Can bugs be an alternative or adjuvant to drugs in schizophrenia?

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ABSTRACT

Schizophrenia (SPR) pathophysiology is complex and uncertain, with growing evidence highlighting the role of gut microbiota (GM) in its etiopathogenesis. Trillions of gut bacteria found to be influencing the brain by crossing the blood–brain barrier through various pathways. Gut dysbiosis in particular has been linked to SPR, which has opened up new avenues for the prevention and treatment of SPR by maintaining the gut bacterial diversity with the supplementation of living organisms in adequate proportions termed psychobiotics. In this paper, we reviewed the most shreds of evidence and concepts relating GM through the vagus nerve, neurotransmitters, and microbial by-products to various conceivable pathways leading to and ameliorating SPR. Both animal and human trials have been reviewed to discover the effects of probiotics in modulating endocrinal, inflammatory, immunochemical, and neuronal changes in modifying the physiological and psychopathological states of an individual, which assisted in identifying their physiological basis to improve mood and cognitive abilities and reduce anxiety in both healthy people and SPR patients. Currently, probiotic supplementation and fecal microbiota transplantation are the most recommended interventions. However, the present literature is scarce to conclude specific microbial species or probiotics that can benefit SPR through modification of the microbiota–gut–brain axis. Further evidence from the clinical trials is essential to discover novel gut microbial species that can maintain the diversity of the gut microbial population and benefit SPR disease.

INTRODUCTION TO SCHIZOPHRENIA (SPR)

SPR is a leading cause of disability [1]. It is characterized by delusions, hallucinations, disordered speech and behavior, and other symptoms that cause social or occupational impairment [2]. As per the World Health Organization (WHO), SPR affects about 24 million individuals globally, with a prevalence of 1 in 300 individuals. The most common age for onset is late adolescence and early adulthood. Patients with SPR often have a poor long-term prognosis, which includes psychotic symptoms, poor social functioning, and low quality of life [3–5]. The global burden of disease is substantial since the ailment impairs several life domains and generally lasts for decades [6].

SEARCH STRATEGY

A systematic literature search of the PubMed database from the beginning to January 1, 2018, i.e., the previous 5 years, was carried out. The following key phrase was entered into the search bar: [probiotics or gut microbiota (GM) or dysbiosis] and SPR. The search covered both original research articles and review articles that dealt with both human and animal investigations. Each article's references were also examined. Additionally, cross-references and manual searches were evaluated to find other related articles that might have been overlooked during the initial database search.

ETIOLOGICAL FACTORS RELATED TO SPR

The specific cause of SPR is still unknown, although it is considered that a mix of genetic, physical, physiological, and environmental factors are to blame despite advances in science and technology. It is believed that this condition develops in utero. The increased risk of SPR in adulthood has been related to poor pregnancy circumstances, emergency cesarean sections, and low birth weight [7]. Prenatal and postnatal environmental vulnerabilities and immune system responses are the best-

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studied etiopathogeneses of SPR. For instance, maternal immune activation (MIA), which is the term for the stimulation of the mother's immune system in reaction to infection or infection-like impetuses, has recently been proposed as a "neurodevelopmental primer" [8]. A cascade of cytokines and immunologic changes are passed down to the fetus, resulting in negative phenotypes, particularly in the central nervous system (CNS) [9]. These studies also suggested that maternal respiratory infections, influenza, *Toxoplasma gondii* infections, and other infections might be biomarkers of MIA and the inflammatory response. It is crucial to keep in mind that some environmental variables, such as advanced paternal age, prenatal insults, obstetric problems, early stress, or illegal substances, are known to raise the risk of psychosis. These factors may also work by altering the gut flora [10].

GUT MICROBIOTA

The GM is the collective name for the enormous number of microbes found in the human gastrointestinal (GI) tract (GIT), other than bacteria there are also viruses, protozoa, fungi, and archaea in it, whereas the genes carried by these cells make up the human microbiome [7].

Both disease-causing and disease-preventing microbes can be found in the microbiome [11]. GM has about 1,000 to 5,000 distinct species, 99% of which are phyla. *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia* are the major phyla of the microbiota. These microorganisms are essential for maintaining homeostasis, and an imbalance can result in several diseases [12]. An estimate indicates that the number of bacteria in the human gut at about 3.8×10^{13}, which is somewhat higher than the total number of human cells [13]. These bacteria have a significant role in the physiologies of both health and sickness. These bacteria not only benefit the metabolic process but also help us avoid illnesses, strengthening, and control of our immune system (adaptive and innate) in the process. They also carry out vital tasks including the metabolism of xenobiotics, the synthesis of vitamin B, the promotion of digestion, and the acceleration of neuronal activities [7]. It directly or indirectly

affects the majority of our physiological activities through these imperative acts [14].

A newborn is sterile until delivery because the inside of the uterus is a sterile environment, with bacterial invasion beginning shortly after delivery, while the infant transits the birth canal. There is the colonization of the conjunctiva, oral cavity, digestive tract, and skin. The neonatal microbiome differs depending on the mode of birth, with vaginally born children having a microbiome similar to the vaginal microbiome and those delivered through abdominal delivery having a microbiome similar to the maternal skin microbiome. *Bifidobacterium*, *Lactobacillus*, and *Prevotella* are the most common genera found in the GM acquired following vaginal birth. The most common bacteria found in neonates delivered by cesarean section are *Staphylococcus*, *Corynebacterium,* and *Clostridioides difficile* [15] indicating the mode of technique has a significant impact on the early microbial settling. The GM will further differ depending on whether breastfed or not, the use of antibiotics, and diet gut [15,16].

THE MICROBIOTA–GUT–BRAIN AXIS (MGBA)

The gut–brain axis is a bidirectional communication system that connects the central and enteric nervous systems (ENS), linking the brain's affective and cognitive centers with digestive processes [17]. The GIT, the microorganisms that live there, and the peripheral and CNS all have sophisticated communication systems. This continuous transfer and interpretation of information from the periphery to the brain and back are termed the MGBA.

The MGBA has a significant impact on mood and behavior [18]. This axis consists of two-way communication between the brain and GM through immunological and inflammatory pathways, neurotransmitters, microbial by-products, neuroendocrine and enteroendocrine signaling [vagus nerve and ENS, cortisol, and hypothalamic–pituitary–adrenal (HPA) axis], the stress response, and the vagus nerve; however, the processes are still being understood [19]. There is a healthy resting inflammatory state that exists under normal physiological and homeostatic settings, and the GM triggers the synthesis of cytokines and chemokines that maintain
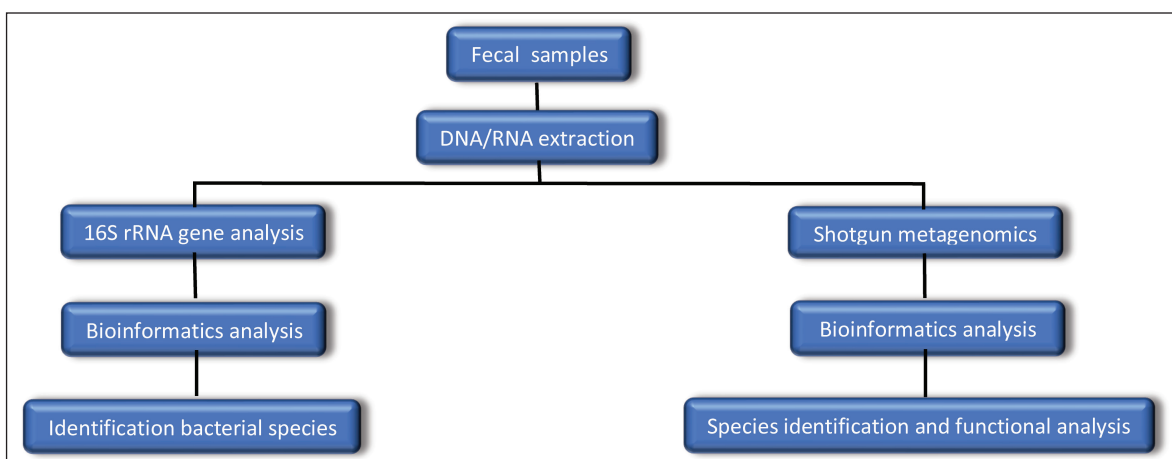

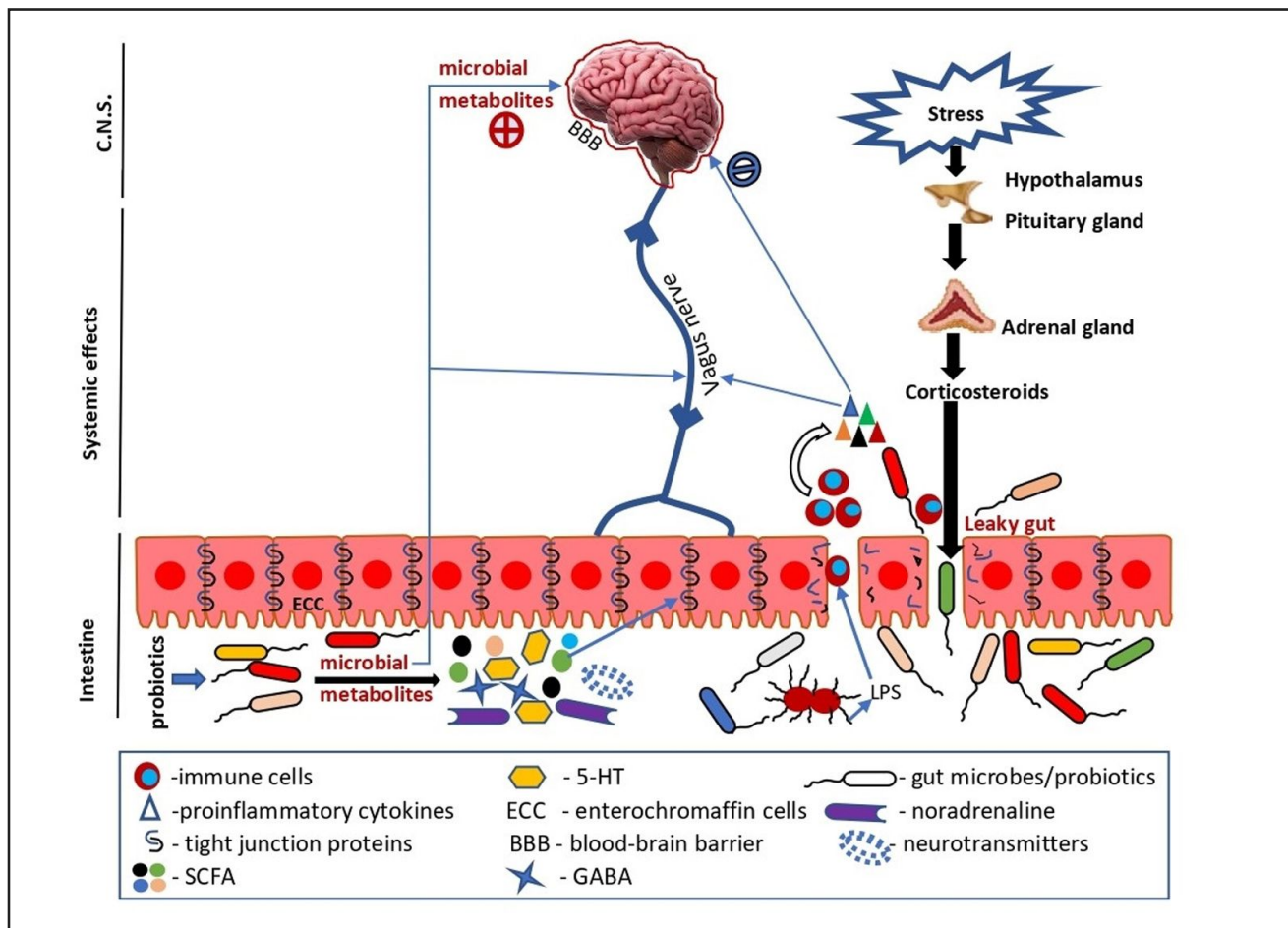**Figure 1.** Laboratory methods for fecal sample analysis.

Figure 2. GM and brain are interconnected by the vagus nerves. Stress and emotions disrupt the HPA axis and ultimately the gut barrier integrity through the release of corticosteroids causing a leaky gut, leading to the migration of bacteria with immune cells releasing proinflammatory cytokines and triggering inflammation. These cytokines further compromise the BBB's integrity and allow potentially harmful and inflammatory substances to enter, which alters mood and cognition and leads to SPR. Probiotics work by (1) Increasing production of SCFA, neurotransmitters, and CA. (2) Inducing expression of tight junction proteins. (3) Restoring the integrity of the gut barrier and BBB and also modulating the neurotransmission in the ENS. (4) Releasing anti-inflammatory cytokines.

microbial inhabitants in the gut [20]. The luminal–mucosal interface, which is predominantly made by the epithelial layer of the GI system, is where most host-microbiota interactions occur. The innate immune response is necessary for this interaction to occur. Intestinal enterocytes produce chemokines and cytokines, contain innate immune receptors, and can affect regional immune cells [21]. As many GI bacteria have a polysaccharide coating, the host immune system may also keep an eye on the GM through Toll-like receptors (TLRs), recognizing prospective infections if exposed [22].

DYSBIOSIS

      A microbial population imbalance caused by alterations to the gut microbiome is frequently referred to as gut dysbiosis or microbial dysbiosis [23].

GUT AND BRAIN BARRIERS

- The blood–brain barrier (BBB) and the intestinal epithelial barrier are two distinct, specialized vascular barriers that connect the gut and brain [24].

- Studies show that gut bacteria can increase the production of cytokines and chemokines, which can affect the HPA axis and cause a neuroinflammatory response. These cytokines and chemokines can then travel to the brain via the bloodstream, lymphatic system, and vagus nerve and increase BBB permeability [19]. When the BBB is compromised, cytokines can have an impact on the hypothalamus and circumventricular organs, among other areas of the brain. The HPA axis can also be stimulated by IL-1 and IL-6 [25]. In investigations on animals, it was shown that the GM regulates the integrity of the BBB, and a varied GM is crucial for maintaining and maturing microglia [19].

- It is found that both fetal and adult mice can have altered BBB permeability due to alterations in the microbiota. In comparison to pathogen-free (PF) mice, who have normal GM, germ-free (GF) animals have a larger BBB permeability, which is also true for the GF mice's embryos. The cause of this anomaly is decreased expression of occludin and claudin-5—which control BBB activities in endothelial tissue and are involved in tight junction disorganization. When the GM of PF mice

was transferred to adult GF mice, they exhibited increased expression of tight junction proteins and a reduction in BBB permeability. Transferring microorganisms that synthesize short-chain fatty acids (SCFAs) or feces of PF animals to GF animals aids in maintaining the BBB's integrity [24].

Major factors and pathways altering the MGBA their connection with SPR

Stress

- Stress on the body, both psychological and physical, affects the makeup of the gut microbiome.
- Stress can lead to elevation of inflammation which further can lead to damage to the intestinal barrier (leaky gut), causing bacterial infiltration and a rise in plasma lipopolysaccharide (LPS) [19] (Fig. 2).
- Stress changes the way mucus is secreted, and this can have a serious effect on the growth of intestinal microbes that prebiotics and dietary fibers promote [26].
- The HPA axis is activated by stress, which has a significant impact on GM through the process of microbial dysbiosis. In SPR, there is more neuroinflammation after extended HPA axis activity [19] (Fig. 2).

Leaky gut

- Stress can weaken the epithelium and make it porous thereby increasing the intestinal barrier's permeability, resulting in a "leaky gut" [24] and loss of bacteria and their by-products [16,27].
- It leads to the translocation of gram-negative microbes LPS, which activates the immune system (TLRs) and regulates the production of proinflammatory cytokines [IL-6, IFN-γ, C-reactive protein (CRP), and TNF-α] [24,27] (Fig. 2).
- In SPR, this bacterial translocation can result in autointoxication, which fuels the chronic inflammatory state. SPR patients have been found to have elevated inflammatory cytokines in addition to LPS, which may help to modify gut permeability and create a "leaky gut" [19].
- Gliadin, b-lactoglobulin, and casein IgA levels were shown to be higher in SPR patients [28]. In large research on SPR patients and nonpsychiatric comparative controls, SPR patients exhibited moderate-to-high levels of IgA antigliadin antibodies (AGA). In another study, patients who had recently developed psychosis and those with numerous-episode of SPR exhibited greater levels of IgG and IgA antibodies to gliadin [28,29].

VAGUS NERVE AND SEROTONIN INTERCONNECTION

Vagal afferent fibers are directly contacted by enteroendocrine cells (EECs) (Fig. 2) by the production of serotonin, which activates 5-hydroxytryptamine-3 ($5\text{-}HT_3$) receptors, or indirectly through gut hormone. In addition to having SCFA receptors, EECs also express TLRs, which are involved in the detection of microbes. Thus, by controlling GI motility, secretion, and food intake, these cells can detect bacterial chemicals and indirectly affect vagal afferent fibers.

TLR4, which is triggered by LPS, allows bacteria to directly detect the vagus nerve. LPS stimulates cytokines like IL-1, which then cause disease by activating the vagus nerve. The induction of cytokines is stopped after vagotomy [30].

VAGUS NERVE AND GAMMA-AMINOBUTYRIC ACID (GABA) INTERCONNECTION

Antibiotic combinations cause gut dysbiosis by altering GABA-mediated neurotransmission [8], indicating the significance of normal GM in relating N-Methyl D-Aspartate Receptor (NMDAR)-GABA activity to hippocampus (HPC) memory, motor control, and cognitive flexibility; reduction in NMDAR-GABA levels can cause impairment in cognitive function similar to SPR and can increase psychotic symptoms because GM development is essential to fuel brain plasticity via the expressions of the adequate NMDA and brain-derived neurotrophic factor (BDNF) receptors [7,8].

VAGUS NERVE AND CYTOKINES INTERCONNECTION

The vagus nerve transmits environmental signals that the CNS continually reacts to maintain homeostasis. Peripheral cytokine synthesis sets off the vagal anti-inflammatory reflex, which produces acetylcholine and stops excessive cytokine release from causing tissue damage [31] (Fig. 2). Recent studies have shown that people with depression, anxiety disorders, and SPR have altered gut microbiome as well as vagal tone. Some probiotics, including *Bifidobacterium*, use vagal pathways to communicate with the brain. While certain probiotics lose their beneficial effects on the brain and behavior when the vagus nerve is severed [32].

Microbial by-products

Numerous bioactive compounds, including bacteriocins, bile acids, choline, and SCFAs, can be secreted by bacteria present in the gut microbiome (Fig. 2). SCFAs are the resultant of the fermentation of polysaccharides by inducing the synthesis of neurotransmitters. They contribute to neurological and mental conditions like SPR [19] (Fig. 2).

The cecum and colon contain GM, which metabolizes fiber, protein, and peptides that are not broken down by digestive enzymes in the upper gut. SCFAs, such as acetate, propionate, and butyrate, are their major products [33], although fermented foods can be a supplemental source. The host's metabolism of long-chain fatty acids, the conversion of pyruvate to acetate, and the degradation of proteins by the microbiota are all endogenous sources of SCFAs. SCFAs are digested by cells through the Krebs cycle of citric acid to provide energy [34]. The main energy source for colonocytes is butyrate, which prevents inflammation by preventing histone deacetylases [35]. Butyrate has been used as an experimental medication for neuropsychiatric diseases because it affects particular receptors and transporters, whereas all SCFAs have inhibitory effects on histone deacetylase [34].

The CNS appears to be affected by SCFAs produced by the GM that act on glial cells, including microglia and astrocytes, although the precise effect varies on the kind of SCFA and the target cell. It has been claimed that butyric acid caused the LPS-

induced microglial cells in rats to exhibit anti-inflammatory properties [36]. According to another study, SCFA treatment of GF mice led to the restoration of microglial malformation and immaturity when FFAR2 was activated [37]. Propionic acid had an impact on cytoskeletal integration and elevated glial fibrillary acidic protein in cultured astrocytes in rat studies [38]. Rats were given injections of propionic acid made from bacteria, which resulted in cognitive and motor damage [39].

Inflammation

SPR is linked to chronic systemic and GI inflammation, oxidative stress, and metabolic dysfunction [40]. According to several research, SPR is linked to increased serological indicators of microbial translocation [41–43], implying higher intestinal lumen permeability affecting physiological functioning. Inflammation and neuropsychiatric diseases are inextricably related [44]. Patients with SPR have elevated levels of proinflammatory cytokines, inflammation-inducing molecules like damage-associated molecular patterns, activated sensors like TLR, inflammasomes, acute-phase proteins like CRP, and adhesion molecules in their blood and cerebrospinal fluid [30]. Similarly, two different studies on mice demonstrated attenuation of the main signs of ketamine-induced SPR, and enhanced memory by modifying the oxido-inflammatory and neurotransmitter-related pathways, with the use of *Carpolobia lutea* extract and diosmin [45,46]. Chronic inflammation brought on by the GM can develop as a result of structural damage to the intestine. A study of SPR patients' autopsies identified several inflammatory bowel diseases that might cause instability in the intestinal wall structure [47].

Research also indicates that around 30% of individuals with SPR have higher AGA of the IgG type, suggesting that this subset of individuals may also have increased gut permeability. AGA IgG in particular suggests that these antibodies may be crossing the BBB, leading to neuroinflammation since new findings have demonstrated a strong correlation of IgG-mediated antibodies between the peripheral and cerebral spinal fluid in SPR but not healthy controls. Other aberrant translational indicators have been strongly linked to SPR, suggesting greater intestinal permeability in this condition. Metabolic syndrome (MS) is seen in >1 in 5 and persistent low-grade peripheral inflammation in 1/3rd of people with SPR. This inflammation has also been linked to SPR-major depression and is a reliable indicator of central inflammation [48].

Immune system and neuronal inflammation in SPR

The intestinal immune system keeps up immunity to dangerous bacteria and tolerance to commensals, and an imbalance between the host immune system and microbiota can influence inflammation and lead to several disorders. Many bacterial substances, including peptidoglycan, lipoteichoic acid (a component of Gram-positive bacteria's cell wall), LPS, flagellum (which facilitates bacterial motility), pilus (which mediates bacterial attachment to cells), DNA, and cell wall fragments, are regarded as pathogen-associated molecular patterns. Pattern-recognition receptors and nonpattern-recognition receptors, which are crucial elements of the immune system, can identify the molecular patterns associated with pathogens. Immune receptors'

detection of pathogen-associated molecular patterns starts a chain reaction of signaling pathways that turns on several transcription factors and increases the production of inflammatory mediators, which are necessary for the eradication of invasive pathogens, including cytokines, chemokines, and antimicrobial peptides. This host-immune response raises intestinal permeability, which makes it easier for drugs to enter circulation [30]. It also causes a systemic inflammatory response, which raises BBB permeability and activates microglial cells. Cytokines in the blood compromise the immune system. The TLR-4 receptor, which is abundantly present in brain monocytes, macrophages, and microglia, is responsible for recognizing LPS. It has been documented that the GM in inflammatory bowel syndrome patients with depression activates TLR-4-mediated inflammatory responses [26,49]. Alterations in circulation levels of proinflammatory and anti-inflammatory cytokines can result from gut bacteria and probiotics' indirect effects on the innate immune system, which in turn have direct effects on brain functioning [26].

Growing evidence points to the immune system's important role in SPR via changing innate and adaptive defensive systems. Immune cells may invade the brain and mediate neuroimmune interaction to cause neuroinflammation by releasing inflammatory cytokines and reactive oxygen species, leading to neurodegenerative and neuro-progressive alterations in SPR [7]. SPR and other associated psychoses exhibit a variety of immune system disorders that often overlap with one another.

A highly intriguing new result from a human investigation revealed a correlation between alterations in the right middle frontal gyrus volume and certain bacteria linked with SPR, suggesting a possible relationship between GM and brain anatomy in SPR. Data on altered GM in SPR sufferers are contradictory overall, especially when it comes to *Proteobacteria* and *Firmicutes* (at the family level) and *Clostridia* (at the class level). The most consistent observation so far seems to be an increase in *Lactobacilli* abundance [24].

Neurotransmitters

Gut microbiome bacteria generate a variety of neurotransmitters like GABA (*Lactobacillus* and *Bifidobacterium*), norepinephrine (*Escherichia coli*, *Bacillus*, and *Saccharomyces* spp.), dopamine (*Bacillus*), acetylcholine (*Lactobacillus*), and serotonin (*Escherichia*, *Enterococcus*, *Candida*, and *Streptococcus*), tryptophan (*Clostridium*, *Burkholderia*, *Streptomyces*, *Pseudomonas*, and *Bacillus*), a precursor to serotonin are the most frequently generated neuroactive substances which interact with various host systems to maintain homeostasis. There are distinct metabolizing bacterial routes found to differ between healthy and those with SPR [19]. Through enterochromaffin cells (ECs) and enteric nerve receptors, these neurotransmitters can communicate with the CNS [26].

GABA

Lactobacillus brevis and *Bifidobacterium dentium* effectively generate GABA, a key inhibitory neurotransmitter whose malfunction is linked to sadness, anxiety, autism, and SPR. In a preclinical investigation, it was proposed that GABA

generated by gut bacteria passes the BBB and reaches the CNS. Mice's anxiety and depression-related behaviors have also been shown to decrease when given *Lactobacillus rhamnosus*, and the concentration of GABA in the HPC has been observed to rise. Given that these effects only manifest when the vagus nerve is unharmed, it is conceivable that gut bacteria indirectly control GABA transmission (discovered as an SPR endophenotype]) via the vagus nerve [26].

Histamine

Some gut microbes can make histamine. Histidine decarboxylase is expressed by *Lactobacillus reuteri*, which also produces histamine [50], and suppresses TNF-α by generating histamine in myeloid progenitor cells. Histamine has been proven to play a similar immunomodulatory effect in intestinal lymphoid organs, where it controls *Yersinia enterocolitica* infection. Additionally, it has been discovered that an H_2-receptor blockade decreases mucus production and increases intestinal barrier dysfunction, which may help to facilitate the transfer of germs from the intestinal lumen to the bloodstream [26].

REGULATION OF SEROTONIN AND DOPAMINE BY THE GM

Lower serotonin levels result in less permeability of the gut wall and reduce occludin expression, which raises gut wall permeability. Another study found that giving *Lactobacillus* to GF mice boosted their levels of 5-HT as well as dopamine in the striatum, suggesting the potential for employing bacterial transplants to treat Parkinson's disease. Additionally, the MGBA allows the GM to influence the enzymes that control dopamine production. Data from rat research indicates a small but significant role for the gut lumen in dopamine synthesis [51].

NOREPINEPHRINE

The fact that specific-PF mice had much higher amounts of dopamine and noradrenaline in the cecum than GF mice suggests GM is a source of catecholamine (CA) [52]. A gene for a transcript with a sequence similar to that of tyrosine hydroxylase, the rate-limiting enzyme in the production of noradrenaline and dopamine, can be found in some bacterial species [53]. Dopamine synthesis by *Lactobacillus* bacteria is known to occur during culture [54]. Dopamine generated in the peripheral nervous system cannot cross the BBB, hence there is currently no proof that CA produced by microbes affect the CNS. However, tyrosine levels are found to be lower in GF mice than in ex-GF animals, suggesting that GM may increase dopamine levels in GF mice's brains [55]. Supported another study showing the brains of GF mice had higher levels of CA as compared to ex-GF animals, but that restoring the GM reduced those levels through modulating dopamine and noradrenaline turnover in the brain [56].

GLUTAMATE

Numerous neuropathological illnesses, including SPR, have been linked to neurotransmission dysfunction of glutamate and disturbance in iGluRs signaling. Alterations in glutamate metabolism have been linked to changes in gut flora. For instance, *Campylobacter jejuni* increases the production of glutamate, and its decreased abundance in the GIT influences the synthesis of glutamate, which indirectly affects the metabolism of glutamate [57].

LABORATORY METHODS FOR MICROBIOME ANALYSIS

Numerous instruments are used to examine the processes of communication between the gut microbiome and the brain, as well as the fundamental laboratory procedures needed to detect bacteria in a fecal sample (Fig. 1) [16]. In the past, the only way to study bacteria was through culture procedures that entailed plating samples on the right media and recognizing the bacterial growth that appeared [58]. Numerous microbes were incompatible with culture, which made it difficult to identify them using this approach. All the microorganisms present may now be identified thanks to the development of "metagenomics," a culture-independent method that allows for direct examination of the genetic material in a sample [59].

Microbe, DNA, and mRNA level analyses are the three main forms of analysis. Culturome, amplicon, metagenome, metavirome, and metatranscriptome analyses are some of the relevant study methods. A high-throughput technique for cultivating and detecting bacteria at the microbe level is called culturome. It is the best way to produce bacterial stocks, but it is expensive and laborious [60]. The microbiome of human beings [61,62], mice [63], marine sediment [64], Arabidopsis thaliana [65], and rice [66] have all been studied using this technique.

Almost all sample types can be used for amplified sequencing. For prokaryotes, this approach mostly uses 16S ribosomal DNA (rDNA), while for eukaryotes, it primarily uses 18S rDNA and internal transcribed spacers [60].

While more expensive than amplicon sequencing, metagenomic sequencing offers more information. For "pure" samples like human excrement, 6 to 9 terabytes (GB) of sequencing data per sample is considered acceptable [67].

Technically, metagenome and metatranscriptome analysis are included in metavirome research since viruses have either DNA or RNA as their genetic material. To obtain enough viral DNA or RNA for analysis because of the low biomass of viruses in a sample, virus enrichment [68], or the removal of host DNA [69], is a necessary step.

DIFFERENT OMICS APPROACHES FOR THE IDENTIFICATION OF BACTERIA IN THE GM

Metagenomics is shotgun sequencing of DNA from samples which yields a collection of genomes and genes, analyzed using a metagenomics program. Following the assembly or mapping of sequences to a reference database typically includes an annotation step. The microbiome's ability to function and the identification of bacteria are revealed by this extensive and expensive procedure. Additionally, it helps in gathering genomic information for gut bacteria that cannot be cultured [70].

Metataxonomics is a high-throughput method for characterizing the entire microbiota majority of which relies on the amplification and sequencing of marker genes,

Table 1. Alteration of the GM and their link to SPR—evidence from clinical research.

Reference	Subject type	Investigated factors	Methods	Result
[71]	Human	GM of SPR and healthy controls	16S rRNA sequencing	• In contrast to the healthy stomach, the intestines of untreated SPR included facultative anaerobes such as *Lactobacillus fermentum*, *Alkaliphilus oremlandii*, *Cronobacter sakazakii/turicensis*, and *Enterococcus faecium*
[72]		Differences in fecal microbiota between 64 SPR patients and 53 healthy controls	16S rRNA amplification of V3–V4 region and illumina sequencing	- ↑*Proteobacteria* (class-Gammaproteobacteria)
				- genera *Succinivibrio*, *Megasphaera*, *Collinsella*, *Clostridium*, *Klebsiella*, and *Methanobrevibacter* were particularly abundant
				- Decline in *Coprococcus*, *Roseburia*, and *Blautia* species
[73]		Fecal samples of 28 FEP and 16 healthy patients were analyzed	qPCR and metagenomic analysis	- No difference in α-diversity index between comparable samples
				- Elevated *Lactobacillaceae Halothiobacillaceae*, *Brucellaceae*, *Micrococcineae*, and *Bifidobacterium*
				- The First episode of SPR was associated with significantly lower levels of *Bifidobacterium* spp., *E. coli*, and Veillonellaceae
[74]	Human, mice	GM of 64 SPR patients and 69 healthy controls	- 16S rRNA gene sequencing	- ↓ Microbiome α-diversity index in SPR (similar findings by [75], and [76])
			- Human-to-mice GM transplant	- No significant difference in *Proteobacteria*
		SPR-relevant behavioral phenotypes in GF mice	- Whole-genome shotgun sequencing of the cecum	- ↓ Disturbances of GM composition Veillonellaceae and Lachnospiraceae families associated with SPR severity
				- GF mice receiving SPR microbiome
				- HPC ↓glutamate and ↑glutamine and GABA
		SPR versus healthy control mice microbiota analysis	- Non-targeted metabolomics analysis	- SPR-relevant behaviors similar to those with GLU hypofunction
[77]	Human	Examination of unweighted UniFrac and Bray-Curtis dissimilarity	16S rRNA sequencing of the V4 region	↓ Proteobacteria (phylum level); ↓*Haemophilus*, ↓*Sutterella*, ↓*Clostridium* ↑*Anaerococcus* (genus level) in SPR patients vs healthy controls
				Ruminococcaceae—correlated with lower severity of negative symptoms
				Bacteroides—with worse depressive symptoms; and *Coprococcus* was related to a greater risk for developing coronary heart disease
				Verrucomicrobia was shown to be favorably associated with overall mental well-being
				No difference in the α-diversity index between comparable samples
[78]	Human	Compared the differences in the fecal microbiota between 82 SPR patients and 80 normal controls	16S rRNA sequencing of the V3–V4 region	Phylum: ↑Actinobacteria, ↓ *Firmicutes*, ↑ genus level of *Collinsella*, *Lactobacillus*, *Succinivibrio*, *Mogibacterium*, *Corynebacterium*, undefined *Ruminococcus*, and *Eubacterium*
				↓ genus level of *Adlercreutzia*, *Anaerostipes*, *Ruminococcus*, and *Faecalibacterium* in SPR patients versus normal controls
[79]	Human	GM study of 81 high-risk individuals, 19 ultra-high risk, and 69 health controls	Magnetic resonance spectroscopy and 16S rRNA analysis of fecal samples	No statistically significant difference in the number of bacteria between the two groups
				No significant differences in alpha diversity (species richness and diversity) between the groups
				↑ *Lactobacillus* group bacteria in FEP patients-linked with the severity of the symptoms
				Extremely high-risk subjects showed ↑ *Clostridiales*, ↑ *Lactobacillales*, and ↑ *Bacteroidales* as compared to the other two groups
[80]	Human	Metagenomic analysis to characterize bacteriophage genomes in the oral pharynx of 41 SPR and 33 healthy individuals	Generated >100 million sequence reads from each sample and the mapping of these reads to databases	*Lactobacillus phage phiadh* was considerably more common in the oropharynx of schizophrenic patients. Which was also linked to immunological disorders
[81]	Human	Metabolic parameters, superoxide dismutase, high-sensitivity CRP, and GM analysis	GM of 41 patients with risperidone administration and 41 controls were assessed for 24 weeks	Following therapy, a notable drop in fecal *Clostridium coccoides* and *Lactobacillus* and a large rise in *Bifidobacterium* and *E. coli* were discovered. This suggests abnormalities in the makeup of the GM in the initial episode of the disease

such as the bacterial 16S rRNA gene, which contain both conserved and variable sections. Community-wide taxonomic classifications are made possible by metataxonomic tree-based hierarchical clustering studies, which also show the evolutionary relationships among all collected sequences. The organization and make-up of the bacterial communities in the guts of humans, mice, and insects have therefore been clarified via metataxonomics. It can be used for gut archaeal community studies in addition to bacterial microbiota identification [70].

Metatranscriptomics—its goal is to study how RNA is expressed and regulated in complex organisms in their natural habitats. In general, samples' RNA is extracted after synthetic clonal DNA is created, and then high-throughput sequencing is applied.

Other omics approaches: Gut microbial activity is tracked by transcriptional and proteomics principles to produce gut metabolites. Studies on GM usually employ the omics technique known as metabolomics, which identifies the metabolite profiles in any particular strain or solo tissue. The measurement of all metabolite amounts (or concentrations) and locations in cells or tissues is known as metabolomics [70].

Various software along with their links used in amplicon and metagenomic sequencing: QIIME (http://qiime.org/), QIIME 2 (https://qiime2.org/), USEARCH (http://www.drive5.com/usearch), VSEARCH (https://github.com/torognes/vsearch), Trimmomatic (http://www.usadellab.org/cms/index.php?page=trimmomatic), Bowtie 2 (http://bowtie-bio.sourceforge.net/bowtie2), MetaPhlAn2 (https://bitbucket.org/biobakery/metaphlan2), Kraken 2 (https://ccb.jhu.edu/software/kraken2), HUMAnN2 (https://bitbucket.org/biobakery/humann2), MEGAN(https://github.com/husonlab/megan-ce), MEGAHIT (https://github.com/voutcn/megahit), metaSPAdes (http://cab.spbu.ru/software/spades), MetaQUAST (http://quast.sourceforge.net/metaquast), MetaGeneMark (http://exon.gatech.edu/GeneMark/), Prokka (http://www.vicbioinformatics.com/software.prokka.shtml), CD-HIT (http://weizhongli-lab.org/cd-hit), Salmon (https://combine-lab.github.io/salmon), metaWRAP (https://github.com/bxlab/metaWRAP), and DAS Tool (https://github.com/cmks/DAS_Tool) [60].

ALTERATION OF THE GM AND THEIR LINK TO SPR—EVIDENCE FROM CLINICAL RESEARCH

One of the initial precise documentation of GI inflammation related to SPR was where an autopsy examination showed that 41 of the 82 SPR patients had gastritis, and 73 and 76 had enteritis, and colitis, respectively (Table 1). Interestingly, reports of mental comorbidities among persons with intestinal diseases with an inflammatory component show the opposite trend [7]. The first study that discovered disturbed GM in SPR patients had 25 chronic SPR patients who differed from 25 healthy controls [28].

The frequency of SPR and autism has been observed to be greater in *C. difficile* infected. A phenylalanine derivative generated and released by the same bacteria in the stomach that is known to control CA levels in the brain helped to explain this link. Twin and adoption genetic studies further support the SPR and GM relationship. In monozygotic twins, the microbial commonality is higher than in dizygotic twins, which is consistent with the frequency of SPR in twin studies. Premature infants have also been found to have a higher chance of having SPR later in life [82]. Several investigations found an increase in species belonging to the gram-negative bacteria *Fusobacterium*, *Megasphaera*, and *Prevotella* genera [4] in SPR.

ALTERATION OF THE GM AND THEIR LINK TO SPR—EVIDENCE FROM PRECLINICAL RESEARCH

When GF animals were given the SPR microbiome showed altered glutamate/glutamine and GABA levels in the HPC, demonstrating SPR-like behaviors comparable to previous mouse models of SPR with glutamatergic hypofunction [76,83] (Table 2).

An *in vitro* study that looked at how LPS affected intestinal epithelial cells discovered that acute LPS injection changed and decreased the distribution of tight junctions. LPS has been demonstrated to be a successful mouse neurodevelopmental model of SPR [4].

The majority of preclinical research on the MGBA has focused on unfolding how GM may impact brain function through immunological, neural, and endocrine mechanisms [84].

ANTIPSYCHOTICS IN SPR

A wide array of typical and atypical antipsychotics are used for the treatment of SPR. The introduction of second-generation antipsychotics, often known as "atypical" antipsychotics, was spurred by the fact that typical antipsychotics like haloperidol and fluphenazine did not have an impact on

Table 2. Alteration of the GM and their link to SPR—evidence from preclinical research.

Reference	Methods and investigated factors	Results
[51]	• Elucidate the involvement of GM in the generation of luminal CA by comparing the findings among specific PF mice, GF mice, and gnotobiotic mice	• Serum tryptophan levels rose in the GF mice, indicating an immunological route mediates the effect of GM on 5-HT activity in the brain
	• CA and β-glucuronidase activity analysis	• Tph2 gene expression (converts serotonin from tryptophan), was normal despite the increased 5-HT turnover in the GF animals
[86]	• Behavioral tests, western immunoblotting, expression of genes analysis using qPCR	• GF mice showed increased DA (corpus striatum); anxious and agitated phenotype
	• GF and PF mice were raised in isolated cages until they reached 8–10 weeks of age	• Mice behavior was similar to SPR with severe cognitive loss (> in male mice i.e., consistent with SPR epidemiology)

the negative and cognitive symptoms and the severity of their side effects. Atypical antipsychotics heralded a significant shift and the development of fresh strategies in psychiatric pharmacology. When compared to "typical" or "conventional" antipsychotics, they exhibit distinct prominent features, which is what the name "atypical" refers to. Contrarily, there are significant variations in the risk of specific adverse effects among antipsychotic medications. Atypical antipsychotics like olanzapine, risperidone, and clozapine appear to have much fewer adverse effects like prolactinemia, pseudoparkinsonism, and dystonia after being taken on a long-term basis, but they are still effective in reducing positive symptoms [85]. The variations in antipsychotic medication efficacy across different drugs, at least at group levels, are rather minimal, except for clozapine, which is more successful in SPR [87,88]. The risk of metabolic problems, such as hypoglycemia and weight gain, is increased with certain atypical antipsychotics [87].

The clinical antipsychotic trials of intervention effectiveness [89] and cost utility of the latest antipsychotic drugs in SPR study [90] revealed no differences in the treatment of psychotic symptoms and quality of life by either type of antipsychotic drugs, in contrast to initial enthusiasm and optimism for the therapeutic advantage of atypical over typical antipsychotics. A meta-analysis separating antipsychotic drugs into typical and atypical antipsychotic groups revealed that amisulpride, clozapine, olanzapine, and risperidone, four atypical antipsychotics, were more effective in treating SPR than the typical antipsychotics studied. In terms of how they attach to dopamine receptors, they are different from traditional medicines. These antipsychotics are more potent at D_2 receptors due to their affinity for 5-HT$_2$A receptors, which raises their overall effectiveness [7]. Additionally, there is mounting proof that antipsychotic usage affects gut microbial species via their antimicrobial action [19].

EFFECT OF ANTIPSYCHOTICS ON THE GUT MICROBIOME

The antipsychotic drug olanzapine has been demonstrated to increase *Firmicutes* and reduce *Bacteroidetes* in rats. Although not always [81], this effect was replicated in human antipsychotic trials. Antipsychotics do have strong anticommensal action [91]. This has the consequence that if antipsychotic therapy damages the GM, then supplemental therapies meant to restore microbial function would be helpful [92].

A systematic review article published that both olanzapine and risperidone were found to enhance the number of *Firmicutes* in comparison to *Bacteroidetes*. These changes in the GM were connected to weight growth, lipogenesis, and increased levels of free fatty acids and acetate all at the same time. Since this research is primarily based on animal models, clinical trial data are less in line. For instance, following 6-week treatment with olanzapine in individuals with SPR, not many substantial alterations were noted in gut flora. Another research found that when compared to healthy controls, drug-naive individuals with first-episode psychosis (FEP) had higher concentrations of *Proteobacteria* and lower concentrations of bacteria that produce SCFAs (*Faecalibacterium* and

Lachnospiraceae) [93]. Importantly, several changes in GM have been linked to regional brain volume. For example, drug-naive FEP individuals have decreased *Actinobacillus* and *Veillonellaceae abundance* [83].

Atypical antipsychotics may be able to affect serotonin, which is disrupted in SPR. A meta-analysis of autopsy studies on SPR found that there were more prefrontal 5-HT1A receptors and fewer prefrontal 5-HT2A receptors. Moreover, the regulation of brain serotonin levels and the gut-brain axis is interlinked. Dopamine and serotonin levels were shown to be higher in research using GF mouse models, indicating that the CNS is influenced by gut microorganisms. In a similar vein, higher serotonin levels have been seen in the hippocampi of GF mice. Another research claimed that by stimulating the synthesis of serotonin in ECs in the GIT, gut microorganisms have contributed to elevated blood levels of the neurotransmitter [7,94].

Patients with SPR who were taking risperidone had significantly reduced fecal *Bifidobacterium* spp., *E. coli*, and *Lactobacillus* spp., when compared to untreated controls. Suggestive of metabolic changes due to risperidone therapy and it alters specific fecal microbes. Okubo *et al.* [95] looked into how the probiotic *Bifidobacterium breve* A-1 affected SPR patients' symptoms of anxiety and depression as well as how it affected immune substances including cytokines and chemokines. Four weeks of probiotic therapy helped SPR patients perform better on anxiety/depression tests, signifying probiotics' role in SPR. Individuals on either risperidone or olanzapine had different levels of *Akkermansia*, *Sutterella*, and *Lachnospiraceae* than healthy controls [96].

The first rats to receive olanzapine had an altered microbial composition [30,97]. A rise in the relative abundance of *Firmicutes*, and a drop in *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* were all detected. Body weight gain, a rise in the amount of adipose tissue, and changes in inflammatory and metabolic markers all occurred in conjunction with the changes in microbial composition. Some of the impacts were gender-specific, i.e., stronger in females than in males [97]. Administration of olanzapine reduced total microbial diversity decreased *Bacteroidia* abundance and raised *Erysipelotrichia*, *Actinobacteria*, and *Gammaproteobacteria* abundances. It is noteworthy to note that olanzapine-induced weight growth was reliant on the presence of GM, it was supported by the lack of a substantial rise in body weight in GF mice treated with the drug and a quick weight gain after microbial colonization [98]. Due to decreased energy expenditure, risperidone therapy led to excessive weight gain in rats, and this effect was linked to changes in the gut flora. More specifically, it was shown that *Firmicutes* were more prevalent than *Bacteroidetes* [99].

PROBIOTICS

In the early twentieth century, Elie Metchnikoff, popularly known as the "father of modern probiotics," discovered that frequent intake of lactic acid bacteria found in fermented dairy products was linked to improved health and longevity in humans. This prompted scientists to investigate the microbiology of human processes further. In 1953, Werner

Kollath coined the term "probiotics", combining the Greek terms "pro," which means "for," and "biotos," which means "life". Fuller recommended probiotics as "live microbial supplements that benefit the host by enhancing its microbial balance." Later in 2001, the United Nations Food and Agriculture Organization and the WHO agreed on a consensus definition of probiotics. The 2001 definition is still valid today, with slight changes. Probiotics are currently described as "live organisms that, when administered in adequate amounts, confer a health benefit to the host" [100]. Bacteria and yeasts are among the biologically active organisms found in probiotics. *Lactobacillus* and *Bifidobacterium* are the two types used Gram-positive lactic acid bacteria found in probiotics [101]. These two species are often utilized in animal and human investigations. *Lactococcus*, *Bacillus*, *Pediococcus*, and *Streptococcus* are among the other microbes commonly used in probiotic research. Other genera include *Kluyveromyces*, *Pichia*, and *Candida*, as well as common yeasts like *Saccharomyces boulardii* [102]. Today, probiotics may be found in a variety of foods, including yogurt, cheese, wafers, fermented milk or other beverages, pills, capsules, sachets, and even chocolates. You may get them from pharmacies, drugstores, supermarkets, health food stores, or online sites [28].

Even though commensal microbes in the gut are frequently the source of probiotic strains, these strains cannot be deemed probiotics unless they have been secluded, and recognized, and a convincing argument for their health aids has been made [103]. A probiotic supplement should list the genus, species, and strain of the microorganisms it contains. This is significant because, one species or strain of *Bifidobacterium* or *Lactobacillus* may be useful for reducing anxiety or elevating mood, while another strain may not. The product should also list the number of colony-forming units (CFU), or live bacteria, that are present. The majority of human psychobiotic trials employ products containing at least one billion CFU/day, even though optimum probiotic doses have not yet been measured [16]. They are used alone or in combination as cocktails of different bacterial species and strains.

Overall, the available information on microbiome changes in SPR is extremely erratic and insufficient to draw any firm conclusions about whether these changes are linked to an elevated risk of the condition or are merely the product of environmental variables or medical interventions. Pro/prebiotic supplementation has shown some hopeful outcomes, although there is conflicting data about its effectiveness in treating SPR.

PSYCHOBIOTICS

The term "psychobiotics" was coined by Dinan *et al*. [104] and is commonly described as any "living organism that, when consumed in sufficient proportions, improves the health of individuals suffering from psychiatric disorder. As a result, psychobiotics are defined as a subclass of probiotics with a focus on mental health. GABA, CA, and 5-HT regulate the brain–gut axis and mental health, and most psychobiotics may produce or promote their endogenous synthesis [50]. Hence, all microbiota-targeted therapies, such as probiotics and prebiotics, that affect the connections between bacteria and the brain and have an impact on mood, anxiety, and cognitive performance are referred to as "psychobiotics" [105]. Psychobiotics also influence metabolism and hormone production, strengthen the immune system, and more [106,107]. Today, these are being thoroughly studied as a complementary therapy for mental diseases.

Effect of psychobiotics on MGBA

Since GM is a target that can be altered and can alter epigenetic processes [108], it may be utilized to treat and lessen the symptoms of mental illnesses. Prebiotics, probiotics, live bacteria, antibiotics, synbiotics (combinations of pre and probiotics), postbiotics (bacterial fermentation products such as SCFAs, and fecal microbiota transplantation (FMT) are some of the methods that might change the MGBA [109].

CLINICAL EVIDENCE ON OUTCOMES OF PROBIOTICS SUPPLEMENTATION IN SPR

The first probiotic investigation was carried out by Benton *et al*. [110] utilizing milk with *Lactobacillus casei* strain *Shirota* (*LcS*) in depressed and normal participants (Table 3). Primary conclusions were that it enhanced cognition in all individuals and mood in 1/3rd of the patients with depression. In a 3-fortnight human experiment, probiotic yogurt (which comprise *B. lactis* and *Lactobacillus acidophilus*) and probiotic capsules (which comprise *L. casei*, *L. acidophilus*, *L. rhamnosus*, *L. delbrueckii bulgaricus*, *Bifidobacterium breve*, *Bifidobacterium longum*, and *Streptococcus thermophiles*) significantly reduced anxiety and depression compared to placebo groups [111]. In another two investigations, a probiotic mixture of *B. longum* and *Lactobacillus helveticus* alleviated anxiety and depression [112]. Probiotics can significantly reduce depression in subjects with mental illnesses, according to a meta-analysis [113], although it is yet unknown if the reduction continues once probiotic use is stopped [48].

It has been discovered that *Lactobacillus* supplementation in asymptomatic obese people reduces the fat content of the subcutaneous and visceral belly. Probiotics' ability to cure obesity and dyslipidemia may have the same promise for schizophrenic patients, as they also have the likelihood of developing MS [82]. Probiotics and prebiotics have been found to be effective in treating psychotic diseases in recent years. Probiotics are routinely given for gut inflammation as they have anti-inflammatory qualities. A study revealed that probiotic supplements might stimulate the vagus nerve and cause cytokines to have immunomodulatory effects [7].

According to a systematic review of 32 studies on the eating habits of SPR patients analyzed these individuals tend to consume diets that are of poor quality, with excess calories and processed foods, foods high in saturated fats, refined sugar, and salt, and little fruit and fiber consumption [114]. These variables have been associated with the emergence of MS in the general population. Patients with SPR may find it difficult to maintain a balanced diet due to several variables, including the weight gain brought on by antipsychotic medications, poor socioeconomic position, unpleasant symptoms, and drug addiction, particularly

Table 3. Clinical evidence on outcomes of probiotics supplementation in SPR.

Reference	Investigated factors and methods	Result
[95]	Hospital anxiety and depression scale (HADS) score, PANSS, and fecal microbiome composition *Bifidobacterium breve* strain A-1 (10^{11} CFU/day) given for 4 weeks followed by 4 weeks of observation 16S rRNA sequencing of the V3–V4 region	Improved HADS and PANSS scores, ↓ anxiety and depression symptoms (although a placebo effect cannot be ruled out)
[113]	• Effect of probiotics on GM in treating SPR was assessed. • 29 patients were given *B. breve* A-1 for 4 weeks. Patients with 25% or more reduction in the HADS—"responders" and those <25%—nonresponders • 16S rRNA sequencing of the V3–V4 region	• Responders ($n = 12$) had higher baseline lipid and energy metabolism than non-responders ($n = 17$). • Emphasizing the necessity of baseline GM functional gene measurement prior to probiotic use
[116]	• PANSS score and biochemical parameters • 50,000 IU vitamin D3 every 2 weeks plus 8×10^9 CFU/day probiotic for 12 weeks	↑ PANSS scores and plasma antioxidant levels, and a decline in metabolic abnormalities and circulating CRP indicating reduced inflammation
[41]	• Anti-*Saccharomyces cerevisiae* IgG antibodies • Patients were selected based on criteria defined by DSM-IV and analyzed for IgG antibodies to *S. cerevisiae*	Found food antigen antibodies and GI inflammation in patients with SPR
[112]	• A combination of *L. helveticus* and *B. longum* administered over a month	Improved HADS score, and urine cortisol levels
[117]	• *Lactobacillus rhamnosus* strain GG and *Bifidobacterium animalis* subsp. *lactis* strain Bb12 given for 14 weeks • PANSS assessed biweekly	Participants reported feeling happier and had lower levels of free cortisol in their urine, which suggests less stress
[84]	• *Lactobacillus rhamnosus* strain and *Bifidobacterium animalis* subsp. *lactis* formulation given for 14 weeks • Assesesment of Anti-*C. albicans* IgG levels and • Anti-*Saccharomyces cerevisiae* IgG antibodies	• No significant difference in psychiatric symptom severity, but bowel difficulty reduced in probiotics group • In another study, the same probiotic showed improvements in markers of intestinal epithelial integrity, immunomodulatory effects, and ↑ BDNF [118]

cigarettes. According to a new meta-analysis, dietary therapies can improve the physical health of those with severe mental illnesses [115].

A pilot study's findings suggest a link between *Candida albicans* infection and worsening positive mental symptoms, and this connection was later verified in a bigger group of 384 men with SPR. Then concluded that probiotics can benefit many men with *C. albicans*-related GI pain and normalize their *C. albicans* antibody levels. GI epithelial and immunological diseases were improved by probiotics in SPR patients, they are also capable of restoring the GM and reducing the growth of *Candida* spp. [84].

It would be advisable to conduct a thorough metabolic and immunological study of people who have taken probiotics because various pieces of research imply that the role of probiotics in stress and mood regulation may be strain-specific.

Several pieces of research suggest that probiotics in stress and mood regulation might be strain-specific, detailed metabolic and immunological examination of probiotics-treated individuals would be advised. For instance, male and female participants given *LcS* for 3-week reported increased levels of happiness than depression. Similarly, to this, 40 male and female healthy patients administered probiotics showed less rumination and aggressive cognition than those who took placebos [119]. It is unknown precisely which probiotic strain is in charge of these behavioral modifications. Consuming

LcS and *Lactobacillus gasseri*, respectively, led to reduced academic stress in students and improved mood in student-athletes. Students who were given probiotics in the classroom had lower plasma cortisol levels than the placebo group, indicating less stress. A probiotic-fed athletic group of kids performed better, demonstrating the influence of probiotics on fundamental life functions. *Bifidobacterium longum* was shown to be able to reduce the excitability of enteric neurons in a different investigation [120].

There are very few clinical studies for SPR, and those that have been done have always included antipsychotics along with dietary changes and probiotics [104,121]. Dietary modifications can alter the gut microflora's makeup and activity. However, the inability to produce a meaningful shift in SPR patients indicates the use of probiotics and/or prebiotics [104]. To obtain better outcomes in terms of health, functionality, and quality of life, psychiatry must adopt a multimodal strategy in the future [122]. Dietary change is the best strategy; however, it is quite difficult when dealing with SPR. More effective interventions than those based on a single nutrient can be achieved with formulations that mix multiple nutrients [115].

As a treatment for mental illness, modulating the MGBA with probiotics appears to promise, but there are still several obstacles to overcome. First, randomized control trials reported so far exhibit methodological variation and

comparatively small sample sizes. Numerous studies also rely solely on selfreported symptomatology characteristics without adequately evaluating patients, confirming a clinical diagnosis, or screening for comorbidities. Second, not every patient may see the same results from probiotics. For instance, a clinical trial has demonstrated that, depending on the environment in the gut, ingested probiotics may change and adapt in a good or harmful way under specific circumstances. Probiotics are live things that go through natural selection. For instance, *E. coli Nissle* probiotic increases the use of mucin in low diversity conditions, which may harm the gut lining [123]. The wide range of investigated strains and strain combinations is a significant factor in the high degree of results variability in probiotic investigations. Different strains of the same species, for instance, have shown conflicting results regarding mental symptoms: *L. rhamnosus* did not affect mood or anxiety levels in healthy males [124], *LcS* improved mood in those with low baseline mood scores [110].

PRECLINICAL RESEARCH

Preclinical research has shown that probiotics can restore corticosterone, norepinephrine, and BDNF levels, as well as immune regulation when they are given chronically

(Table 4). In an animal model of separation from the mother, the probiotic *Bifidobacterium infantis* was given, and when compared to a placebo, it regulated the immunological response, corrected behavioral impairments, and returned norepinephrine levels in the brain stem to normal [30]. Studies examining the impact of probiotics on the HPC BDNF, which was linked to lower levels of hippocampal BDNF in a model of low-grade colitis (AKR mice), revealed that the aberrant behavior was restored. They show that in rats probiotics enhance the expression of the neurotrophin under circumstances of chronic stress, inflammation, and aging, most likely through lowering microglia activation [28].

Unresolved is the question of whether probiotic colonization of the gut is a permanent process or only a temporary one. Prebiotics and probiotics are frequently advised to be used continuously to maintain a healthy GM and increase natural immunity; however, more thorough research is needed to determine their effectiveness, identify the most potent microbial strains, and determine the right number of fiber/prebiotics to promote their growth. Furthermore, probiotics and prebiotics must be evaluated using cutting-edge technologies such as genotype identification, epigenetic marker focusing, neuropsychology, biochemical marker

Table 4. Preclinical evidence of probiotics in SPR.

Reference	Methods and investigating factors	Result
[125]	Rats were given *B. infantis* for 14 days and assessed for forced swim test, and also for immune, neuroendocrine, and central monoaminergic activity	• Reduction of pro-inflammatory immune responses, and elevation of tryptophan • Supports the proposition that probiotics may possess antidepressant properties by modifying social behavior by stimulating the vagus nerve or modifying cytokine levels
[126]	Mice were given *L. rhamnosus* for 28 days and tested for various behavioral analysis tests along with *in situ* hybridization	• Region-dependent alterations in $GABA_{B1b}$ mRNA (brain) with increases in cingulate and prelimbic regions and reductions in expression in the HPC, amygdala, and locus coeruleus • Reduced $GABA_{A\alpha2}$ mRNA expression in the prefrontal cortex and amygdala, but increased $GABA_{A\alpha2}$ in the HPC and reduced stress-induced corticosterone and anxiety, depression-related behavior; these effects were not found in vagotomized mice
[127]	• Rats were fed with high-fat diet ×12 weeks, at week 13 to 24 weeks *L. paracasei* administered • Rats were assessed for metabolic parameters, brain mitochondrial function, hippocampal plasticity, oxidative stress, and apoptosis	• Probiotics reduced HPC oxidative stress, apoptosis, and glial activation while restoring cognitive performance in obese, insulin-resistant mice
[128]	• Specific PF rats were subjected to 21 days of restraint stress followed by behavioral testing and biochemical analysis. Supplemental *L. helveticus* was given every day during stress, and citalopram served as a positive control	• Improved chronic restraint stress-induced behavioral and cognitive dysfunction • Decreased levels of post-restraint anxiety and improved post-restraint object-recognition memory, also reduced levels of corticosterone and adrenocorticotropic hormones and higher levels of IL-10, as well as HPC BDNF mRNA, serotonin, and noradrenaline
[129]	• Mice were given *L. rhamnosus* × 4 weeks • Using magnetic resonance spectroscopy assessed glutamate + glutamine, total N-acetyl aspartate + N-acetyl aspartyl glutamic acid (tNAA), as well as GABA levels for 4 weeks and were subjected to MRS weekly and again 4 weeks after cessation of treatment to check temporal changes in these neurometabolites	• Glutamate and glutamine levels rose after 2 weeks of treatment and remained increased for 6 weeks • GABA concentrations increased only during the treatment • tNAA increased after 2 weeks of treatment and persisted during treatment until dropping back to baseline at the end of treatment. These results imply that the probiotic effect is momentary and has no permanent effects

evaluation, and other techniques to determine their efficacy as an adjunctive therapy to recognize the associated pathways, GI activity, metabolism disparities, and cognition dysfunction in SPR subjects. Environmental aspects of this therapy (food, hydration, age, gender, and comorbidities) should also be taken into consideration.

INTERCONNECTION BETWEEN CONSTIPATION-SPR AND PROBIOTICS

Constipation is a frequent sign of SPR. Probiotics have been demonstrated to relieve constipation in a variety of groups, but SPR has not yet been the subject of research on them [28]. Probiotics may thus be used as an additional therapy for SPR, particularly in those with high intestinal permeability.

Constipation is lessened by butyrate generated by *Clostridium butyricum*, which stimulates the release of intestinal hormones [130]. An improvement in insulin resistance with probiotics may have been indicated by the sequential shift in the triglycerides (TGs)/HDL-C ratio from 3.44 at baseline to 2.00 at 1 month and 2.05 at 2 months. Even more powerful than the total cholesterol/high-density lipoprotein cholesterol (HDL-C) ratio and low-density lipoprotein cholesterol/HDL-C ratio, the TG/HDL-C ratio is an atherogenic index that is a highly significant independent predictor of myocardial infarction. This is because it has been demonstrated that in people with insulin resistance, TG levels rose while HDL-C levels fell. This outcome is consistent with the finding that probiotics may improve dysbiosis and stimulate insulin signaling. Promising outcomes have been observed as more research on probiotics' impact on metabolism and constipation has been conducted in recent years. However, animal research accounts for the vast bulk of the available data. A combination probiotic may help SPR patients with constipation and insulin resistance, according to this early clinical trial.

Regulating bowel movements and treating metabolic problems are two advantages of probiotics. SPR subjects were given *Streptococcus faecalis* 2×10^8 CFU/day, *Bacillus mesentericus* 1×10^7 CFU/day, and *C. butyricum* 5×10^7 CFU/day mixture for constipation for 60 days and found improvement in excretion score and there was substantial relief from constipation at the end of the therapy [131].

According to some research using animal models, probiotics may enhance brain activity and signaling and hence aid in the treatment of psychiatric illnesses [132]. Probiotics' effectiveness in treating SPR and their capacity to lessen illness symptoms must yet be shown via extensive research. Before prescribing probiotics as a treatment for SPR, it may also be wise to take strain-specific and temporary effects into account. Because bacteria do not receive enough nourishment to proliferate quickly and sustain their population in the gut, probiotics may only have a temporary effect. Additionally, it is never easy for a new strain to establish itself in an equilibrium microbial community since it would have to remove autochthonous strains with likely stronger adaptation advantages in their particular niche. Daily fluctuations in GM can be managed by providing them with dietary fiber/prebiotics [7].

In SPR, bacteria from the Lactobacillaceae family and the genus *Lactobacillus* had beneficial health outcomes. One

explanation might be that various species in this genus have varied effects. One study found that the rise in psychosis and SPR was due to a subspecies not normally found in a healthy gut [133]. Increased *Lactobacillus*, on the other hand, has been linked to antipsychotic usage in the past. This was partially confirmed here since four psychosis and SPR studies that found increased *Lactobacillus* were done in medicated groups, whereas the one that indicated decreased *Lactobacillus* was undertaken in a treatment-naive group [134].

FECAL MICROBIOTA TRANSPLANTATION

FMT: This procedure entails the transfer of fecal microorganisms from a healthy subject to a receiver [135].

A study showed FMT from SPR individuals and healthy controls in GF mice resulted in decreased glutamate levels, whereas glutamine and GABA levels were increased in the HPC. Furthermore, recipient animals exhibited behaviors reminiscent of mice with glutamatergic hypofunction in SPR [76]. Another study using a similar experimental technique found that FMT from drug-naive SPR subjects to antibacterial-treated mice causes behavioral complications in recipient animals, including hyperactivity, poor learning, and memory functions. These behavioral impairments were accompanied by higher values of baseline extracellular dopamine in the prefrontal cortex and 5-HT in the HPC, as well as activation of the kynurenine–kynurenic acid pathway in peripheral tissues and the brain. According to the same study transplantation of *Streptococcus vestibularis* (a bacterium prevalent in people with SPR) causes hyperkinetic behavior and affects social interactions in mice [83].

In a human investigation, a decline in the number of *Faecalibacterium* can cause an increase in gut TH17 cells in SPR patients. It has been suggested that these cells may pass the BBB and stimulate the microglia in the HPC, causing aberrant behavior [93].

Experimental research unequivocally demonstrates that the GM of untreated SPR patients may be transplanted into GF mice and cause a variety of behavioral as well as altered neurotransmission. Clinical study results, however, do not substantiate probiotics as supplemental therapy for SPR [83]. To get a conclusion on the causal relationships between GM and SPR/psychosis, longitudinal research is still required.

CONCLUSION

Numerous studies in the last decade have emphasized the importance of GM in brain function and dysfunction. The to-and-fro interaction between microbes and the brain (interconnected by the vagus nerve) modulates immune, enteroendocrine, and neuronal inflammation system pathways impacting stress response, behavior, mood, and cognition. It can be hypothesized that probiotics can be targeted at neurobehavioral disorders. As several preclinical and clinical trials showed the efficacy of probiotics in alleviating the symptoms of SPR and other mental diseases by modulating the MGBA. Even though the preclinical studies have promising data on the interconnection between gut dysbiosis and SPR, further comprehensive human studies are needed to conclude on the efficacy and usage of probiotics both in preventing and treating

SPR and other psychiatric disorders. Effects of probiotics in modulating endocrinal, inflammatory, immunochemical, and neuronal changes in both physiological and pathological states should be evaluated to identify their thorough mechanism of action in preventing/treating SPR. This will assist in determining the probiotics supplement's dose, duration, and side effects. It is critical to discover novel gut microbial species that can maintain the diversity of the gut microbial population. As the present literature is insufficient to conclude specific microbial species or probiotics that can benefit SPR.

FMT, an emerging microbiome-focused strategy of one-time transplantation of completely new human GM is prophesied to be the future of GM-targeted strategies in SPR.

We also noticed a grey area in gene-specific microbial therapy. Since the role of genetics and epigenetics in SPR is not clearly understood. Hence, we stress the identification of functional genes which might lead to the recognition of individualized probiotic treatment of psychiatric disorders through GM analysis in the future.

AUTHOR CONTRIBUTIONS

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Syed Mushraf and Veena Nayak. The first draft of the manuscript was written by Syed Mushraf and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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All the authors have consent for publication.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects. Hence ethical clearance is not required.

DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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