REVIEW ARTICLE

Microbiome therapeutics: exploring the present scenario and challenges

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Abstract

Human gut-microbiome explorations have enriched our understanding of microbial colonization, maturation, and dysbiosis in health-and-disease subsets. The enormous metabolic potential of gut microbes and their role in the maintenance of human health is emerging, with new avenues to use them as therapeutic agents to overcome human disorders. Microbiome therapeutics are aimed at engineering the gut microbiome using additive, subtractive, or modulatory therapy with an application of native or engineered microbes, antibiotics, bacteriophages, and bacteriocins. This approach could overcome the limitation of conventional therapeutics by providing personalized, harmonized, reliable, and sustainable treatment. Its huge economic potential has been shown in the global therapeutics market. Despite the therapeutic and economical potential, microbiome therapeutics is still in the developing stage and is facing various technical and administrative issues that require research attention. This review aims to address the current knowledge and landscape of microbiome therapeutics, provides an overview of existing health-and-disease applications, and discusses the potential future directions of microbiome modulations.

Key words: microbiome therapeutics; human microbiome; engineered microbiome; probiotics; prebiotics; fecal microbiome transplantation

Introduction

The human microbiome enlists all the microorganisms and their related metabolites/products identified in and on the human body [1]. Technological advancement has enabled the assessment of the pleiotropic effects of the human gut microbiome in health and diseases [2]. With the extensive role of microbes in human health, they hold the enormous potential to be used as therapeutics for disease management. Microbiome therapy holds great promise to treat any severe type of disease condition and acts as the potential source to achieve the objective of personalized therapy by overcoming key issues like interpersonal variability and stability in every type of environment [3]. The high-resolution data analysis enabled

the development of modifiers for microbiome engineering [4, 5]. As microbial dysbiosis is associated with the majority of human diseases, various strategies are now being applied to restore the native microbiota for efficient disease management [6]. There is now an emerging interest in developing and delivering synthetic microbial consortia for health benefits [7]. Strategies such as fecal microbiota transplantation (FMT) or probiotics that rely on the administration of exogenous microbes could be used to manage dysbiosis-related disorders [6]. Recent advancements in synthetic biology have developed the possibility of targeted cell therapeutics through probiotic engineering that targets specific cells, tissues, or pathways [8]. Genetic switches are being prepared to modulate the microbiome-
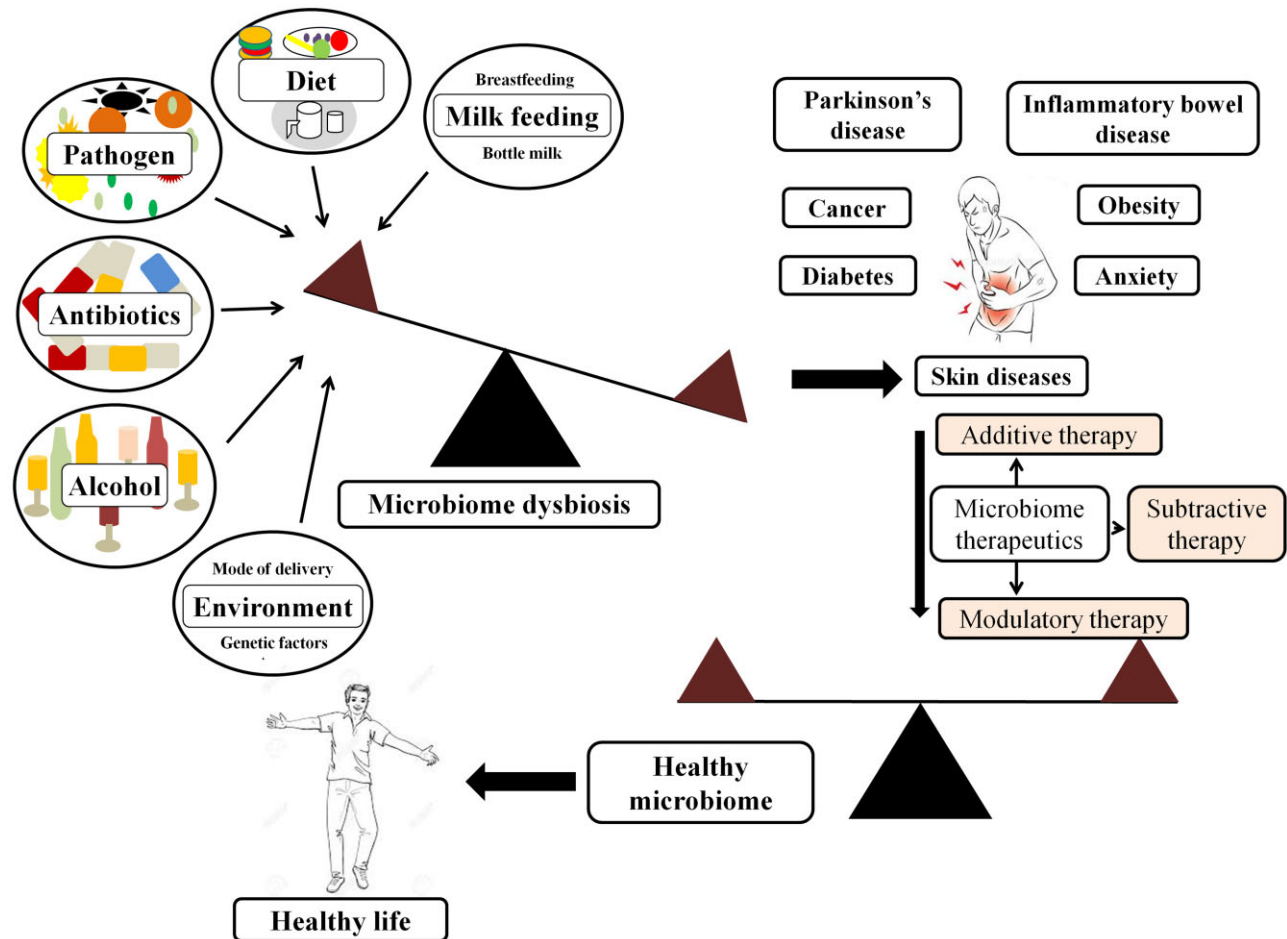
Figure 1. Role of microbiome augmentation in the maintenance of healthy life

related pathways [9]. The individual microbe or entire microbial consortia could be manipulated to generate certain therapeutic molecules or antitoxins [10]. Gut-microbiome engineering leads to the development of chemical entities to advance personalized medicine and improve human healthcare [11–14]. Similarly, bacteriophages could be used or even engineered to add or delete specific functions into the microbial community [11]. Certain non-living agents such as microbial metabolites and peptides could be engineered to be used as small-molecule modulatory therapy for microbial disease management [12]. The response to therapeutics could vary from individual to individual depending upon the disease and type of medications [13]. However, the field of microbiome therapeutics faces some major challenges such as the proper identification of disease-causing microbial signatures, lack of consideration of ethical and safety issues, and lack of clinical trials, as most of the experiments have been done in rodents. Thus, more experimental trials need to be done to provide efficacy in the microbiome therapeutics through the study of the interaction between therapeutics and the host. In this review, we took efforts to summarize the current research progress in the field of microbiome therapeutics (Figure 1), as well as tried to showcase the health implications of microbiome therapeutics.

Why is microbiome therapeutics significant?

It is believed that conventional therapies have resulted in antibiotic resistance among pathogens, resistance to chemotherapy,

drug non-responsiveness, and poor specificity. These manifestations are posing a serious threat to the health of the human population. Microbial therapy overcome these drawbacks of modern medicines [15, 16]. Microbes are natural residents within the body that increase their therapeutic capacity without any side effects. Additionally, microbes can be engineered genetically to improve their efficacy and safety. A resilient human gut microbiome has an important role in the maintenance of human health, while its dysbiosis could result in disease onset [17]. Microbes harbor the potential to overcome the onset of the diseases by interacting with the host and thus are useful for microbiome therapeutic development [18]. *Christensenella* sp. is known to reduce depression and anxiety-like behavior [19]. *Akkermansia muciniphila* relieves metabolic disorders and cooperates with the use of metformin in cancer therapeutics [20] as well as protects against atherosclerosis by reducing gut permeability and preventing inflammation [21]. *Lactobacillus johnsonii* protects against cancer [22]. *Bifidobacterium longum* reduces the severity of Crohn's disease [23] and repairs the integrity of the mucus layer impaired due to a high-fat diet [24]. *Oxalibacterium formigenes* prevents kidney stones by the homeostasis of oxalic acid [25]. *Bacteroides* spp. protects against adiposity [26]. With increased information about the potential of gut microbes, the scope in the field of therapeutics emerged with a new hope of disease diagnosis, test methods, and new ways of data collection and manipulation [27]. Live biotherapeutics are being developed to introduce microbes into the host [28]. Microbiome modulation by the addition of exogenous
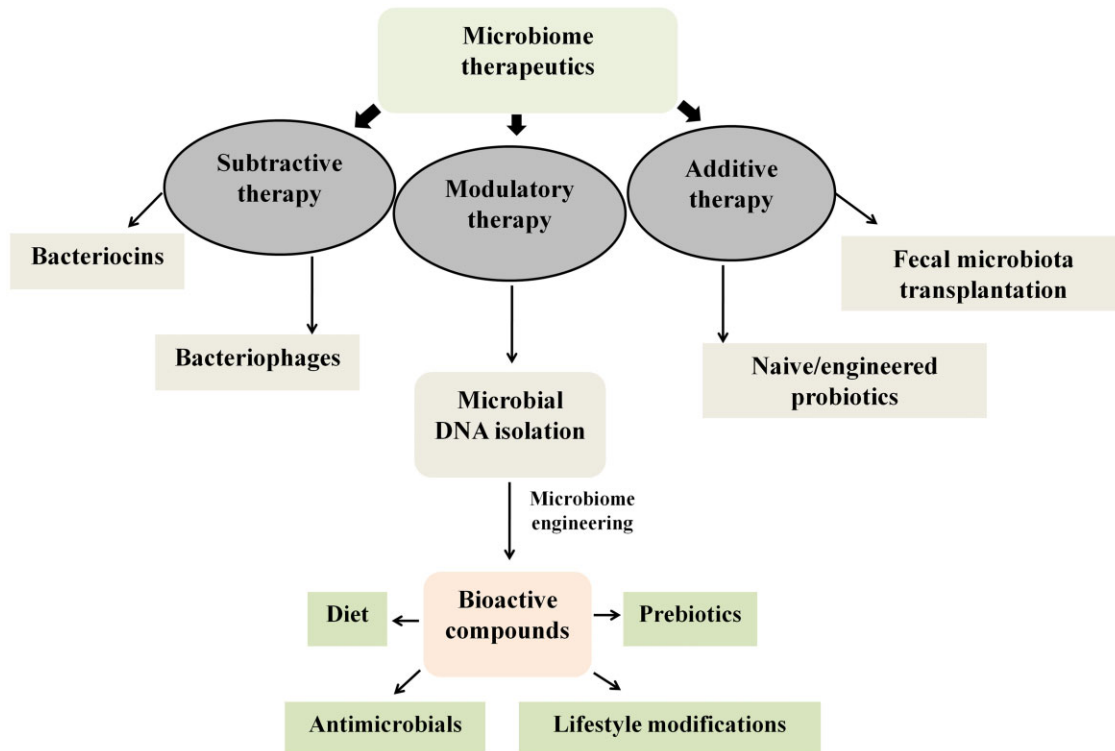
Figure 2. Overview of the strategies used as microbiome therapeutics

microbes has been fascinating for the previous decade [12]. The need of the hour is the small-molecule therapeutics to manipulate the host microbiota. The small molecules should be able to alter the functions of the microbes to prevent the cause of diseases [12].

How to implement microbiome therapeutics

Efforts were made to harness the benefits of the host–microbiome interaction for microbiome therapeutic development [4]. Additive therapy, subtractive therapy, and modulatory therapy are commonly used strategies for microbiome therapeutics (Figure 2) [4]. Additive therapy includes the addition of microbial strains or microbial consortia, while subtractive therapy is aimed to remove the lethal pathogens known for the onset of a specific disease [29]. Modulatory therapy is primed to modify or manipulate the host–microbiome interaction for certain functions using certain non-living agents [30].

Additive therapy

Additive therapy is the administration of individual strain or microbial consortium to harness the health-promoting benefits either as probiotics or through FMT (Figure 2) [29]. Microbes used in additive therapy could be either natural or genetically engineered to produce therapeutic molecules [31].

FMT

In general, FMT involves the administration of the therapeutic microbial population. FMT is a beneficial method to replace the disease-causing microbes with beneficial microbes. It involves the transfer of healthy microbes from healthy donors to recipients through various modes of delivery. The donor must be screened using strict guidelines [32]. To reduce the transmission

of infection from the donor, suitable stool and blood examinations need to be done within 4 weeks before transplantation [32]. For the immunotolerance of the recipient against the donor's microbes, a close relative of the recipient should be preferred as the donor [33] whereas an unrelated donor should be the choice in the case of a genetic disorder such as inflammatory bowel disease [34]. It was found that mere fecal filtrate containing bacterial debris, metabolites, DNA, etc. was enough to treat recurrent *Clostridioides difficile* infection (CDI) [35, 36]. Pure culture of intestinal bacteria from a single healthy donor was used to treat recurrent CDI [37]. FMT has been successfully used to treat antibiotic-induced dysbiosis in *C. difficile* infection [38, 39]. FMT has been used efficiently to treat recurrent infections of *C. difficile* (with a spectacular percentage of recovery) [40] and research is ongoing to test whether FMT can be used for other diseases (with the percentage of success much lower than those observed with CDI) [41]. Restoration of butyrate-producing bacteria and improved insulin production were achieved on the transfer of fecal microbes from lean to obese mice [42]. Antibiotic-resistant pathogens associated with recurrent urinary-tract infections were drastically reduced post FMT [43]. Certain disorders such as alcoholic hepatitis and cirrhosis are associated with a deficiency in mucosa-associated invariant T-cells. Restoration of these T-cells was observed in patients after FMT [44]. Similarly, alcohol-induced loss of Bacteroidetes was restored after FMT [45]. With successful efficacy, several FMT clinical trials have been done in the case of liver disorders, the progression of fibrosis, hepatic encephalopathy, and alcoholic hepatitis [46]. FMT has been successfully studied in various neurological disorders such as autism, sclerosis, and Parkinson's disease [47]. The transfer of fecal microbes from healthy to cancer patients improved the response to immune checkpoint inhibitors [48]. The success of the FMT approach depends on the heritability of microbes once transferred, which depends on the

Table 1. Health attributes of fecal microbiota transplantation in various diseases

Serial no.	Target disease	References
1	*Clostridium difficile* infection	[35]
2	Recurrent *Clostridium difficile* infection	[38]
3	Irritable bowel syndrome	[54, 55]
4	Insulin sensitivity	[42]
5	Recurrent urinary-tract infection	[43]
6	Alcoholic liver disease	[44]
7	Autism	[47]
8	Multiple sclerosis	[56]
9	Parkinson's disease	[57]
10	Cancer	[48]
11	Pseudomembranous colitis	[58]
12	Ulcerative colitis	[59, 60]
13	Crohn's disease	[61, 62]
14	Hepatic encephalopathy	[63]
15	Alcoholic hepatitis	[64]
16	Diarrhea	[65]
17	Aging	[66, 67]
18	Stroke	[68]
19	Alzheimer's disease	[69, 70]
20	Sepsis	[71]

host genes related to the immune system [49]. FMT has shown success in the treatment of ulcerative colitis but was accompanied by the chance of remission [50, 51]. Similarly, the FMT treatment was not found effective in recurrent Crohn's disease [52]. Immune-compromised people are prone to ill effects of fecal transfer, as was reported in the case of the transfer of β-lactam-resistant *Escherichia coli* [53] (Table 1). The adverse effects of FMT and the risk posed due to bad stools [72] urged an alternative FMT approach in the form of the artificial stool where commensals from stool can be fermented and grown *in vitro* under conditions similar to those in the human gut and then encapsulated in the living form [73]. Additionally, the patient's stool can be restored to re-establish the native gut microbes in case of severe infections.

Probiotics

Probiotic therapy is one of the most convenient methods of additive therapy. The use of probiotics is based on the employment of either natural or genetically engineered therapeutic microbes as monotherapies. As per guidelines given by the FAO/WHO working group in 2002, probiotics are defined as "Live microorganisms which when administered in adequate amounts confer a health benefit to the host." An ideal probiotic should be species-specific, free from pathogens, have a beneficial impact on the host, and be able to survive within the human body [74]. *Lactobacilli*, *Bifidobacteria*, and *E. coli* have been successfully used to treat a range of diseases [75, 76]. Probiotics work through the competitive exclusion of pathogens by producing bacteriocins, competing for attachment sites and nutrients, altering pathogen functions, and improving the immune-stimulatory and nutritional status of the host [77]. Recognized health benefits are pathogen defense, stimulation of the host immune system, and antimicrobial production posing potential efficacy within the human body [78]. One of the most significant effects of probiotics is the improvement in gut-microbiome composition. Thus, now the intestinal microbes are being used as next-generation probiotics to improve the intestinal microbiome composition [79]. The gut microbes respond to

the live microbes given orally as diet and thus the probiotic microbes have a positive impact on the human gut microbial composition [80], thereby improving nutritional health and status [81]. Probiotics have been efficiently used to treat several diseases such as inflammatory bowel disease [82], diarrhea [83], Crohn's disease [84], ulcerative colitis [85], and cancer [86]. During inflammatory bowel disease, ulcers, or fistula, the gut barrier is destroyed. The leaky gut lining is more prone to pathogens and pH fluctuations [87]. Now, efforts are being made to engineer these probiotics to express certain biotherapeutics. Engineered probiotics are being developed for disease diagnosis and treatment (Table 2). This approach works based on re-modulating the diseased environment to healthy environmental conditions by augmenting with an exogenous functional trait.

In general terms, FMT can be considered as super probiotic, as the fecal microbiota consists of a microbial consortium that has a complex network/support mechanism for long-term survival within the host (Figure 3).

Subtractive therapy

Subtractive therapy has emerged to be a fascinating tool in the field of microbiome engineering [124]. This therapy aims to reduce the deleterious pathogens from the microbiome with the help of the antimicrobial activity of bacteriocins and bacteriophages (Figure 2). Antibiotics were traditionally used for the removal of unwanted pathogens but due to the development of antibiotic resistance among the gut microbes, therapies such as bacteriocins and bacteriophages are being used to target the pathogens with minimal effects on the other members of the microbiome. Bacteriocins are ribosomally synthesized peptides exhibiting antimicrobial activity [125]. Bacteriocins work against pathogens in multiple ways, such as membrane rupture, toxins, inhibition of the respiratory mechanism, and overall cell lysis [126]. Bacteriocins could be either lanthionine-containing [127] or non-lanthionine-containing bacteriocins [128]. The non-lanthionine-containing bacteriocins act against *Clostridium*, *Enterococcus*, *Pediococcus*, *Lactobacillus*, and *Leuconostoc* [129] whereas lanthionine-containing enterocin and nisin have antibacterial activity against *Bacillus cereus, Geobacillus stearothermophilus*, and *Clostridium botulism* [130]. Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria are known to produce bacteriocins [131, 132]. Commensals use bacteriocin to successfully survive the niche competition within the gut [133]. They prevent pathogen colonization, inhibit the defensins, and have an overall positive effect on host immunity [134] (Table 3). Bacteriocins have been used for the preservation of dairy products [156]. Bacteriocins are used to preserve meat, vegetables, beverages, etc. [157]. Pediocin and nisin are commercial food preservatives [158]. Bacteriocins produced by *Pediococcus acidilactici* BA28 are used to treat peptic ulcers [159]. Ferrmenticin HV6b produced by *Limosilactobacillus fermentum* HV6b has antimicrobial and spermicidal activity, and thus is used in vaginal creams [160]. Bacteriocins such as nisin are also used in the veterinary industry to control microbial infections [156]. Similarly, *Enterococcus faecalis* SL-5-produced ESL5 is used as a lotion to prevent acne lesions caused by *Propionibacterium acnes* [161]. Bacteriocins are also used for oral care. Macedocin produced by *S. macedonicus* is used for mouthwash and maintaining oral health [162]. Similarly to the development of antibiotic resistance, microbes may develop bacteriocin resistance by adapting to the environmental conditions or degradation of bacteriocins [163]. Efforts

Table 2. Applications of naive/engineered probiotics to achieve a desired physiological trait for better health

Serial no.	Engineered/ naive strain	Cloned gene	Desired target	Effect	References
1	*Escherichia coli*	CsgA-TFF + trefoil factor	Gut epithelium during colitis	Treated colitis with mucosal healing and immunomodulation	[88]
		Deletion of negative regulator of L-arg biosynthesis and insertion of a feedback-resistant L-arg biosynthetic enzyme	High concentration of ammonia in blood	Conversion of ammonia to arginine	[89]
		Phenylalanine metabolizing enzyme	Phenylalanine concentration in blood	Conversion of phenylalanine to *trans*-cinnamate and phenylpyruvate treating phenylketonuria	[90]
		Antibiofilm protease DegP	Biofilm inhibition of other *E. coli* strains, *S. aureus*, and *S. epidermidis*	Inhibition of the growth of pathogens	[91]
		Antibiotic microcin H47	Pathogen-growth inhibition	Displaced *Salmonella enterica* from gut	[92]
		Detecting and utilizing tetrathionate and Microcin	Inhibition of *Salmonella* sp.	Inhibition of *Salmonella* sp. in presence of tetrathionate	[93]
		β-galactosidase and luciferase	Tumor detection	Liver metastasis detection with luciferin detection in urine	[94]
		Thiosulfate and tetrathionate sensor	Detection of tetrathionate	Detection of gut inflammation	[95]
		Lysine and pyosin	Sense biofilms of *Pseudomonas aeruginosa* through quorum sensing	Inhibition of the growth of *Pseudomonas aeruginosa*	[96]
		Quorum sensing with CRISPRi technology	The presence of *Vibrio cholerae*	*Vibrio cholera* detection	[97]
		Sense and detect inflammatory signal from nitric oxide	Detection of gut inflammation due to nitric oxide	Inflammatory signals cause activation of DNA recombinase to detect and respond to NO signals	[98]
		Two-component regulatory system to detect tetrathionate	Detection of tetrathionate	Detection of inflammatory signals	[99]
2	*Lactococcus lactis*	IL-10	Intestinal inflammation during colitis and Crohn's disease	Anti-inflammatory IL-10 production	[100]
		Human Trefoil Factor 1	Oral mucosa	Reduced severity of oral mucositis	[101]
		GAD65370–575-encoding plasmid	Reversal of diabetes	Tolerance induction in Type 1 diabetes	[102]
		Proinsulin and IL-10	Reversal of autoimmune diabetes	Tolerance induction in Type 1 diabetes	[103]
		Glucagon like Peptide-1	Oral delivery of Glucagon like Peptide-1	Efficacy in treatment of Type 2 diabetes	[104]
		Ligand-binding domain and signal transduction domain of *Vibrio cholera*	Sense the presence of *Vibrio cholerae*	Detection and suppression of pathogen *Vibrio cholera*	[105]
		MT1 or MT1–MT1 nanobody with a HisG and Myc-tag	Intestinal inflammation associated with colitis	Anti-inflammatory action against colitis	[106]

Table 2. (continued)

Serial no.	Engineered/ naive strain	Cloned gene	Desired target	Effect	References
				through secretion of anti-mTNF antibodies	
3	*Bacteroides ovatus*	Transforming growth factor-β1	Intestinal inflammation during colitis	Improvement of colitis treatment by production of transforming growth factor-β	[107]
		Keratinocyte-growth factor-2 with xylanase promoter	Intestinal inflammation during colitis	Anti-inflammatory action against colitis through secretion of human growth factors in response to dietary xylan	[108]
4	Lactic-acid bacteria	Elafin	Intestinal inflammation during inflammatory bowel disease	Improved treatment against intestinal dysfunction	[109]
5	*Lactobacillus gasseri* ATCC 33323	Glucagon like Peptide-1	Intestinal cells to become glucagon-responsive insulin-secreting cells	Reduced hyperglycemia	[110]
6	NS8		Attenuation of neuroinflammation and metabolism of 5-hydroxytryptamine	Prevention of cognitive decline and anxiety-like behavior during hyperammonemia	[111]
7	*Lactobacillus acidophilus*, group N Streptococcus, *Bacteroides distasonis*, *Escherichia coli* var. *mutabilis*, *Clostridium* sp., *Streptococcus faecalis*, *Lactobacillus salivarius*, and an EOS fusiform bacterium		Urease activity	Reduced hyperammonemia	[112]
8	*Limosilactobacillus reuteri*	Interleukin-22	Increased expression of REG3G	Reduction in ethanol-induced steatohepatitis	[113]
9	*Lactobacillus acidophilus*, *Lacticaseibacillus casei*, and *Bifidobacterium bifidium*		Change in biochemical measures of depression and anxiety	Depression	[114]
10	*Lacticaseibacillus casei*, *Lactobacillus acidophilus*, and *Bifidobacterium longum*		Hypocholesterolemic effects	Obesity	[115]
11	*Limosilactobacillus fermentum* NCIMB 5221		Anti-proliferative effect against cancer	Colorectal cancer	[116]
12	*Lactiplantibacillus plantarum* L67		IL-12 and IFN-γ production	Allergy	[117, 118]
13	*Levilactobacillus brevis* DPC6108 and *Bifidobacterium dentium*		γ-aminobutyric acid production	Anxiety and depression	[119]
14	*Levilactobacillus brevis* W, *Bifidobacterium lactis* W, *Lactobacillus acidophilus* W37, *Bifidobacterium bifidum* W2, *Ligilactobacillus salivarius* W2, *Lacticaseibacillus casei* W5, and *Lactococcus lactis*		Central nervous system	Neurodegeneration	[120]

(continued)

Table 2. (continued)

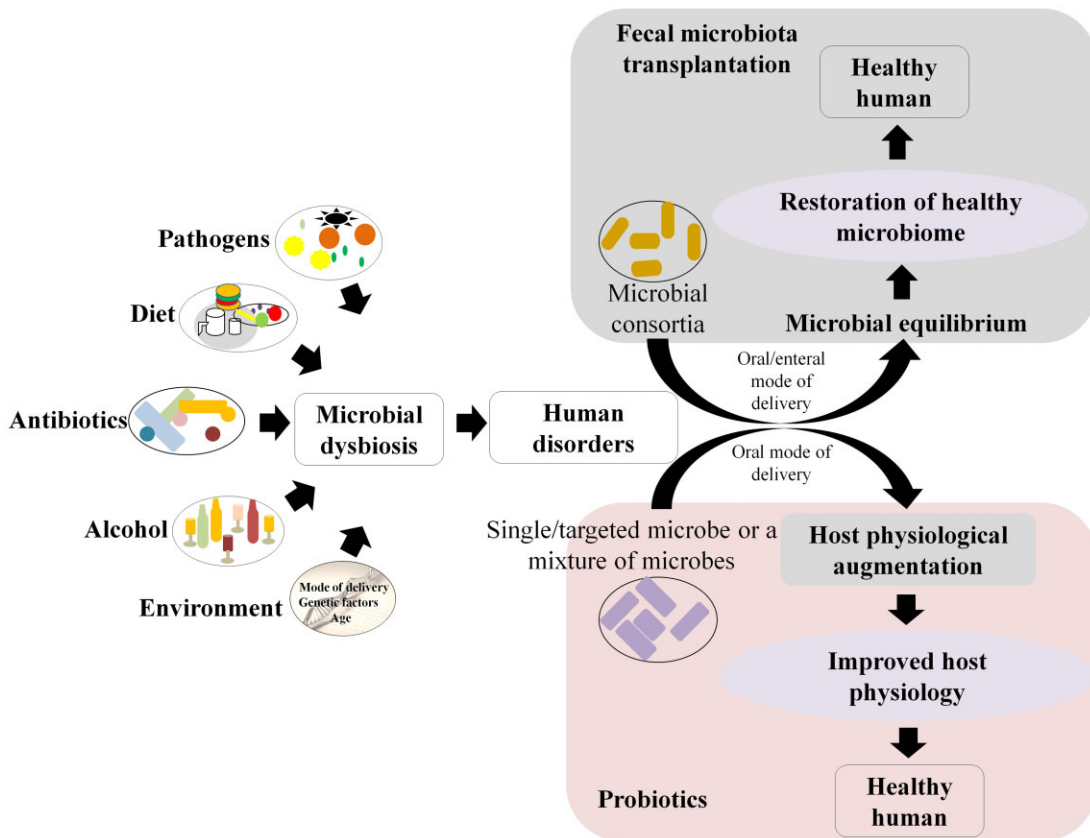| Serial no.	Engineered/ naive strain	Cloned gene	Desired target	Effect	References
15	*Lacticaseibacillus rhamnosus, Limosilactobacillus reuteri,* and *Bifidobacterium lactis*			Diarrhea	[121]
16	*Bifidobacterium, Lactobacillus,* and *Streptococcus thermophilus*			Ulcerative colitis	[122, 123]

Figure 3. The differential functions of fecal microbiota transplantation and probiotics in treating human disorders

should be made to improve the potency of bacteriocins to work in the direction of therapeutics.

Bacteriophages are viruses that are specific to a bacterium. As bacteriophages insert their genome within their specific bacteria and cause bacterial membrane disintegration, bacteriophages are used to target antibiotic-resistant pathogens [164]. Phage and phage products are used to treat several diseases caused by antibiotic-resistant microbial pathogens [165]. Bacteriophage therapy successfully eradicated methicillin-resistant *Staphylococcus aureus* [166], thus treating osteomyelitis [167]. The φMR299-2 and φNH-4 have been successful in the treatment of Pseudomonas-induced lung infection [168]. *Propionibacterium acnes* bacteriophage is successfully used in acne treatment [169]. Bacteriophage sb-1 from *Staphylococcus* was used to heel foot ulcers [170]. Bacteriophages selectively reduce the colonization of an *E. coli* strain responsible for inflammation

[171–173]. Phage treatment relieved the colitis symptoms in *E. coli* strain LF82-colonized mice [174]. Engineered phages used with a CRISPR-Cas system help in the more specific killing of pathogens by sensing the strain-specific determinants [175]. Phages are host-specific and function against their specific hosts without affecting the environment with no side effects in the host. Also, the phages can be administered through various routes, which facilitates the treatment. Additionally, phages can mutate to prevent the development of resistance within the host. Despite these advantages, phage therapy suffers some drawbacks. Bacteria may develop resistance to phages by adopting restriction modification, spontaneous mutations, or using adaptive immunity by the CRISPR-Cas system [176]. Phage therapy should be preceded by the correct identification of the bacterial pathogen [16]. Certain cases of phage therapy have shown no efficacy [16]. Also, phage therapy requires a neutralized environment that is

Table 3. Therapeutic potential of bacteriocin-producing human gut microbes

Serial no.	Host strain	Bacteriocin produced	Target organism	Host benefits	Reference
1	*Enterococcus faecalis*	Bacteriocin 21	Multi-drug-resistant Enterococcus	Limiting infections	[133]
2	*Ligilactobacillus salivarius*	Abp118	Listeria monocytogenes	Anti-infective activity	[135]
		Salivaricin P	Listeria monocytogenes	Anti-infective activity	[136]
		Bacteriocin L-1077	Campylobacter jejuniL-4	Antimicrobial activity	[137]
3	*Streptococcus salivarius*	Salivaricin A2 and Salivaricin B	Streptococcus pyogenes	Pathogen inhibition	[138]
4	Engineered R-type bacteriocins	Avidocin	*Clostridium difficile*	Anti-infective activity	[139]
5	*Lactococcus lactis*	Nisin Z	*Clostridium difficile*	Anti-infective activity	[140]
		Nisin A	*Clostridium difficile*	Bactericidal activity	[141]
		Nisin V	*Clostridium difficile*	Bactericidal activity	[142]
		Lacticin	*Clostridium difficile*	Antimicrobial activity	[143]
6	*Streptococcus mutans*	Mutacin B-Ny266	*Staphylococcus aureus*	Anti-infective activity	[144]
		Mutacin H-29B	*Neisseria gonorrhoeae, Helicobacter pylori, Campylobacter jejuni*	Antimicrobial activity	[145]
7	Probiotic mixture of Lactobacillus, Bifidobacterium, and Lactococcus/ Streptococcus	Mixture of bacteriocins	*Salmonella enterica* and Listeria monocytogenes	Inhibition of pathogen growth	[146]
8	*Pediococcusacidilactici UL5*	Pediocin PA-1	Listeria monocytogenes	Pathogen inhibition	[147]
9	*Bacillus thuringiensis DPC 6431*	Thuricin CD	*Clostridium difficile*	Bactericidal activity	[148]
10	*Planobisporarosea*	LFF571	*Clostridium difficile*	Antimicrobial activity	[149]
11	*Actinoplanesliguria*	Actagardine A (DAB)	Gram-positive pathogens including *Clostridium difficile*	Antimicrobial activity	[150]
12	*Bacillus sonorensis*	Sonorensin	*Staphylococcus aureus* and Listeria monocytogenes	Inhibition of spoilage bacteria	[151]
13	*Escherichia coli* strain H22	ColicinIb, E1, and Microcin C7	Enterobacter, Escherichia, Klebsiella, Morganella, Salmonella, Shigella, and Yersinia	Antimicrobial activity	[152]
14	*Brevibacillus* sp. strain SKDU10	Laterosporulin10 (LS10)	Cancer cells like MCF-7, HEK293T, HT1080, HeLa, and H1299	Antibacterial and anticancer activity	[153]
15	*Enterococcus faecium*	Bacteriocin E50–52	*Campylobacter jejuni*	Antimicrobial activity	[154]
16	*Enterococcus* sp.	Enterocin E-760	*Campylobacter* sp.	Antimicrobial activity	[155]

not obtained within the digestive tract due to the influence of gastric secretions [177].

Modulatory therapy

Modulatory therapy includes the modulation of the gut microbes or their associated interactions with the human host for human health. It considers the restoration of the depleted microbiome and the transformation of existing microbes for a healthier microbiome (Figure 2). Restoration/modulation of gut microbiota can happen through various modulations such as diet, exercise, and antibiotics that impact the composition of the gut microbiome [178, 179]. The microbiome sustains what we eat, thus diet is a major target for modifying the gut microbiome. Dietary modification has a great effect on the gut microbiome. Physical exercise is also associated with a healthy microbiome and consequent short-chain fatty acid (SCFA) production [180]. Athletes consume more proteins that have an impact on the gut microbiome [181]. Marathon runners were examined with an increase in *Veillonella* promoting exercise endurance [182]. A gluten-free diet [183]; reduced fiber intake [184]; fermentable oligo-, di-, or monosaccharides, and polyols [185];

and increased protein intake [181] affect the gut-microbiome composition. Dietary fibers improve disorders such as chronic constipation [186]. Limited or inadequate dietary intake renders the gut microbes having to use the glycans in the host mucus layer, which disturbs the integrity of the mucus layer [187]. Vitamin D is a key factor in determining microbiota composition [188]. Dietary modifications could improve the production of microbial metabolites such as SCFAs [189] and help in the continued growth of beneficial microbes [190]. Diet modification along with other therapy for diabetes improves the glycemic index [191]. Administration of a ketogenic diet decreased the abundance of gut *Eubacterium rectale*, Bifidobacteria, and Dialister in children with severe epilepsy [192]. Thus, a ketogenic diet was used as an alternative for drug-resistant epilepsy [192]. The administration of long-chain fatty acids restored the *Lactobacillus* and improved the pathological conditions in ethanol-induced liver disease [193]. Similarly, butyrate concentration was corrected by the administration of glycerol tributyrate [194] to have a positive effect on health [195]. The antioxidant tempol was used to change the bacterial composition towards non-obese conditions, thus treating obesity [196]. Prebiotics increase the beneficial microbes and remove the pathogens such as fibers, galactooligosaccharides that increase the *Bifidobacterium* abundance [197]. Other factors such as alcohol consumption [198], smoking [199], and drugs [200] also impact the gut-microbiome composition. Some medicines/drugs may impact the gut-microbiome composition and may potentially increase antibiotic-induced resistance [201]. Alcohol consumption increases the content of gram-negative bacteria [202], decreases SCFA production [203], and increases intestinal permeability [204]. Alcohol increases the abundance of Bacteroidetes and reduces the *Lactobacilli* content [205] as well as the abundance of Proteobacteria [206]. Increased alcohol uptake results in the increased abundance of Proteobacteria and decreased *Faecalibacterium* in the human stool [203]. Smoking also induces alterations in the oral, airway, and gut-microbiome composition [207]. Smoking cessation alters the intestinal microbiome composition by increasing the abundance of Firmicutes and Actinobacteria with a simultaneously decreased abundance of Bacteroidetes and Proteobacteria [208]. There exists an association between smoking, dysbiosis, and the onset of an illness. The increased abundance of Bacteroidetes in CD patients contributes to the disease development and severity [209]. Antibiotics also affect the gut microbiome negatively by decreasing the microbial diversity, altering the metabolic activity, and developing the antibiotic resistance that ultimately leads to antibiotic-associated diarrhea and CDI infections [210].

Psychobiotics

Psychobiotics are the group of agents that may be probiotic, postbiotic, prebiotic, or synbiotic and target the gut–brain axis and confer mental health [211] (Table 4). Psychobiotics have a psychotropic effect on anxiety, depression, and stress [216]. Brain and gut microbes communicate through vagus nerves, immunoregulatory pathways, and the neuroendocrine system [217]. Psychobiotics work through a strategy by affecting the cognitive and emotional pathways, targeting the hypothalamic–pituitary–adrenal (HPA) axis for inflammatory molecules that are directly related to depression [218], or targeting the neurotransmitters as well as proteins that are a part of the brain functions [219]. Human microbes such as *Lactobacillus GG* and *Bifidobacterium infantis* 35,624 increase the interleukin-10 and thus, by reducing pro-inflammatory cytokines directly or indirectly, they help in maintaining the integrity of the blood–brain barrier [220] (Table 5). Strains of *Lactobacillus* such as *Lactobacillus odontolyticus* and *Lactiplantibacillus plantarum* produce acetylcholine [232]. Similarly, spore-forming human gut microbes increase the biosynthesis of serotonin from the enterochromaffin cells [233]. Psychobiotics, mainly FMT, have shown beneficial results in the case of various mental disorders such as Parkinson's disease [229], Alzheimer's disease [234], Tourette syndrome [235], autism [236], and insomnia [237]. FMT was found to be successful in relieving depression and anxiety [238]. Thus, psychobiotics have emerged as a solution to various neurodegenerative disorders. They can be a useful and promising strategy for healthy well-being. Although the results are promising, human studies are still lacking. Further research in the area of psychobiotics needs to be done to make them an alternative therapy for neurodevelopmental and neurodegenerative disorders.

Challenges in the field of microbiome therapeutics

Microbiome therapy establishes a native gut microbial environment for healthy gut functioning and preventing dysregulation.

Table 4. Various types of significant psychobiotics

Psychobiotics	Definition	Examples	Reference
Probiotics	Live microbes that when consumed or applied in adequate amounts to the body provide health benefits	*Escherichia coli*, *Lactococcus lactis*, *Bacteroides ovatus*, lactic-acid bacteria, *Lactobacillus gasseri*, *Lactobacillus helveticus*, etc.	[212]
Postbiotics	Inanimate microbes and/or their components that confer health benefits to the host	Microbial cell lysates, cell fractions, short-chain fatty acids (SCFAs), polysaccharides (EPS), peptidoglycan-derived muropeptides, teichoic acid, metabolites, etc.	[213]
Prebiotics	A non-digestible food component that stimulates the host's health by improving the growth or activity of one or more colon microbes	Fructans, Galacto-Oligosaccharides, Starch, and Glucose-derived Oligosaccharides	[214]
Synbiotics	A mixture of prebiotics and probiotics that affect the host's health by improving the growth/activity of beneficial microbes present in the gut	A mixture of probiotics such as *Lacbobacilli*, *Bifidobacteria* spp., *S. boulardii*, *B. coagulans*, etc., with prebiotics such as fructooligosaccharide, xyloseoligosaccharide, inulin, etc.	[215]

Table 5. Attributes of psychobiotics in mental health

| Serial no.	Psychobiotic	Effect	Target disease	Reference
1	*Lacticaseibacillus rhamnosus* JB-1	Regulation of emotional behavior and central GABA-receptor expression	Depression and anxiety	[216]
2	Galactooligosaccharide mixture	Waking cortisol response	Depression	[221]
3	Sodium butyrate	Central serotonin neurotransmission and brain-derived neurotrophic factor (BDNF) expression	Depression	[222]
4	Bifidobacterium or Lactobacillus	Restoration of gut-barrier integrity	Stress	[223]
5	*Lactiplantibacillus plantarum* PS128	Inflammation and corticosterone level	Depression and anxiety	[224]
6	*Lactobacillus helveticus* NS8	Levels of serotonin, norepinephrine, and BDNF	Anxiety, depression, and cognitive dysfunction	[225]
7	*Bifidobacterium longum* NCC3001	BDNF expression	Depression	[226]
8	*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175		Anxiety and depression	[227]
9	*Lactiplantibacillus plantarum* MTCC1325	Improves cognitive behaviors, gross behavioral activities, and restores the level of acetylcholine	Alzheimer's disease	[228]
10	Mixture of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Limosilactobacillus reuteri*, and *Limosilactobacillus fermentum*	High-sensitivity C-reactive protein and malondialdehyde levels	Parkinson's disease	[229]
11	*Lactobacillus acidophilus*	Self-control and attention	Attention deficit hyperactivity disorder	[230]
12	Heat-killed *Levilactobacillus brevis* SBC8803	Wakefulness and night-time wheel-running activity	Insomnia	[231]
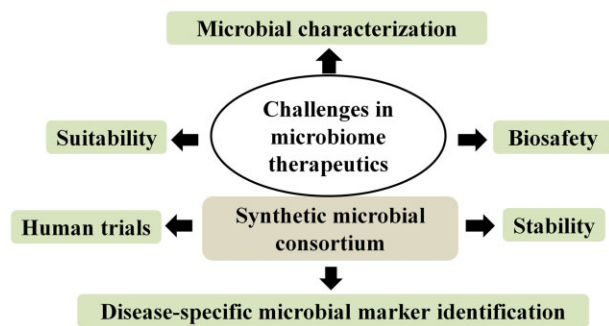
Figure 4. Challenges associated with the field of microbiome therapeutics

The microbes as therapy aim to restore dysbiosis and improve the host survival by affecting the metabolic, nutritional, as well as physiological pathways. The success of microbiome therapeutics is promising but usually suffers from a few challenges. The major challenge in the field of microbiome therapeutics is the identification of the microbes to address disease complexities (Figure 4). Different microbial strains are suitable for different therapeutic approaches based on their survival within the body. The *Bacteroides* sp. [107], *Lactobacillus* sp. [110], *E. coli* Nissle 1917 [239], and *Lactobacillus lactis* [102] have been used as therapeutic vectors. *Bacteroides sp.* colonizes the colon and caecum successfully while *Lactobacillus sp.* and *E. coli* Nissle successfully enrich within the small intestine. *Lactobacillus lactis* cannot colonize the intestine [240]. Thus, the disease biogeography decides the suitability of the probiotic used for the treatment. Proper characterization of microbes based on their functional benefits needs to be

done before choosing them for treatment. The efficacy of microbiome therapeutics has, for a long time and under various circumstances, become challenging. Additionally, microbiome therapeutic research was primarily carried out using rodent models and efforts are required for human trials. The stability and robustness of the clinically relevant microbial strains ensure successful microbiome therapeutics (Figure 4). To understand the environmental conditions faced by the microbes and the mutual interactions among microbes that affect functions, chemostats need to be developed [241]. Similarly, 3D intestinal scaffolds [242], organoids [243], and gut-on-a-chip models [244] have been used to study the interactions between hosts and probiotics. Various safety and regulatory issues need to be examined for successful clinical trials of microbiome therapeutics. A regulatory framework needs to be designed to address the biosafety of therapeutics to reduce the negative effects and release of the engineered microbes into the environment. The safety of engineered probiotics needs to be assessed for prolonged therapeutic efficacy (Figure 4). The horizontal transfer of the recombinant DNA from the engineered microbiome to the native microbiome is a major concern [245]. Similarly, the environmental release of recombinant probiotics could have harmful effects [246]. Thus, auxotrophic microbes that lose viability in the absence of a particular substrate need to be used as therapeutics [247], as they are not able to colonize the outer environments. Thus, synchronized research and regulatory mechanisms need to be used for a safe therapeutic approach, in addition to therapeutic maintenance, as engineered phages may lead to their loss of function [248]. Thus, further efforts should be done to reduce the burden on cellular therapies for the long-term stability of therapeutics [246].

Current scenario

As an estimate, the global microbiome therapeutics market size was valued at USD 34.1 million in 2019, which is expected to reach to USD 838.2 million by 2026 [249]. Several companies are virtually using microbiome therapeutic approaches to treat and diagnose diseases [250]. In 2020, the fecal microbiome suspension with the name SER-109 completed its third-phase trial. It was found to be effective against CDIs. Companies such as Rebiotix and Ferring pharma are using the bacterial suspension as RBX2660 [251]. This suspension with live spores of bacteria was the first to enter clinical trials. Additionally, companies are targeting immune-system arousal through the use of checkpoint inhibitors to treat tumors. Vedanta Biosciences has developed a microbe–drug combination to induce helper T-cells against tumors [252]. This trial is currently in phase 1. Merck along with Evelo Biosciences and 4D Pharma is currently working in this direction with the use of the drug Keytruda [253]. There are certainly several advantages in using this subset of bacteria for disease treatment but it is often associated with risk factors such as the presence of pathogens in the stool sample that may lead to disease initiation. To avoid this, certain companies such as Seres are using purified suspensions devoid of pathogens [254]. Microbiotica [255] and Vedanta [256] are currently working on a strategy to isolate gut microbes and identify detailed genomic information for the healthy bacteria and pathogens and then prepare a list of microbes associated with the disease or human health. However, there is a risk of contamination at every step of purification. Thus, companies are trying to isolate the specific bacterial products for therapeutics and scaling them up through fermentation. Companies like 4D Pharma use single microbes for immunomodulation [257]. 4D Pharma in association with Merck have invested in developing a microbial vaccine. Evelo Biosciences is also working on the strategy of the single-microbiome approach [258]. Also, Second Genome [259], Kaleido Biosciences [260], and Enterome [261] are focusing on the biologically active molecules of microbes. Enterome has targeted a signaling pathway that causes Crohn's disease and developed a product that is currently in a phase 2 trial. Certain microbial molecules that may inhibit inflammation or initiate immunity against tumor development are also being targeted by the company (Table 6).

Conclusion and future perspectives

Global human microbiome therapeutics is expected to grow spontaneously by 2027 and acquire a market size worth USD 1,731 million [265]. Advancement in synthetic biology and microbiome ecology has inspired the use of additive, subtractive, and modulatory therapies of microbiome engineering in clinics. Although research efforts have proven the efficacy of microbiome therapeutics, additional research to understand the microbiome and its interaction with the host needs to be done to move this concept of microbiome therapeutics into clinical trials to create a guide for efficient treatment. This era of microbiome therapeutics along with the combined efforts may help in disease treatment with clinical applications. The use of bacterial suspensions poses a risk to patients' health, as it may lead to the entry of pathogens within the recipient; thus, strategies to avoid contamination need to be used. Live therapeutics is the need of the hour to treat patients with the hand-picked healthy microbial group. Additionally, before the administration of bacteria into patients, proper genomic characterization of the bacterial groups needs to be done to discriminate disease-specific signature microbes from healthy microbes. Companies should now increase the manufacturing of bacteria-specific products to avoid the broad range of negative impacts of the microbes and, for that, companies should invest more. Efforts need to be made to prepare pills with single-microbe species that may improve the immune response in patients and treat patients better. Thus, the microbiome therapeutic companies need to unite with the pharma industries to improve the efficacy of the treatments. The studies and results obtained through the clinical trials on gut bacteria should further be explored for autoimmunity and neurological disorders to expand the field of microbiome therapeutics.

Table 6. Strategies adopted by the various institutions to overcome microbial disorders

Serial no.	Disease target	Strategy	Outcome	Reference
1	IBD	Fimbrialadhesin (Fim H) inhibitor	Blockage of *Escherichia coli* binding to intestinal epithelium	[12]
2	Irritable bowel syndrome	SYN-010 containing modified lovastatin	Reduction in methane production to provide relief in IBS	[12]
3	Hyperammonemia	Drug KB195	Reduction in nitrogen metabolism to provide relief in hyperammonemia	[12]
4	Inhibition of drug-resistant pathogen	Bioactive products	Improvement in beneficial microbes and inhibition of methicillin-resistant *Staphylococcus aureus*	[262]
5	*Clostridium difficile* infection	Vaccine oral capsule CP-101	Improved treatment of CDI	[263]
6	Cancer	Microbial consortium of commensal bacteria (VE800)	Improvement in the ability of T-cells to infiltrate tumors, suppress tumor growth, and potentially improve patient survival	[263]
7	Obesity	Oxygen pills	Oxygen pills improve the aerobic/facultative aerobic bacteria for effective treatment of obesity	[262]
8	Multiple disorders	Live biotherapeutics	Single strain of gut bacteria improves the microbial dysbiosis	[264]

Authors' Contributions

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Conflict of Interest

None declared.

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