Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 6 Issue 12 December 2023

Review Article

Exploring the Microbiome through Probiotics and Psychobiotics: Deciphering the Gut-Brain Link

Rimjhim Pathak[1], Prem Saran Tirumalai[2]* and Seema Kashyap[1]

[1]*Dayalbagh Educational Institute, Department of Home Science, Dayalbagh, Uttar Pradesh, India*

[2]*Dayalbagh Educational Institute, Department of Agricultural sciences (Botany), Food and Dairy Testing Lab Dayalbagh, Uttar Pradesh, India*

***Corresponding Author:** Prem Saran Tirumalai, Dayalbagh Educational Institute, Department of Agricultural sciences (Botany), Food and Dairy Testing Lab Dayalbagh, Uttar Pradesh, India.

DOI: 10.31080/ASMI.2023.06.1315

Received: September 11, 2023

Published: November 07, 2023

Abstract

The journey from the discovery of normal flora to gut microbes and the resulting field of probiotics and psychobiotics has been a long and fascinating one. From the time normal flora and gut microbes came to be known researchers began to realize that these microorganisms played an important role in maintaining human health playing a crucial role in digestion, immunity, and even mental health. This led to the development of probiotics, which when ingested in adequate amounts, confer health benefits to the host. Probiotics have been shown to help treat a variety of health conditions, including gastrointestinal disorders, allergies, and even depression and anxiety. More recently, the field of psychobiotics has emerged, which refers to the use of probiotics and other dietary interventions to improve mental health. This field is still in its early stages, but initial studies suggest that certain probiotics may be effective in treating depression, anxiety, and other mental health disorders. This article reviews the emergence of Psychobiotics and discusses related topics and findings in context.

Keywords: Psychobiotics; Probiotics; Gut Microbes; Gut-Brain Axis

Abbreviations

GBS: Gut Brain Axis; CNS: Central Nervous System; ENS: Enteric Nervous System; HPA: Hypothalamic Pituitary Axis; GABA: Gamma-aminobutyric Acid; ANS: Autonomic Nervous System; IBS: Irritable Bowel Syndrome; VIP: Vasoactive Intestinal Peptide; GI: Gastrointestinal; GLP-1: Glucagon Like Peptide; PYY: Peptide Tyrosine Tyrosine; CCK: Cholecystokinin; SCFA: Short Chain Fatty Acid; LDL: Low-Density Lipoprotein; GABA: Gamma-aminobutyric Acid; 5HT: 5- Hydoxy Tryptophan; sCSDS: Sub Chronic and Mild Social Defeat Stress

Introduction

In the 19th century Theoder Escherich observed bacteria in the intestine of human individuals, ever since the microbial existence as inhabitants of human body has remained perplexing. Research pursuits to understand the relationship between the human host and the residing microbial flora, commonly referred to as the Normal flora, has given rise to horde of reports that has led to emergence of varied applications and concurring fields, such as the science of Gut Microbes, Probiotics and Psychobiotics. Psychobiotics, the most recent of all, has unraveled much to support the phrase 'you are what you eat', paving way for unforeseen applications.

Gut microbes

The term Normal Flora of humans is not nascent though, the understanding of the denizens of the normal flora in terms of their role and relevance as incumbents of the flora continues to intrigue us more than ever. While the collection of voluminous data that indicate specific roles of individual species of the normal flora persists, new and dynamic branches of applied science emerge that may have a large impact on the human populace in the future. Human intestinal microbiota, which is a part of the normal flora, also referred to as Gut Microbiota or Gut Flora, are microorganisms that inhabit our digestive tract. The quantity of microbial cells present in our body is equivalent to number of human cells [1], needless to mention the relentless pursuit to understand their role.

Gut microbiota as normal flora at different stages of life

Gut microbiota begins to establish during the first few years of life and during these early years of life, microbiota is highly dynamic, low in diversity and instable. Gut colonization by microbes that begins right from birth, continues to persist, with modulation, throughout the lifespan of individuals (Figure 1) [2] Every individual's microbiome is unique, it is synonymous to one's fingerprint and continues to evolve through the lifespan [3]. The Gut microbial community of human beings consists of thousands of species belonging to five major phyla; Firmicutes, Bacteroidetes, Actinobacteria, Verrucomicrobia and Proteobacteria. Eckburg and colmicrobiomwleagues were in consensus with the findings of the past studies [4] which showed majority of bacteria harbouring the gut of children belonged either to the phylum Firmicutes or Bacteroidetes, followed by Proteobacteria and Actinobacteria. Similar phylum distributions were also detected among healthy adults as reported by others [5]. In elderly population a general decrease in species diversity was found for *Bacteroides*, *Prevotella*, *Bifidobacteria*, and *Lactobacillus* spp., and an increase in species numbers within the *Enterobacteriaceae*, *Staphylococci*, *Streptococci* and *Candida albicans* relative to healthy young adults. With an overall lower diversity, decreased levels of key species such as *Faecalibacterium prausnitzii*, and increased levels of *Proteobacteria* were also observed in aged population than most adults [6]. Similarly, during youth, bacteria belonging to the phylum *Bacteroidetes* have been reported to dominate the gut, which gets suppressed among the aged, when the bacteria of phylum *Firmicutes* increasingly dominates [7]

It is evident that the composition of the gut microbiota is dynamic and can vary among individuals and within an individual at different time periods and this variation could be attributed to a number of factors. The composition of the gut microbiota is likely to be shaped by a gamut of factors including environmental factors genes, mode of delivery and diet [8], stress [9], medications such as antibiotics [10] and gastrointestinal infections [11].

The role of gut microbes in sustaining and regulating human

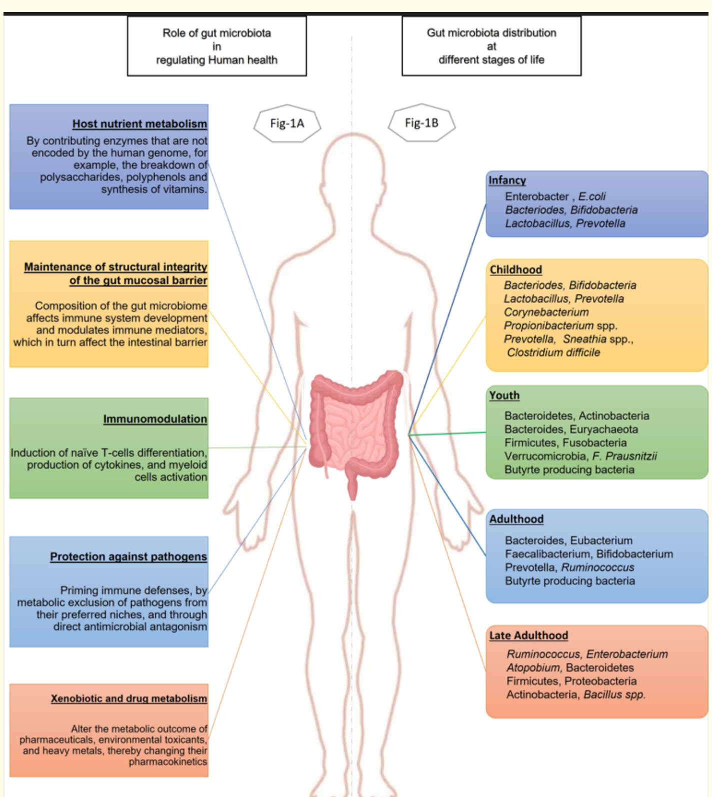

Figure 1: A) Distribution of Gut microbiota at different stages of Life. **B)** Mechanism of regulatory functions of Gut microbiota towards human health.

health

When in balance, the gut microbiome confers significant immunological, endocrinological and neurological benefits to the host [12]. Gut microbiota strongly affect the host's health and disease status (Figure 1) [13].

When the microbial community in the gut ecosystem is imbalanced this leads to a condition known as "Dysbiosis". Researchers have confirmed that altered microbiota (Dysbiosis) attributes to causing several diseases including those that are psychiatric in nature [14]. Gastrointestinal disorders such as the Inflammatory Bowel (Disease IBD), peptic ulcer, Crohn's disease, chronic diarrhea, constipation, besides intestinal motility disorders have been linked to dysbiosis [15]. Also, reports indicate that the changes in microbiota composition and its function may contribute to the host's susceptibility to various diseases like Multiple sclerosis [16] certain type of cancers [17], allergies [18] and ischemic stroke [19]. Studies have also found possible linkage between immune function, gut health, mood, and mental health [20]. Gut flora has been linked to depression, anxiety [21], Parkinson's Disease [22] and Alzheimer's Disease [23]. Besides sustaining health by their gut presence, microbes have also been reported to regulate human health via nutrient metabolism, maintenance of the gut mucosal barrier, immunemodulation, protection against pathogens and xenobiotic metabolism.

Among the various roles of the gut microbiota and their relationship with the host, one of the intriguing aspect is that of their influence on the brain functioning. It is in this context relevant to understand the gut-brain nexus, the Gut-Brain Axis.

Gut-brain axis and gut microbiota

The Gut-Brain Axis (GBA) is the physical and biochemical signaling that happens between the gastrointestinal tract and the central nervous system. It is a bidirectional communication system that connects the gut and the brain through a complex network of nerves, hormones, and signaling molecules (Figure 2). This communication occurs through the vagus nerve, neural pathways, and the release of various molecules such as neurotransmitters, hormones, and immune system factors. The gut-brain axis mediates constant communication and interaction between the gut and the brain, influencing various physiological processes and behaviors. It plays an important role in maintaining homeostasis of the body and is also known to be involved in several important processes, including regulating inflammation, immunity, digestion, endocrinology, and metabolism. The gut-brain axis is involved in the coordination of numerous bodily processes, including energy balance, reward, taste perception, emotion, memory, and cognitive functions.

The GBA also involves the interplay of the gut microbiota. Strong evidence have been established that indicate that the gut microbiota can influence the gut-brain axis and, in turn, impact various aspects of brain function and behavior. This bidirectional communication is thought to occur through several mechanisms. The GBA includes several pathways, such as the endocrinal, immunological, and neural pathways. The brain to gut direction of communication are via the Autonomic Nervous System (ANS) [24], the Enteric Nervous System (ENS) [25] and the Hypothalamic Pituitary Axis (HPA) [26] and on the other hand the gut microbiota influence the emotional and cognitive functioning of the brain via the expression of bioactive molecules or by regulating enteric cells to release modulating molecules that in turn modulate cells in the brain, which includes neurotransmitters, such as serotonin, dopamine, and gamma-aminobutyric acid (GABA) [27]. Microbes can influence the gut to Brain communication via Expression of Neurtotransmitters [28], hormones [29], enteric immune regulation [30] and expression of metabolites [31].

The bidirectional communication between the brain to gut is regulated via 1) Stress response on gut microbiota 2) Neuroendocrine HPA axis 3) Immunoregulatory pathways, and the gut to brain via 1) Neurotransmission 2) Gut harmones secretion 3) En- teric immune regulation 4) Microbial metabolites.

Probiotics

The dynamics of gut microbial population is largely influenced by the diet, in other words our diet shapes our gut microbiota by diversifying or narrowing down the species abundance and therefore also influencing the individual and collective roles played by them [32]. One major contributing factor to the role of diet is the inclusion and ingestion of probiotics. Probiotics, as the name goes, are microbes that are 'pro' to our health and wellbeing (biotic). Probiotics include beneficial species of live organisms which confer a large and lasting impact on the physical and mental wellbeing of the consumers by modulating gut flora. Use of Probiotics is to support and reset the colonization of microbial species in the gut

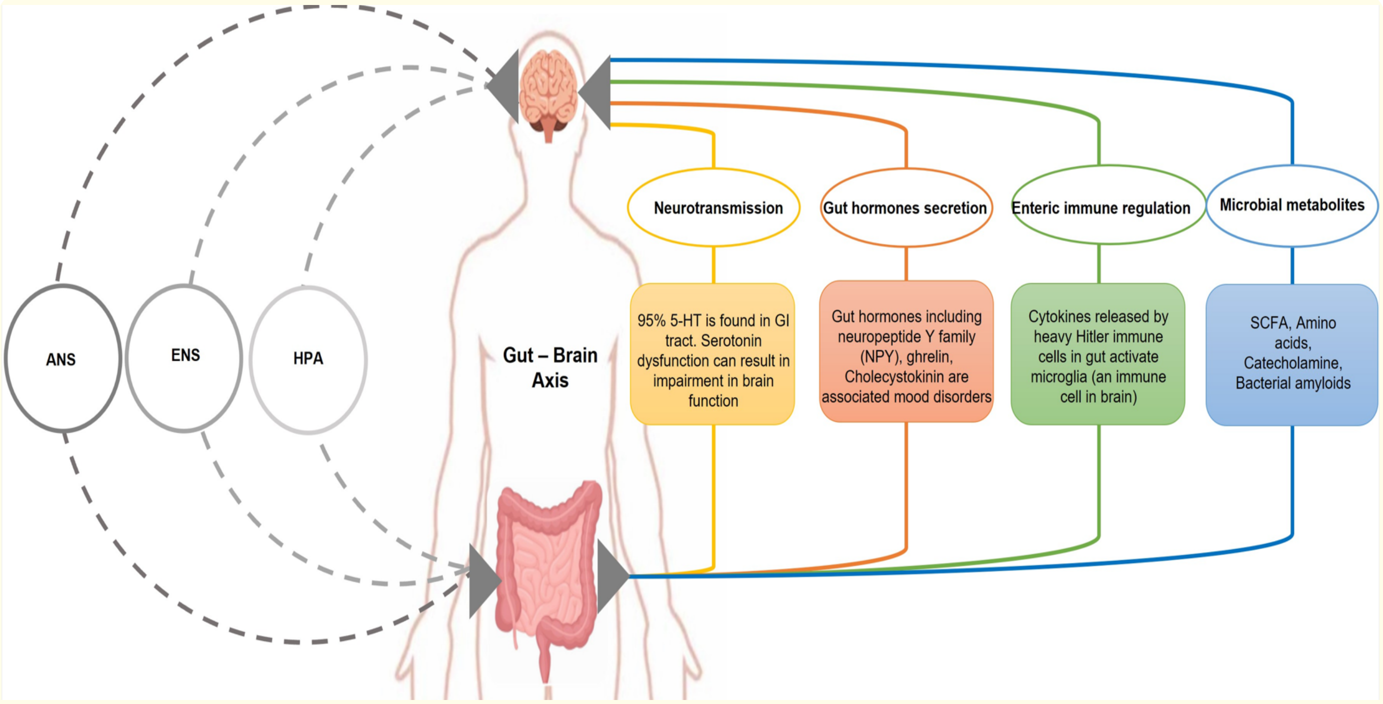

Figure 2: Gut-Brain Axis and the gut microbiota influence on the brain.

by way of addressing dysbiosis. Probiotics are live microorganisms used in their active state as a supplement to confer health benefits both to animal and human subjects, however this definition has been broadened with the advent of new terminologies like para-biotics, para-psychobiotics and post-biotics which make use of in-active and non-viable form of probiotics also. A list of organisms that have been categorized as probiotics is given in Table 1.

Probiotics have been associated with various health benefits such as cholesterol regulation, immune and hormonal regulation and skin and gut barrier function. In addition, they also mitigate the risk or severity of diseases such as Inflammatory bowel disease, Acute respiratory tract infection, diarrhea, cardiovascular diseases and Type 2 diabetes, and pschological and mood disorders.

Our understanding of the role of gut microbiota in human health paved way to use of probiotics in the diet, which confer specific health benefits as has been discussed in a series of reports in the recent past. Recent research suggests that probiotics reduce the risk of developing a variety of medical conditions, thus in the mainteinenance of good health. Probiotics support in sustainenace via digestive health [34], immune system support [35], heart health [36] and mental health [37]. Besides support in sustaining health, probiotics have also been reported to have relevance in the mitigation of diseases related to the gastrointestinal and respiratory systems, metabolism and psychological disorders. An extensive and elaborate significance of probiotics in the sustenance of human health is as shown in Figure 3.

Influence of the normal flora of the gut and the probiotic supplements on the Brain

Much could be derived on the significance of the gut microbiota and the probiotics from the earlier sections. Microbes have influence on various functions of the body, including the brain. Gut microbes and probiotics can have a variety of effects on brain activity and cognition, including changes in neurotransmitter levels, synaptic plasticity, neuroplasticity, and memory. As discussed, studies have shown that gut bacteria have ability to produce neu-

Phylum	Order	Family	Genus	Species
Actinomycetota	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	*B. adolescentis*
				B. bifidum
				B. bifidum
				B. breve
				B. lactis
				B. longum
				B. infantis
				B. animalis subsp. lactis
	Propionibacteriales	Propionibacteriaceae	Propionibacterium	*P. freudenreichii*
Bacillota	Bacillales	Bacillaceae	Bacillus	*B. subtilis*
				B. coagulans
				B. pumilus
				B. licheniformis
				B. subtilis
				B. cereus var. toyoi
				B.clausii
				B. laterosporus
	Lactobacillales	Lactobacillaceae	Lactobacillus	*L. sacidophillus*
				L. bulgaricus
				L. casei
				L. ferementum
				L. casei
				L. feremetum
				L. lactis
				L. acidophilus
				L. paracasei
				L. rhnamosus
				L. delbrueckii subsp. Bulgaricus
				L.brevis
				L. brevis
				L. johnsoni
				L. plantarum
				L. salivarrius
				L. fermentum
				L. kefir
		Streptococcaceae	Streptococcus	*S. thermophilus*
			Lactococcus	*L. lactis subsp. lactis*
		Enterococcaceae	Enterococcus	*E. durans*
				E. faecalis
				E. faecium
Ascomycota	Saccharomycetales	Saccharomycetaceae	Saccharomyces	*S. cerevisiae*
				S. boulardii

Table 1: Microbial species identified and listed as Probiotics.

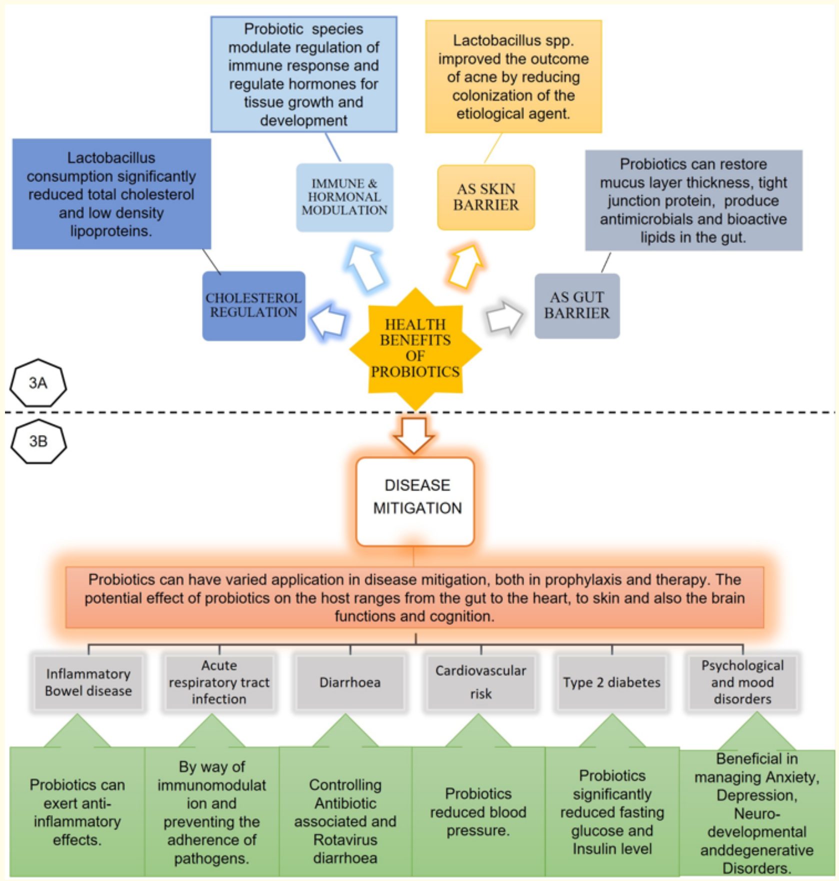

Figure 3: Health benefits of Probiotics.

rotransmitters, such as serotonin, dopamine, and norepinephrine, which influence the brain. Also, studies have shown that probiotics contribute to cognitive health by improving overall cognitive performance, especially in people with neurological and psychological disorders, such as autism, Alzheimer's disease, and depression. Furthermore, probiotics have to been observed to modulate the inflammation in the intestinal tract, leading to reduction in reducing symptoms associated with anxiety and depression.

The influence of the microbes as psychobiotics on the brain activity can be through 3 major mechanisms as shown in the Figure 4. Gut dysbiosis has been proposed to alter behavioural and psychological health of individuals [37]. Gut microbiota influences the brain development and its physiology and perseverance, which is regulated by production of secondary metabolites; Short chain fatty acids (SCFA), tryptophan metabolites and neurotransmitters including their precursors.

Influence of microbial SCFA on brain

Among most examined microbiota derived SCFA 95% consist of acetate, butyrate and propionate. Low level of SCFAs has been reported in models with changed brain physiology and behaviour including anorexia nervosa [38], Alzheimer disease [39], Parkinson's disease [40] and chronic stress [41]. In addition low level of butyrate and acetate were seen in fecal of children with autism spectrum disorder [42]. SCFAs found in colon are transported across gut epithelium into bloodstream. It affects blood brain barrier (BBB) integrity, functioning of glial cells and modulates neurotropic factors. SCFA has been reported to ameliorate microglia immaturity and physiology in mice [43]. It is believed that neurons are activated by SCFA induced vagus nerve signaling in CNS. More work is needed to understand the activation of specific neuron by SCFAs in the brain that result in behavior change.

Microbial short chain fatty acids (SCFAs) produced in the colon by the microbial metabolism of dietary fiber are one of the primary sources of energy for the host. Currently, several studies have demonstrated a close connection between the gut microbiota, metabolic activities, and the central nervous system, suggesting that the metabolite-sensing capability of the gut-brain axis is important for regulating normal physiology. SCFAs, particularly acetate, propionate, and butyrate, are the main end-products of microbial fermentation of dietary fiber [44]. These SCFAs will then enter the circulation to be metabolized in peripheral tissues and taken up by the brain. Acetate is thought to be the primary source of energy, as it is more rapidly converted to acetyl-coenzyme A than are the other SCFAs [45]. Propionate and butyrate appear to modulate inflammation and metabolism, while also influencing behavior and stress-related affects.

In the brain, short-chain fatty acids (SCFAs) engage in direct interactions with various targets, including receptors on neurons, oligodendrocytes, astrocytes, and microglia. Additionally, they exert indirect effects by serving as a source of energy for specific activities and by initiating transcription-dependent neuroinflammatory processes. Direct SCFA binding at the cell surface and intracellular signaling has been shown to influence the firing of neural pathways, stimulate neurogenesis, and activate neuroimmune processes, as well as metabolically supporting the oxidative energy needs of the brain [46]. There is growing evidence that SCFAs may play a role in neurotransmission and the neuropsychological health of the individual. It is well known that SCFAs can regulate neuronal responses in the hippocampus, amygdala, and hypothalamus, and that they can be neuroprotective due to their antioxidative effects [47]. Further, SCFAs can directly bind to G-protein coupled receptors that are involved in the regulation of synaptic and astroglial functions, including those involved in the maintenance of homeostasis, biota are not just a fuel source for the body, but also modulate brain functions in an important manner. The modulation of these functions by SCFAs could offer potential treatments for a variety of neurological and psychiatric disorders.

Influence of Microbially secreted neurotransmitters on brain

The mechanism that implicitly play role in maintaining the brain physiology is the expression of neurotransmitters by gut microbiota [48]. Number of bacteria reside in GI tract are recognized to produce catecholamine such as norepinephrine and dopamine that are similar in chemical structure to those found in host [49]. Both gut microbiota and host have ability to convert the glutamate to Gamma amino-butyric acid (GABA) which is the major inhibitory neurotransmitter of host nervous system [50]. Several *Lactobacillus spp.* have known to produce GABA in the intestine lumen [51]. Lower level of GABA was reported in germ free mice compared to mice with microbiota [52]. The Human microbiome project revealed that Glutamate decarboxylase is responsible for the conversion of glutamate to GABA. Their precursor reaches to BBB and participates in the synthesis cycles of various neurotransmitters in brain, further influences functional capability of neurons. GABAergic neurons are found in hippocampus, thalamus, basal ganglia, hypothalamus, and brainstem, modulating brain's response to different stressors. The GABA producing species had been seen to alleviate visceral hypersensitivity in rats [53].

The fate and course of microbial neurotransmitters from the gut is complex and varied. As microbes produce neurotransmitters, they can be taken up into the body by the bloodstream or other routes and exerts their action on the nervous system. These microbial neurotransmitters may also interact directly with the enteric nervous system, the nerves that line the gastrointestinal tract. Alternatively, small molecules from the microbiota can be transformed into new substances with novel biological activities, including metabolites that act as neurotransmitters throughout the body. In some cases, microbial metabolites can become more concentrated in certain organs, allowing them to be transported to other regions of the body. Ultimately, the fate and course of microbial neurotransmitters from the gut depends on many factors, such as the type of microbe, the environmental conditions, the body's physiological processes, and the type of molecule being produced. Microbial neurotransmitters from the gut, such as Dopamine, influence the brain in various ways. Inhibitory or excitatory neurotransmission, regulating mood, sleep and appetite, controlling learning, motivation and pleasure are some of the actions linked to microbial neurotransmitters. Microbial neurotransmitters are produced in the enteric nervous system, which is located in the gut. They are then released into the bloodstream and transported to the brain where they influence the functioning of neurons. The exact mechanisms through which gut microbial neurotransmitters influence the brain are not yet fully understood, but are thought to involve various pathways, such as the hypothalamus-pituitary-adrenal-axis, the vagus nerve, and the immune system. Changes in these pathways have been linked with depression and anxiety.

In addition, some research has suggested that changes in the composition of the gut microbiota influences the activities of microbial neurotransmitters. An elevation in the concentration of beneficial bacteria, particularly Bifidobacteria, enhanced the production of neurotransmitters, thereby exerting a discernible influence on the intricate functioning of the brain [54]. Microbial neurotransmitters from the gut may play an important role in the development of various mental health problems, such as depression, anxiety, and bipolar disorder [55]. Therefore, further research is needed to explore the potential therapeutic benefits of targeting microbial neurotransmitters in the treatment of such disorders.

Influence of Microbially secreted Tryptophan metabolites on brain

Microbial tryptophan metabolites produced in the gut have been shown to influence brain activity through a variety of mechanisms, including modulation of the neurotransmitter serotonin [56]. Tryptophan is absorbed from the gut and enters the bloodstream, where it is converted into serotonin and then degraded into a variety of metabolites including the regulatory tryptophan metabolites 3-hydroxykynurenine, kynurenine, and kynurenic acid.

Microbial serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter produced by the gut microbiome. It is shared by humans and animals and is believed to play a role in both the gut-brain axis and the immune system. Microbial serotonin is involved in a range of neurological and physiological processes, including the regulation of mood and appetite. 95% of body's Serotonin (5HT) is produced in gastrointestinal tract [57]. The gut microbiota influences hosts GI serotonergic system. Serotonin is derived from tryptophan metabolism; tryptophan can also enter in kyunerinine pathway that leads to less availability of serotonin in body [58]. A study reported that decrease in *L. reuteri* lead to chronic stress with increase in kyunerinine concentration [59]. Tryptophan derived metabolites have been implicit to control glial cells in CNS [60]. The hormone of darkness, Melatonin is produced from serotonin provides circadian and seasonal signals to host, great interest in therapeutics of diseases particularly sleep disorders [61]. The interconnection of the metabolic pathways of gut and brain reveals to be influenced by gut microbiota.

Microbial 5HT is broken down by the gut microbiome into indole, skatole and 5-hydroxytryptamine and then transported to the epithelial cells along with dietary tryptophan-derived indole and skatole via transporter proteins. In the epithelial cells, 5-hydroxytryptamine is further broken down by monoamine oxidase and other enzymes, releasing 5-hydroxyindoleacetic acid and other metabolites, which are then transported to the blood and elsewhere in the body, where it is used as a neurotransmitter or a hormone [62]. These microbial metabolites can cross the blood-brain barrier and influence the brain in various ways including influencing the levels of dopamine and other neurotransmitters, modulating neural excitability, and altering the expression of genes related to neuronal development and synaptic plasticity. Microbial tryptophan metabolites modulate the production of inflammatory molecules and even alter the permeability of the blood-brain barrier itself. In summary, the microbial serotonin produced in the gut has an important role in regulating the gut-brain connection. It impacts both the gastrointestinal tract and mental well-being, as well as the immune system. Since microbial serotonin is absorbed in the bloodstream, it plays an important role in the modulation of systemic inflammation, which is linked to disease. Thus, microbial serotonin has the potential to influence a broad range of physiological functions and mental states in both humans and animals.

The fate and course of microbial tryptophan metabolites from gut to brain, as well as their influence on the brain, are very complex and are still being studied. The exact mechanisms through which these microbial metabolites influence the brain are likely to vary by species and by individual.

Emerging evidence on the impact of probiotics as psychobiotics

Probiotics are being increasingly studied to see if they could be used to treat certain mental health problems. Probiotics act by reducing inflammation, stimulating the production of certain neurotransmitters, and directly affecting parts of the brain. Probiotics may also help improve absorption of key vitamins and minerals, which can have a positive effect on mental health including mood. Probiotics are thought to help improve biomarkers related to depression, anxiety, and stress. Psychobiotics, a relatively new term, was coined to describe bacteria and other microorganisms that benefits mental health and well-being as stated above. More research is underway to determine exactly how probiotics could be

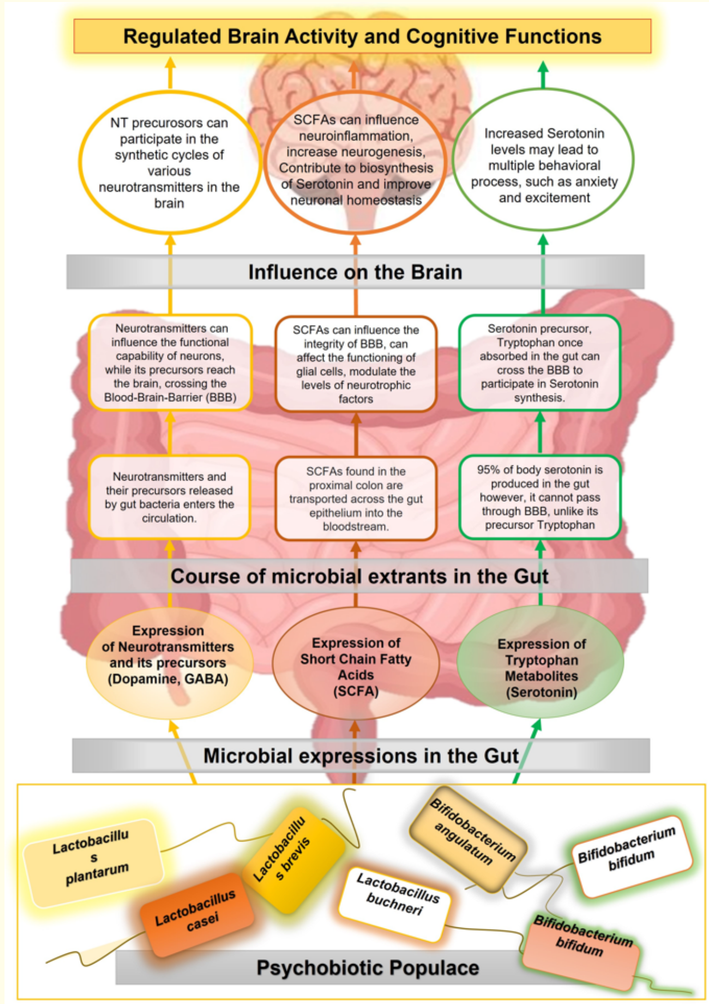

Figure 4: Mechanism of the microbial (gut microbiota/probiotics) influence on the brain activity.

used as psychobiotics. Emerging evidence on this purview hitherto is being discussed here.

Studies on animal models

Animal model experiments for psychobiotics have largely focused on examining the effects of certain probiotic or prebiotic treatments on both the gut microbiome and related mental health outcomes. To study the effects of psychobiotics, researchers have used rodent models.In one study, researchers used a mouse model to investigate the effects of *Lactobacillus rhamnosus* on anxiety and stress-related behavior. Results showed that the mice given the probiotic exhibited fewer anxious behaviors than those without them [63]. Another earlier study used a rat model to measure the effects of an oral probiotic supplement containing *Lactobacillus helveticus* and *Bifidobacterium longum* on depression-like behaviors. It was found that probiotic supplementation was able to reduce depression-like symptoms in the rats [64].

There have been a number of animal model experiments suggesting that certain probiotic treatments can be effective in improving mental health outcomes in rodents and other animals, prompting the need for further research to validate these findings, as well as to investigate other potential effects of these treatments in humans.

An exhaustive list of reports and mounting evidence on the significance of Psychobiotics, using animal models is detailed in Table 2.

Table 2: Studies on the effect of Psychobiotics on Animal Models.

Probiotic strain	Animal model	Behaviour effect
B. breve	Adult male mice	Reduced anxiety like behaviour and induced weight loss[65]
B. breve	Male 10 year week old mice	Prevented amyloid-beta-induced dysfunction in Alzheimer's disease model mice[66]
B. infantis	Pregnant Sprague Daw- ley dams	Reversed behavioural deficits in maternal separation model of depression[67]
B. longum	Male mice(6-8 weeks of age)	Reduced anxiety like behaviour, Normalization of the behaviour of mice with induced colitis[68]
B. longum subsp. Infantis	Male adult C57BL	Reduced depressive behaviour of mice subjected to chronic unpredictable mild stress[69]
L. acidophilus EG004, L. paracasei EG005 and L. rhamnosus EG006	rats	Improved cognitive ability by cognitive-behavioural tests[70]
L. helveticus/B. longum	Male wistar rats	Reduced anxiety like behaviour, Normalization of the behaviour of mice with induced colitis[64]
L. plantarum	Adult zebrafish	Reduced anxiety-related behaviour and altered GABAergic and serotoner- gic signalling in the brain [71]
Lactobacillus casei strain Shirota	rats	Plasma corticosterone were significantly suppressed[72]
Lactobacillus fermentum NS9	ampicillin-treated rats	Reduced the anxiety-like behaviour and alleviated the ampicillin-induced impairment in memory retention [73]
Lactobacillus helveticus NS8	Adult rats	Improved chronic restraint stress induced behavioral (anxiety and depres- sion) and cognitive dysfunction[74]
Lactobacillus helveticus strain MCC1848	sub chronic and mild social defeat stress (sCSDS) model mice	Improved anxiety- or depressive-like behaviour, positive effect on stress- related disorder [75]
Lactobacillus plantarum 90sk and Bifidobacterium adolescentis 150	mice for 2 weeks	Reduced depressive-like behaviour in the forced swimming test; the effect was similar to that of fluoxetine [76]
Lactobacillus plantarum strain PS128	Adult mice	Influenced neurochemicals related to affective disorders in prefrontal cortex[21]
Lactobacillus reuteri NK33, Bifido- bacterium adolescentis NK98	Mice with anxiety/ de- pression and colitis	Suppressed the occurrence and development of anxiety/depression[77]
Lactobacillus rhamnosus GG	6 female mice	Relieved anxiety-like behavior [63]

Studies on human subjects

Experiments have been conducted to determine if probiotic supplementation could have an effect on mental health and, specifically, depression. A study published in 2019 found that probiotics had a beneficial effect on depression, anxiety, and stress in healthy adults who had recently experienced a stressful life event. The probiotic supplement was associated with significantly lower levels of all three measures, compared to the placebo [64].

Recent studies published in 2021 have ratified the relevance of pyschobitoics using human subjects. A group of researchers examined the effect of a probiotic mixture on depressive symptoms in healthy adults. They found that the probiotic mixture significantly reduced self-reported symptoms of depression and anxiety, compared to the placebo [78]. Subsequently, as per a study published in Frontiers in Psychiatry, researchers tested the effect of probiotic supplementation on depressive symptoms in older adults. They found that probiotic supplementation was associated with an improvement in depressive symptoms, compared to the control [79]. Similarly, a study published in Nutrients reported that probiotic supplementation improved anxiety, depression, and stress in college students. The probiotic supplement was associated with significantly lower levels of all three measures, compared to the placebo [80].

A growing number of experiments has demonstrated that probiotic supplementation is associated with improved mood and mental health, particularly for those experiencing depression, stress, and anxiety. An elaborate list of studies conducted on human subjects and their findings are shown in Table 3. Future research will be needed to determine which probiotics and delivery methods may be most beneficial for these conditions.

Table 3: Studies on the effect of Psychobiotics on Human Subjects.

Probiotics Strains	Human model	Psychological effects
L. helveticus R0052, *B. longum*R0175	66 Healthy men/women	Alleviated psychological stress in healthy volunteers[64]
L. helveticus R0052, *B. longum*R0175	7 Female 3 Male(18-19 year old)	Significant Improvement in sleep quality[79]
B. longum NCC3001	44 adults with IBS and diarrhoea	Reduced depression[81]
Bifidobacterium animalis subsp. *lactis* BB-12	21 Chongqing young divers	Improved cognitive state anxiety, somatic state anxiety, and anxiety emotion[82]
Bifidobacterium breve CCFM1025	70 MDD patients	Reduced the serum serotonin turnover compared with the placebo[83]
Bifidobacterium. longum 1714	22 healthy volunteers	Reduced stress and improved memory[84]
Bifidobacterium breve A-1	29 patients with schizophrenia	Improving anxiety and depressive symptoms in patients with schizophrenia[85]
Lactobacillus casei strain Shirota	Healthy medical students	Salivary cortisol levels and the incidence rate of physical symptoms were significantly suppressed[72]
Lactobacillus plantarum	111 subjects	Reduced symptoms of stress, anxiety, and total psychological scores[86]
Lactobacillus plantarum P8	103 subjects	Reduced scores of stress[87]
Lactobacillus gasseri CP2305	60 Japanese medical students	Reduced anxiety and sleep disturbance[88]
Lactobacillus plantarum 299v	79 patients with MDD	Improved cognitive performance and decreased KYN concentration[89]
Lactobacillus plantarum	90 patients	Improved symptoms of depression, anxiety, and cognitive and somatic components[90]
Lactobacillus Plantarum PS128	200 patients	Improved the anxiety symptoms of patients[91]
Lactobacillus plantarum PS128	11 patients	Reduced Depression and Somatic symptoms[92]
Lactobacillus plantarum PS128	40 participants	Decrease in Beck Depression Inventory-II scores, fatigue levels, brainwave activity, and awakenings during the Deep sleep stage[93]
Lactobacillus rhamnosus CNCM I-3690	46 healthy students	Stress-preventative effect[94]

Findings in support of Psychobiotics

Anxiety

Multiple studies have reported the association of probiotic strains with anxiety like behaviour in animal as well as in human models. For instance, Liu., *et al.* (2016) reported reduction of anxiety like behaviour in mice when they were subjected to open field test after being ingested *L. plantarum* strain PS128. The altered dopamine and serotonin level were observed in prefrontal cortex that promising the role of *L. plantarum* in gut-brain signalling. The strain *L. plantarum* were also reported to reduce anxiety related behaviour in zebra fish model by validating behaviour tests. Modulation in GABAergic pathway were found to be associated with anxiety like behaviour including up regulation of GABA- A- alpha 1 receptor and serotonin transport encoding genes in *L. plantraum* treated zebrafish [71]. *B. adolescentis, Bifidobacterium adolescentis NK98, Lactobacillus helveticus* NS8*, Lactobacillus helveticus strain* MCC1848*, Lactobacillus fermentum, Lactobacillus reuteri* NK33*, Lactobacillus rhamnosus* have also shown similar pattern with suppression the occurrence and development of anxiety, and influencing biochemical pathways on murine models. A study on human model involved 111 participant recruited with moderate stress level, they were given *L. plantarum* for 12 weeks. The symptoms of anxiety were reduced as early in 8 weeks among adults as compare to control, accompanied by biochemical change in plasma cortisol, plasma pro-inflammatory cytokines, and dopamine and serotonin pathways [86].

Depression

Immobilization stress induced mice were orally administered *L. reuteri* and *B. adolescentis* strains. The organisms synergistically supressed IS-induced depression followed by alleviating gut dysbiosis and immune modulation [77]. *L. helveticus* MCC1848 significantly reduced symptoms related to depression in sub chronic and mild social defeat stress (sCSDS) model mice by ameliorating sCSDS induced gene expression alterations. Schizophrenia patients were given *B. breve* strain A-1 for 4 weeks. Depression score was significantly improved in 4 weeks baseline with the increase in the expression of IL-22 and abundance of parabacteriodes [85]. Interestingly, *Lactobacillus plantarum* 90sk was found to be positively associated with depression as equal to fluoxetine. In addition *L. plantarum* was assessed with combination SAMe (*S*-adenosylmethionine) [90]. This warrants further research to study the treating psychological illnesses with pychobiotic species along with reducing dosage of antidepressant drugs.

Sleep

Probiotic studies are being conducted for psychological problems which also include exploring its association sleep quality. Effect of *Lactobacillus plantarum* PS128 was assessed on 20 to 40 year old age group with insomnia. Questionnaires and miniature-polysomnography recordings at baseline and on the 15th and 30th days of taking capsules showed that the strain decreased brain wave activity and awakening period during deep sleep stage after 30 days [93]. *Lactobacillus gasseri* CP2305 shortened sleep latency and wake time after sleep onset and increased the delta power ratio in the first sleep cycle, and modulation in gut microbial composition in Sixty Japanese medical students (41 men and 19 women) [85]. Similarly, *B. longum* also improved duration of sleep and sleep cycle of students with exam stress. However, no changes were found with working memory compared to placebo. This association of probiotic strains with the circadian rhythm warrants further research.

Cognition

Lactobacillus helveticus NS8 improved cognitive function in mice by lowering plasma corticosterone and adrenocorticotropic hormone levels with higher plasma interleukin-10 (IL-10) levels, and restoring hippocampal serotonin and norepinephrine levels, and more hippocampal brain-derived neurotropic factor (BDNF) mRNA expression compared to control [74]. *L. acidophilus* EG004*, L. paracasei* EG004*, and L. rhamnosus* EG004 have improved cognitive ability compared to control mice models. However *L. acidophilus* has highest ability to improve cognitive performance than other strains. It was observed that *Firmicutes* and *Proteobacteria* phyla increased in *L. plantarum* fed models which led to alteration in neurotransmission and neurotropic factors. *Lactobacillus spp. and Bifobacterium spp.* have improved cognition in human [70].

Future and scope of psychobiotics

Overwhelming evidences on the impact of gut microbiota and their secretions on the Gut-Brain axis and the brain indicate the implications of Psychobiotics for the future. Pyschobitoics, a term that was coined by Ted Dinan and John Cryan [95], referring to a group of microbes, is the net result of the growing evidence on the impact of the gut microbiota and probiotics on the mental wellbeing of human subjects. The past decade has had mounting reports on the role and relevance of microbes in sustaining mental health, supporting the birth of the science of Psychobiotics. Studies indicating decrease in symptoms of depression [81,85,90,92], reducing stress and anxiety [82,84,87,88,94] decreased risk of cognitive disease(including Alzheimer's disease) [84, 87], positive effect on neurological conditions (including Autism, Schizophrenia, Parkinson's disease etc.) [96] have gathered momentum to the research pursuits in the stream. It is believed that specific bacterial strains can be beneficial in sustaining mental well-being, namely, *Lactobacillus* and *Bifidobacterium* species (Table 3). These findings have been amply supported by studies conducted on rodent models and human subjects both, as discussed in the previous section.

Studies in the context prompt to explore the possibilities of including Psychobiotics in the diet. Researchers have studied foods spiked with specific bacterial strains as a part of clinical trials. Strains of *Lactobacillus casei* [72], *Lactobacillus helveticus* [64], *Lactobacillus gasseri* [88], *Lactobacillus plantarum* [90], *Bifidobacterium animalis* [82] have been included in fermented food products as part of clinical trials and the results reported have shown positive effects on the subjects. Reports from these studies indicate that foods spiked with specific strains improved cognitive functions (in both old and middle aged adults) [74,97], improved sleep quality [98], improved moods and provided protective mechanism against cognitive impairment [99], improved memory [100], decreased stress and anxiety [101] in human subjects. It seems clear that eating foods that contain scientifically proven strains of bacteria with known physiological, physical and mental benefits is close to reality. It is also of relevance and noteworthy to mention in this context that several species of *Bifidobacterium* have made it to mainstream application in medicines and many of them have been listed as GRAS (Generally Recognized as Safe) [102]. Although listed as GRAS, some of the species of *Bifidobacterium* have had negative effects in certain cases [103,104], therefore, this warrants for more in-depth studies giving clarity on specific bacterial strains, prior to their inclusion in diet as part or as supplement and to tread cautiously, seeking application with or without restrain.

Mounting evidence in support of probiotics as pyschobiotics has led to the emergence of the Next Generation Probiotics (NGPs) [105]. Pure cultures of *Akkermansia muciniphila, Faecalibacterium prausnitzii, Bacteroides fragilis, Eubacterium hallii,* and *Roseburia* spp. have been termed as Next Generation Probiotics (NGPs) [106]. NGPs are likely to widen our understanding of the underlying mechanisms of probiotic-host health interactions, molecular mechanisms in animals, applications, and safety. Applied research for better use of NGPs is anticipated. For instance, research pursuits are underway to modify obligate anaerobic strains such as *Faecalibacterium prausnitzii* with anti-inflammatory properties, to be able to survive in oxygen-rich environments of the human gut [107]. Gut microbiome deficient in *F. prausnitzii* has been linked to conditions like type 2 diabetes and cardiovascular disease [108]. However, Molecular studies involving the understanding of the genome and *invivo* expression studies of a candidate psychobiotic strain, validating the activity and effect needs to be carried out, prior to their use as a part of or as supplement to diet. It is pivotal to screen for psychotic potential of the candidate strain at genetic and molecular level, following which their potential in animal/murein models and human subjects need to be ascertained. It is also essential to check for bio-safety and toxicity of the strains. While studies on psychobiotic strains in food matrices with admixtures are underway [109], research findings are yet to yield concrete details on these aspects.

It is interesting to observe that despite the ongoing studies and research pursuits to validate and whether the role of probiotic strains with psychobiotic significance, the market is already set. More than 10 global brands have ventured into the Psychobiotics market and products such as HOWARU Calm © (DUPONT) are already available in the market [110]. As on date, awareness on the benefits of probiotics among the population of the developing and developed nations is growing, it has been reported that in the U.S. alone about 4 million were using Probiotic supplements a decade ago [111]. According to a report by Grand View Research, Inc., the global probiotics market size was valued at USD 58.17 billion in 2021 and is expected to grow at a compound annual growth rate (CAGR) of 7.5% from 2021 to 2030 [112]. also suggest that brain/mood supplement products in the U.S. had grown at the rate of 6.5% from 2013 to 2018 and it is projected that Psychobiotics as supplement will have a market size of value 140.3 million USD in 2023, which will rise to 201.8 million USD in 2033 at a compound annual growth rate (CAGR) of 3.7% globally [113].

Ethics Approval

Not Applicable.

Consent for Publication

Not Applicable.

Availability of Data and Materials

Not Applicable.

Competing Interests

There is no conflict of interests.

Funding

The project was funded by the Dayalbagh Educational Institute.

Authors Contribution

- RIMJHIM PATHAK − The first author contributed to collection and compilation of the material required for the article. The author was involved in proof reading and editing.
- PREM SARAN TIRUMALAI – This author contributed to conceive, collate, proof-read and edit the article. The schematics and figures were prepared by this author.
- SEEMA KASHYAP- This author contributed to Proof reading and editing.

Acknowledgements

The authors acknowledge the Head of the Institute, Dayalbagh Educational Institute for constant support and encouragement.

Bibliography

1. Walker A W and Hoyles L. "Human microbiome myths and misconception". Nature microbiology 8.8 (2023): 1392- 1396.
2. Tanaka M and Nakayama J. "Development of the gut microbiota in infancy and its impact on health in later life". *Allergology International* 66.4 (2017): 515-522.
3. Johnson D., *et al*. "Exploring the role and potential of probiotics in the field of mental health: Major depressive disorder". *Nutrients* 13.5 (2017): 1728.
4. Hold GL., *et al*. "Assessment of microbial diversity in human colonic samples by 16S rDNA sequence analysis. *FEMS Microbiology Ecology* 39.1 (2002): 33-39.
5. Ringel-Kulka T., *et al*. "Lactobacillus acidophilus NCFM affects colonic mucosal opioid receptor expression in patients with functional abdominal pain-a randomised clinical study". *Alimentary Pharmacology and Therapeutics* 40.2 (2014): 200-207.
6. Salazar N., *et al*. "Nutrition and the gut microbiome in the elderly". *Gut Microbes* 8.2 (2017): 82-97.
7. Yatsunenko T., *et al*. "Human gut microbiome viewed across age and geography". *Nature* 486.7402 (2012): 222-227.
8. Conlon M A and Bird A R. "The impact of diet and lifestyle on gut microbiota and human health". *Nutrients* 7.1 (2015): 17-44.
9. Lyte M., *et al*. "Stress at the intestinal surface: catecholamines and mucosa–bacteria interactions". *Cell and Tissue Research* 343 (2011): 23-32.
10. Strati F., *et al*. "Antibiotic-associated dysbiosis affects the ability of the gut microbiota to control intestinal inflammation upon fecal microbiota transplantation in experimental colitis models". *Microbiome* 9 (2021): 1-15.
11. Wardwell L H., *et al*. "Current concepts of the intestinal microbiota and the pathogenesis of infection". *Current Infectious Disease Reports* 13 (2011): 28-34.
12. Qamar A., *et al*. "The role of intestinal microbiota in chicken health, intestinal physiology and immunity". *JAPS: Journal of Animal and Plant Sciences* 31.2 (2021).
13. Nie Y., *et al*. "Dietary nutrition and gut microflora: A promising target for treating diseases". *Trends in Food Science and Technology* 75 (2018): 72-80.
14. Gao R., *et al*. "Dysbiosis signatures of gut microbiota along the sequence from healthy, young patients to those with overweight and obesity". *Obesity* 26.2 (2018): 351-361.
15. Li J., *et al*. "Clostridiales are predominant microbes that mediate psychiatric disorders". *Journal of Psychiatric Research* 130 (2020): 48-56.
16. Zuo T and Ng S C. "The gut microbiota in the pathogenesis and therapeutics of inflammatory bowel disease". *Frontiers in Microbiology* 9 (2018): 2247.
17. Cantarel BL., *et al*. "Gut microbiota in multiple sclerosis: possible influence of immunomodulators". *Journal of Investigative Medicine* 63.5 (2015): 729-734.
18. Vivarelli S., *et al*. "Gut microbiota and cancer: from pathogenesis to therapy". *Cancers* 11.1 (2019): 38.
19. Lynch SV. "Gut microbiota and allergic disease. New insights".

Annals of the American Thoracic Society 13 (2016): S51-S54.

20. Benakis C., *et al*. "Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells". *Nature Medicine* 22.5 (2016): 516-523.
21. Dinan T G and Cryan J F. "Brain-gut-microbiota axis and mental health". *Psychosomatic Medicine* 79.8 (2017): 920-926.
22. Liu Y W., *et al*. "Psychotropic effects of Lactobacillus plantarum PS128 in early life-stressed and naïve adult mice". *Brain Research* 1631 (2016): 1-12.
23. Sampson TR., *et al*. "Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease". *Cell* 167.6 (2016): 1469-1480.
24. Minter MR., *et al*. "Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease". *Scientific Reports* 6.1 (2016): 30028.
25. Marazziti D., *et al*. "The Microbiota/Miscrobiome and the Gut–Brain Axis: How Much Do They Matter in Psychiatry?". *Life* 11.8 (2021): 760.
26. Westfall S., *et al*. "Microbiome, probiotics and neurodegenerative diseases: deciphering the gut brain axis". Cellular and molecular life sciences 74 (2021): 3769-3787.
27. Rea K., *et al*. "Gut microbiota: a perspective for psychiatrists". *Neuropsychobiology* 79.1 (2020): 50-62.
28. Yano JM., *et al*. "Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis". *Cell* 161.2 (2015): 264-276.
29. Lyte M. "Microbial endocrinology: host-microbiota neuroendocrine interactions influencing brain and behavior". *Gut Microbes* 5.3 (2014): 381-389.
30. Sarkodie E K., *et al*. "Influences of stress hormones on microbial infections". *Microbial Pathogenesis* 131 (2019): 270-276.
31. Graversen K B., *et al*. "Short-term amoxicillin-induced perturbation of the gut microbiota promotes acute intestinal immune regulation in brown Norway rats". *Frontiers in Microbiology* 11 (2020): 496.
32. Mittal R., *et al*. "Neurotransmitters: The critical modulators regulating gut–brain axis". *Journal of Cellular Physiology* 232.9 (2017): 2359-2372.
33. Balakrishnan M and Floch MH. "Prebiotics, probiotics and digestive health". *Current Opinion in Clinical Nutrition and Metabolic Care* 15.6 (2012): 580-585.
34. Suez J., *et al*. "Artificial sweeteners induce glucose intolerance by altering the gut microbiota". *Nature* 514.7521 (2015): 181–186.
35. Cunningham-Rundles S., *et al*. "Probiotics and immune response". *The American Journal of Gastroenterology* 95.1 (2000): S22-S25.
36. Ebel B., *et al*. "Impact of probiotics on risk factors for cardiovascular diseases. A review". *Critical reviews in Food Science and Nutrition* 54.2 (2014): 175-189.
37. Ruddick J P., *et al*. "Tryptophan metabolism in the central nervous system: medical implications". *Expert Reviews in Molecular Medicine* 8.2 (2006): 0 1-27.
38. Morita C., *et al*. "Gut dysbiosis in patients with anorexia nervosa". *PloS One* 10.12 (2015): e0145274.
39. Zhang L., *et al*. "Altered gut microbiota in a mouse model of Alzheimer's disease". *Journal of Alzheimer's Disease* 60.4 (2017): 1241-1257.
40. Unger M M., *et al*. "Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls". *Parkinsonism and Related Disorders* 32 (2016): 66-72.
41. Maltz R M., *et al*. "Prolonged restraint stressor exposure in outbred CD-1 mice impacts microbiota, colonic inflammation, and short chain fatty acids". *PloS one* 13.5 (2018): e0196961.
42. Liu S., *et al*. "Altered gut microbiota and short chain fatty acids in Chinese children with autism spectrum disorder". *Scientific Reports* 9.1 (2019): 287.
43. Erny D., *et al*. "Host microbiota constantly control maturation and function of microglia in the CNS". *Nature Neuroscience* 18.7 (2015): 965-977.

44. Baxter N T., *et al*. "Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers". *MBio* 10.1 (2019): e02566-18.

45. Macfarlane S and Macfarlane GT. "Regulation of short-chain fatty acid production". *Proceedings of the Nutrition Society* 62.1 (2003): 67-72.

46. McOrist A L., *et al*. "Fecal butyrate levels vary widely among individuals but are usually increased by a diet high in resistant starch". *The Journal of Nutrition* 141.5 (2011): 883-889.

47. Shukla S and Tekwani B L. "Histone deacetylases inhibitors in neurodegenerative diseases, neuroprotection and neuronal differentiation". *Frontiers in Pharmacology* 11 (2020): 537.

48. Mossad O and Erny D. "The microbiota–microglia axis in central nervous system disorders". *Brain Pathology* 30.6 (2020): 1159-1177.

49. Lyte M. "Microbial endocrinology: host-microbiota neuroendocrine interactions influencing brain and behavior". *Gut Microbes* 5.3 (2014): 381-389.

50. Iyer LM., *et al*. "Evolution of cell–cell signaling in animals: did late horizontal gene transfer from bacteria have a role?". *TRENDS in Genetics* 20.7 (2004): 292-299.

51. Smith D K., *et al*. "Escherichia coli has two homologous glutamate decarboxylase genes that map to distinct loci". *Journal of Bacteriology* 174.18 (1992): 5820-5826.

52. Strandwitz P., *et al*. "GABA-modulating bacteria of the human gut microbiota". *Nature Microbiology* 4.3 (2019): 396-403.

53. Matsumoto M., *et al*. "Impact of intestinal microbiota on intestinal luminal metabolome". *Scientific Reports* 2.1 (2012): 233.

54. Pokusaeva K., *et al*. "GABA-producing Bifidobacterium dentium modulates visceral sensitivity in the intestine". *Neurogastroenterology and Motility* 29.1 (2017): e12904.

55. Luck B., *et al*. "Neurotransmitter profiles are altered in the gut and brain of mice mono-associated with Bifidobacterium dentium". *Biomolecules* 11.8 (2021): 1091.

56. Makizaki Y., *et al*. "Improvement of loperamide-induced slow transit constipation by Bifidobacterium bifidum G9-1 is mediated by the correction of butyrate production and neurotransmitter profile due to improvement in dysbiosis". *PLoS One* 16.3 (2021): e0248584.

57. Halverson T and Alagiakrishnan K. "Gut microbes in neurocognitive and mental health disorders". *Annals of Medicine* 52.8 (2020): 423-443.

58. Foster J A., *et al*. "Stress and the gut-brain axis: regulation by the microbiome". *Neurobiology of Stress* 7 (2021): 124-136.

59. Davis I and Liu A. "What is the tryptophan kynurenine pathway and why is it important to neurotherapeutics?". *Expert Review of Neurotherapeutics* 15.7 (2015): 719-721.

60. Marin IA., *et al*. "Microbiota alteration is associated with the development of stress-induced despair behavior". *Scientific Reports* 7.1 (2017): 43859.

61. Dodd D., *et al*. "A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites". *Nature* 551.7682 (2017): 648-652.

62. Vasey C., *et al*. "Circadian rhythm dysregulation and restoration: the role of melatonin". *Nutrients* 13.10 (2021): 3480.

63. Bravo R., *et al*. "Tryptophan-enriched cereal intake improves nocturnal sleep, melatonin, serotonin, and total antioxidant capacity levels and mood in elderly humans". *Age* 35 (2013): 1277-1285.

64. Zhou B., *et al*. "Lactobacillus rhamnosus GG colonization in early life regulates gut-brain axis and relieves anxiety-like behavior in adulthood". *Pharmacological Research* 177 (2022): 106090.

65. Messaoudi M., *et al*. "Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects". *British Journal of Nutrition* 105.5 (2011): 755-764.

66. Sudo N., *et al*. "Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice". *The Journal of Physiology* 558.1 (2004): 263-275.

67. Bercik P., *et al*. "The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut–brain communication". *Neurogastroenterology and Motility* 23.12 (2011): 1132-1139.

68. Tian P., *et al*. "Bifidobacterium with the role of 5-hydroxytryptophan synthesis regulation alleviates the symptom of depression and related microbiota dysbiosis". *The Journal of Nutritional Biochemistry* 66 (2019): 43-51.

69. Savignac H M., *et al*. "Bifidobacteria exert strain-specific effects on stress-related behavior and physiology in BALB/c mice". *Neurogastroenterology and Motility* 26.11 (2014): 1615-1627.

70. Kobayashi Y., *et al*. "Therapeutic potential of Bifidobacterium breve strain A1 for preventing cognitive impairment in Alzheimer's disease". *Scientific Reports* 7.1 (2017): 1-10.

71. Yunes R A., *et al*. "A multi-strain potential probiotic formulation of GABA-producing Lactobacillus plantarum 90sk and Bifidobacterium adolescentis 150 with antidepressant effects". *Probiotics and Antimicrobial proteins* 12 (2020): 973-979.

72. Davis D J., *et al*. "Lactobacillus plantarum attenuates anxiety-related behavior and protects against stress-induced dysbiosis in adult zebrafish". *Scientific Reports* 6.1 (2016): 1-11.

73. Liang S., *et al*. "Administration of Lactobacillus helveticus NS8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress". *Neuroscience* 310 (2015): 561-577.

74. Wang T., *et al*. "Lactobacillus fermentum NS9 restores the antibiotic induced physiological and psychological abnormalities in rats". *Beneficial Microbes* 6.5 (2015): 707-717.

75. Takada M., *et al*. "Probiotic Lactobacillus casei strain Shirota relieves stress-associated symptoms by modulating the gut–brain interaction in human and animal models". *Neurogastroenterology and Motility* 28.7 (2016): 1027-1036.

76. Jang H M., *et al*. "The preventive and curative effects of Lactobacillus reuteri NK33 and Bifidobacterium adolescentis NK98 on immobilization stress-induced anxiety/depression and colitis in mice". *Nutrients* 11.4 (2019): 819.

77. Maehata H., *et al*. "Heat-killed Lactobacillus helveticus strain MCC1848 confers resilience to anxiety or depression-like symptoms caused by subchronic social defeat stress in mice". *Bioscience, Biotechnology, and Biochemistry* 83.7 (2019): 1239-1247.

78. Jeon S., *et al*. "Positive effect of Lactobacillus acidophilus EG004 on cognitive ability of healthy mice by fecal microbiome analysis using full-length 16S-23S rRNA metagenome sequencing". *Microbiology Spectrum* 10.1 (2022): e01815-21.

79. Vaghef-Mehrabany E., *et al*. "Calorie restriction in combination with prebiotic supplementation in obese women with depression: effects on metabolic and clinical response". *Nutritional Neuroscience* 24.5 (2021): 339-353.

80. Wallace C J and Milev R V. "The efficacy, safety, and tolerability of probiotics on depression: clinical results from an open-label pilot study". *Frontiers in Psychiatry* 12 (2021): 618279.

81. Salleh RM., *et al*. "Effects of probiotics on anxiety, stress, mood and fitness of badminton players". *Nutrients*, 13.6 (2021): 1783.

82. Pinto-Sanchez M I., *et al*. "Probiotic Bifidobacterium longum NCC3001 reduces depression scores and alters brain activity: a pilot study in patients with irritable bowel syndrome". *Gastroenterology* (2017).

83. Dong W., *et al*. "Bifidobacterium animalis subsp. lactis BB-12 improves the state anxiety and sports performance of young divers under stress situations: a single-arm, prospective proof-of-concept study". *Frontiers in Psychology* 11 (2021): 3710.

84. Tian P., *et al*. "Bifidobacterium breve CCFM1025 attenuates major depression disorder via regulating gut microbiome and tryptophan metabolism: A randomized clinical trial". *Brain, Behavior, and Immunity* 100 (2022): 233-241.

85. Allen A P., *et al*. "Bifidobacterium longum 1714 as a translational psychobiotic: modulation of stress, electrophysiology and neurocognition in healthy volunteers". *Translational psychiatry* 6.11 (2016): e939-e939.

86. Lew LC., *et al*. "Probiotic Lactobacillus plantarum P8 alleviated stress and anxiety while enhancing memory and cognition in stressed adults: A randomised, double-blind, placebo-controlled study". *Clinical Nutrition* 38.5 (2019): 2053-2064.

87. Saccarello A., *et al*. "Oral administration of S-Adenosylmethionine (SAMe) and Lactobacillus plantarum HEAL9 improves the mild-to-moderate symptoms of depression: a randomized, double-blind, placebo-controlled study". *The Primary Care Companion for CNS Disorders* 22.4 (2020): 23164.

88. Meng X., *et al*. "Clinical application value of Lactobacillus plantarum PS128 in patients with anxiety disorders". *Clinical Psychopharmacology and Neuroscience* 20.3 (2022): 560.

89. Chen H M., *et al*. "Psychophysiological effects of lactobacillus plantarum ps128 in patients with major depressive disorder: a preliminary 8-week open trial". *Nutrients* 13.11 (2021): 3731.

90. Ho Y T., *et al*. "Effects of Lactobacillus plantarum ps128 on depressive symptoms and sleep quality in self-reported insomniacs: A randomized, double-blind, placebo-controlled pilot trial". *Nutrients* 13.8 (2021): 2820.

91. Wauters L., *et al*. "Lactobacillus rhamnosus CNCM I-3690 decreases subjective academic stress in healthy adults: A randomized placebo-controlled trial". *Gut microbes* 14.1 (2022): 2031695.

92. Dinan TG., *et al*. "Psychobiotics: a novel class of psychotropic". *Biological psychiatry* 74.10 (2013): 720-726.

93. Zou R., *et al*. "Psychobiotics as a novel strategy for alleviating anxiety and depression". *Journal of Functional Foods* 86 (2021): 104718.

94. Liu YW., *et al*. "Effects of Lactobacillus plantarum PS128 on children with autism spectrum disorder in Taiwan: a randomized, double-blind, placebo-controlled trial". *Nutrients* 11.4 (2019): 820.

95. Liu Y., *et al*. "Similar fecal microbiota signatures in patients with diarrhea-predominant irritable bowel syndrome and patients with depression". *Clinical Gastroenterology and Hepatology* 14.11 (2016): 1602-1611.

96. Chung Y C., *et al*. "Fermented milk of Lactobacillus helveticus IDCC3801 improves cognitive functioning during cognitive fatigue tests in healthy older adults". *Journal of Functional Foods* 10 (2014): 465-474.

97. Ohsawa K., *et al*. "Lactobacillus helveticus-fermented milk containing lactononadecapeptide (NIPPLTQTPVVVPPFLQPE) improves cognitive function in healthy middle-aged adults: A randomised, double-blind, placebo-controlled trial". *International Journal of Food Sciences And Nutrition* 69.3 (2018): 369-376.

98. Tillisch K., *et al*. "Consumption of fermented milk product with probiotic modulates brain activity". *Gastroenterology* 144.7 (2013): 1394-1401.

99. Akbari E., *et al*. "Effect of probiotic supplementation on cognitive function and metabolic status in Alzheimer's disease: a randomized, double-blind and controlled trial". *Frontiers in Aging Neuroscience* 8 (2016): 256.

100. Hwang K., *et al*. "Para-psychobiotic Lactobacillus gasseri CP2305 ameliorates stress-related symptoms and sleep quality". *Journal of Applied Microbiology* 123.6 (2017): 1561-1570.

101. Butler M I., *et al*. "Recipe for a healthy gut: intake of unpasteurised milk is associated with increased lactobacillus abundance in the human gut microbiome". *Nutrients* 12.5 (2020): 1468.

102. Meile L., *et al*. "Safety assessment of dairy microorganisms: Propionibacterium and Bifidobacterium". *International Journal of Food Microbiology* 126.3 (2008): 316-320.

103. Esaiassen E., *et al*. "Bifidobacterium bacteremia: clinical characteristics and a genomic approach to assess pathogenicity". *Journal of Clinical Microbiology* 55.7 (2017): 2234-2248.

104. Takiguchi Y., *et al*. "Peribronchial connective tissue infection caused by Bifidobacterium Longum and veillonella species mimicking lung cancer". *Internal Medicine* 60.3 (2021): 453-456.

105. Sharma R., *et al*. "Psychobiotics: The next-generation probiotics for the brain". *Current Microbiology* 78 (2021): 449-463.

106. Zhai Q., *et al*. "A next generation probiotic, Akkermansia muciniphila". *Critical Reviews in Food Science and Nutrition* 59.19 (2019): 3227-3236.

107. Martín R., *et al*. "Functional characterization of novel Faecalibacterium prausnitzii strains isolated from healthy volunteers: a step forward in the use of F. prausnitzii as a next-generation probiotic". *Frontiers in Microbiology* 8 (2017): 1226.

108. Kallassy J., *et al*. "Strains of Faecalibacterium prausnitzii and its extracts reduce blood glucose levels, percent HbA1c, and improve glucose tolerance without causing hypoglycemic side effects in diabetic and prediabetic mice". *BMJ Open Diabetes Research and Care* 11.3 (2023): e003101.

109. Pathak R., *et al*. "Time dependent growth pattern study of Lactobacillus spp. and Bifidobacterium spp. in the presence of Tartrazine; an azo dye". *Journal of Microbiology and Experimentation* 11.1 (2023): 20-24.

110. Lalou K. "DuPont N&B launches Howaru Calm to tackle stress and support mental well-being, nutritioninsight" (2021).

111. National Institute of Health. "Probiotics: What you need to know". National centre for complementery health (2019).

112. Grand View Research. "Probiotics Market Size, Share and Trends Analysis Report By Application (Food and Beverages, Dietary Supplements By End Use (Human, Animal By Sales Channel, By Formulation, And Segment Forecasts, 2019 – 2025" (2019).

113. Future Market Insights. "Psychobiotic Supplements Market: Global Industry Analysis 2016-2020 and Opportunity Assessment 2021-2031" (2021).