in microbiota-Gut-Brain communication. Nature reviews. Gastroenterology & hepatology, 16 (8), 461–478.

24. Lach, G., Schellekens, H., Dinan, T. G., & Cryan, J. F. (2018). Anxiety, Depression, and the Microbiome: A Role for Gut Peptides. Neurotherapeutics: the journal of the American Society for Experimental NeuroTherapeutics, 15 (1), 36–59.

25. Dockray G. J. (2013). Enteroendocrine cell signaling via the vagus nerve. Current opinion in pharmacology, 13 (6), 954–958.

26. Brawman-Mintzer, O., Lydiard, R. B., Bradwejn, J., Villarreal, G., Knapp, R., Emmanuel, N., et.al. (1997). Effects of the cholecystokinin agonist pentagastrin in patients with generalized anxiety disorder. The American journal of psychiatry, 154 (5), 700–702.

27. Willard, F. S., & Sloop, K. W. (2012). Physiology and emerging biochemistry of the glucagon-like peptide-1 receptor. Experimental diabetes research, 2012, 470851.

28. Rinaman L. (1999). Interoceptive stress activates glucagon-like peptide-1 neurons that project to the hypothalamus. The American journal of physiology, 277 (2), R582–R590.

29. Simon, M. C., Strassburger, K., Nowotny, B., Kolb, H., Nowotny, P., Burkart, V., et.al. (2015). Intake of Lactobacillus reuteri improves incretin and insulin secretion in glucose-tolerant humans: a proof of concept. Diabetes care, 38 (10), 1827–1834.

30. Mörkl, S., Butler, M. I., Holl, A., Cryan, J. F., & Dinan, T. G. (2020). Probiotics and the Microbiota-Gut-Brain Axis: Focus on Psychiatry. Current nutrition reports, 9 (3), 171–182.

31. Möhle, L., Mattei, D., Heimesaat, M. M., Bereswill, S., Fischer, A., Alutis, M., et.al. (2016). Ly6C(hi) Monocytes Provide a Link between Antibiotic-Induced Changes in Gut Microbiota and Adult Hippocampal Neurogenesis. Cell reports, 15 (9), 1945–1956.

32. Breton, J., Tennoune, N., Lucas, N., Francois, M., Legrand, R., Jacquemot, J., et.al. (2016). Gut Commensal E. coli Proteins Activate Host Satiety Pathways following Nutrient-Induced Bacterial Growth. Cell metabolism, 23 (2), 324–334.

33. Nilsson, A., Johansson-Boll, E., Sandberg, J., & Björck, I. (2016). Gut microbiota mediated benefits of barley kernel products on metabolism, gut hormones, and inflammatory markers as affected by co-ingestion of commercially available probiotics: a randomized controlled study in healthy subjects. Clinical nutrition ESPEN, 15, 49–56.

34. Rajpal, D. K., Klein, J. L., Mayhew, D., Boucheron, J., Spivak, A. T., Kumar, V., et.al. (2015). Selective Spectrum Antibiotic Modulation of the Gut Microbiome in Obesity and Diabetes Rodent Models. PloS one, 10 (12), e0145499.

35. Hsu, L. T., Hung, K. Y., Wu, H. W., Liu, W. W., She, M. P., Lee, T. C., et.al. (2016). Gut-derived cholecystokinin contributes to visceral hypersensitivity via nerve growth factor-dependent neurite outgrowth. Journal of gastroenterology and hepatology, 31 (9), 1594–1603.

36. Queipo-Ortuño, M. I., Seoane, L. M., Murri, M., Pardo, M., Gomez-Zumaquero, J. M., Cardona, F., et. al. (2013). Gut microbiota composition in male rat models under different nutritional status and physical activity and its association with serum leptin and ghrelin levels. PloS one, 8 (5), e65465.

37. Hwang, I., Park, Y. J., Kim, Y. R., Kim, Y. N., Ka, S., Lee, H. Y., et.al. (2015). Alteration of gut microbiota by vancomycin and bacitracin improves insulin resistance via glucagon-like peptide 1 in diet-induced obesity. FASEB journal: official publication of the Federation of American Societies for Experimental Biology, 29 (6), 2397–2411.

38. Rudzki, L., Ostrowska, L., Pawlak, D., Małus, A., Pawlak, K., Waszkiewicz, N., et.al. (2019). Probiotic Lactobacillus Plantarum 299v decreases kynurenine concentration and improves cognitive functions in patients with major depression: A double-blind, randomized, placebo-controlled study. Psychoneuroendocrinology, 100, 213–222.

39. Bürhan-Çavuşoğlu, P., İscan, E., Güneş, A., Atabey, N., & Alkın, T. (2021). Increased telomerase activity in major depressive disorder with melancholic features: Possible role of pro-inflammatory cytokines and the brain-derived neurotrophic factor. Brain, behavior, & immunity - health, 14, 100259.

40. Bersani, F. S., Lindqvist, D., Mellon, S. H., Penninx, B. W., Verhoeven, J. E., Révész, D., et.al. (2015). Telomerase activation as a possible mechanism of action for psychopharmacological interventions. Drug discovery today, 20 (11), 1305–1309.

41. Niu, K. M., Bao, T., Gao, L., Ru, M., Li, Y., Jiang, L., et.al. (2021). The Impacts of Short-Term NMN Supplementation on Serum Metabolism, Fecal Microbiota, and Telomere Length in Pre-Aging Phase. Frontiers in nutrition, 8, 756243.

42. Manchia, M., Paribello, P., Arzedi, C., Bocchetta, A., Caria, P., Cocco, C., et.al. (2020). A multidisciplinary approach to mental illness: do inflammation, telomere length, and microbiota form a loop? A protocol for a cross-sectional study on the complex relationship between inflammation, telomere length, gut microbiota, and psychiatric disorders. BMJ open, 10 (1), e032513.

43. Agirman, G., Yu, K. B., & Hsiao, E. Y. (2021). Signaling inflammation across the Gut-Brain axis. Science (New York, N.Y.), 374 (6571), 1087–1092.

44. Bonaz, B., Bazin, T., & Pellissier, S. (2018). The Vagus Nerve at the Interface of the Microbiota-Gut-Brain Axis. Frontiers in neuroscience, 12, 49.

45. Wang, W., Xu, H., Lin, H., Molnar, M., & Ren, H. (2021). The role of the cholinergic anti-inflammatory pathway in septic cardiomyopathy. International immunopharmacology, 90, 107160.

46. Warner, B.B. (2019). The contribution of the gut microbiome to neurodevelopment and neuropsychiatric disorders. Pediatr Res 85, 216–224.

47. Winter, Gal.Hart, Robert, & Charlesworth, Richard. (2018). Gut microbiome and depression: What we know and what we need to know. Reviews in the Neurosciences. 29. 10.1515/revneuro-2017-0072.

48. Zacharias, H. U., Hertel, J., Johar, H., Pietzner, M., Lukaschek, K., Atasoy, S., et.al. (2021). A metabolome-wide association study in the general population reveals decreased levels of serum laurylcarnitine in people with depression. Molecular psychiatry, 26 (12), 7372–7383.

49. F Guerreiro Costa, L. N., Carneiro, B. A., Alves, G. S., Lins Silva, D. H., Faria Guimaraes, D., et.al. (2022). Metabolomics of Major Depressive Disorder: A Systematic Review of Clinical Studies. Cureus, 14 (3), e23009.

50. Perić, I., Lješević, M., Beškoski, V., Nikolić, M., & Filipović, D. (2022). Metabolomic profiling relates tianeptine effectiveness with hippocampal GABA, myo-inositol, cholesterol, and fatty acid metabolism restoration in socially isolated rats. Psychopharmacology, 239 (9), 2955–2974.

51. Chen, Z., Luo, J., Li, J., Kim, G., Chen, E. S., Xiao, S., et.al. (2021). Foxo1 controls gut homeostasis and commensalism by regulating mucus secretion. The Journal of experimental medicine, 218 (9), e20210324.

52. Medina-Rodriguez, E. M., Madorma, D., O'Connor, G., Mason, B. L., Han, D., Deo, S. K., et.al. (2020). Identification of a Signaling Mechanism by Which the Microbiome Regulates Th17 Cell-Mediated Depressive-Like Behaviors in Mice. The American journal of psychiatry, 177 (10), 974–990.

53. Kazemi, A., Noorbala, A. A., Azam, K., Eskandari, M. H., & Djafarian, K. (2019). Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: A randomized clinical trial. Clinical nutrition (Edinburgh, Scotland), 38 (2), 522–528.

54. Chen, Z., Luo, J., Li, J., Kim, G., Chen, E. S., Xiao, S., et.al. (2021). Foxo1 controls gut homeostasis and commensalism by regulating mucus secretion. The Journal of experimental medicine, 218 (9), e20210324.

55. Wynendaele, E., Verbeke, F., Stalmans, S., Gevaert, B., Janssens, Y., Van De Wiele, C., et.al. (2015). Quorum Sensing Peptides Selectively Penetrate the Blood-Brain Barrier. PloS one, 10 (11), e0142071.

56. Abisado, R. G., Benomar, S., Klaus, J. R., Dandekar, A. A., & Chandler, J. R. (2018). Bacterial Quorum Sensing and Microbial Community Interactions. MBio, 9 (3), e02331-17.

57. Kim, Y. K., & Shin, C. (2018). The Microbiota-Gut-Brain Axis in Neuropsychiatric Disorders: Pathophysiological Mechanisms and Novel Treatments. Current neuropharmacology, 16 (5), 559–573.

58. Evrensel, A., & Ceylan, M. E. (2016). Fecal Microbiota Transplantation and Its Usage in Neuropsychiatric Disorders. Clinical psychopharmacology and neuroscience: the official scientific journal of the Korean College of Neuropsychopharmacology, 14 (3), 231–237.

59. Sanders, M. E., Akkermans, L. M., Haller, D., Hammerman, C., Heimbach, J., Hörmannsperger, G., et.al. (2010). Safety assessment of probiotics for human use. Gut microbes, 1 (3), 164–185.

60. Zhu, F., Tu, H., & Chen, T. (2022). The Microbiota–Gut–Brain Axis in Depression: The Potential Pathophysiological Mechanisms and Microbiota Combined Antidepression Effect. Nutrients, 14 (10), 2081.

61. Apte, S. (2018). Probiotics as Potentiators of Cancer Immunotherapy. Journal of Excipients and Food Chemicals. 9 (2).