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Oral Health by Using Probiotic Products

Edited by Razzagh Mahmoudi

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Scope of the Series

Biochemistry, the study of chemical transformations occurring within living organisms, impacts all of life sciences, from molecular crystallography and genetics, to ecology, medicine and population biology. Biochemistry studies macromolecules - proteins, nucleic acids, carbohydrates and lipids –their building blocks, structures, functions and interactions. Much of biochemistry is devoted to enzymes, proteins that catalyze chemical reactions, enzyme structures, mechanisms of action and their roles within cells. Biochemistry also studies small signaling molecules, co-enzymes, inhibitors, vitamins and hormones, which play roles in the life process. Biochemical experimentation, besides coopting the methods of classical chemistry, e.g., chromatography, adopted new techniques, e.g., X-ray diffraction, electron microscopy, NMR, radioisotopes, and developed sophisticated microbial genetic tools, e.g., auxotroph mutants and their revertants, fermentation etc. More recently, biochemistry embraced the 'big data' omics systems.

Initial biochemical studies have been exclusively analytic: dissecting, purifying and examining individual components of a biological system; in exemplary words of Efraim Racker, (1913 –1991) "Don't waste clean thinking on dirty enzymes." Today however, biochemistry is becoming more agglomerative and comprehensive, setting out to integrate and describe fully a particular biological system. The 'big data' metabolomics can define the complement of small molecules, e.g., in a soil or biofilm sample; proteomics can distinguish all the proteins comprising e.g., serum; metagenomics can identify all the genes in a complex environment e.g., bovine rumen. This Biochemistry Series will address both the current research on biomolecules, and the emerging trends with great promise.

Contents

Preface

In this book, the importance of using probiotics and probiotic foods in oral and oral health has been studied. The contents of the book include oral health and effective factors, probiotic microarmonts, probiotic foods, probiotics and dental caries, probiotics, and oral fungal infections. The most suitable probiotic strains that can be used in food and that are appropriate for the prevention of oral and dental problems are also discussed.

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Introductory Chapter: Oral Health by Using Probiotic Products

Razzagh Mahmoudi, Sara Moosazad, Katayoon Aghaei

1. Oral health

Oral health is one of the most important health issues and tooth decay raises a great deal of concern about that and with widespread public concern about the use of industrial drugs to reduce dental caries, nature-based treatments are highly welcomed. The second organ that has a diverse microbial community is the mouth, which contains more than 700 species of bacteria. Disruption of the natural microbial flora of the mouth through the consumption of various nutrients can have consequences for our health, such as oral and throat cancer, tooth loss, and periodontal disease [1].

2. Probiotic products

Many publications said using probiotic products can be a factor in improving the health of the digestive system, reproductive organs, and oral hygiene.

Probiotic bacteria should have the ability to adhere to and colonize surfaces in the oral cavity, have a good shelf life, and be nontoxic. Two main groups probiotic bacteria are *Lactobacillus* and *Bifidobacterium*. The *Lactobacillus*, as a member of oral microbial flora, can play an important role in the microcosm balance of the oral cavity. *B. bifidum*, *B. longum*, and *B. infantis* are the probiotic species of *Bifidobacterium*.

Today, with the increasing awareness of people about the beneficial effects of probiotic bacteria, consumption of functional foods is increasing. Functional foods are such food that promotes health [2, 3].

Functional foods must have three items:

1. they have a different effect from a nutrition standpoint;

2. they reduce the risk of pathological illnesses; and

3. they benefit the community and the consumer [4].

New food products are converted into probiotic foods by adding probiotic bacteria. These foods include a variety of different types such as cheese, ice cream, milk-based dessert, baby milk, and mayonnaise. The main thing is the texture of these foods [5].

The fourth mechanism of how probiotic bacteria work in the mouth include:

1. competition between probiotic bacteria and pathogenic bacteria in adhesion to mucus and teeth, which in this way prevents the pathogenic bacteria from attaching;

2. they can produce factors such as peroxide and bacteriocin, which act as antibacterial agents against oral pathogens; and

3. with the presence of probiotic bacteria, oral conditions change and the growth medium of pathogenic bacteria do not occur, such as the reduction of pH or alteration of the structural protein of salivary glands [6, 7].

4. Probiotics can have beneficial effects on dental health by stimulating non-specific immunity and regulating cellular and humoral immune responses.

3. Conclusion

Probiotics are becoming more common due to concerns about oral and dental diseases and increased consumer interest in natural remedies. Based on the research and the effect of probiotics in reducing the number of pathogenic bacteria in these organs, they can be used in foods.

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Chapter 2

Therapeutic Potential of Probiotics and Prebiotics

Nabila Gulzar, Iqra Muqaddas Saleem, Saima Rafiq and Muhammad Nadeem

Abstract

Advancement in technology is continued as the time is passed. The biological active ingredients technology is the challenging issues for both the researchers and manufacturers. In last decade, much research has been happened about the potential health benefits of pro and prebiotic ingredients. *Probiotics* are the live microorganisms when given in adequate amount confer health benefits but the prebiotics are indigestible ingredients that enhance the activity of one or more probiotics in the colon. The therapeutic potential of these two biological active ingredients is the outcome of emerging field of biotechnology. A number of studies indicating therapeutic potential of probiotics regarding gastrointestinal health, cancer, constipation, immunomodulation, *Helicobacter pylori*, liver disease and urogenital infection have been documented. Similarly, prebiotics also improved helpful for many communicable and non-communicable diseases. It can be seen that advancement in the biotechnology field offer great choice to deliver a marvelous health advantages. Although the market for probiotic and prebiotic food and supplements has been increased in this new era but still need to develop food and supplements containing these two active ingredients with the cooperation of legal bodies of the country.

Keywords: therapeutic, probiotics, prebiotics

1. Introduction

This chapter criticizes and presents the role of different probiotics and prebiotics in the prevention of various ailments and improvement of consumer's health. Scientist worked and explored the action of beneficial live microorganisms and non-digestible food components for the improvement of human health. The chapter highlights various sources and mode of action of probiotics and prebiotics. Disease prevention and health intriguing aspects of these therapeutic components is also addressed in this chapter.

2. Probiotics

The term probiotics was characterized by Parker [1] as life forms and some substances which are responsible for the equalization of the intestinal microorganisms. Fuller [2] defined probiotics as feed supplements in the form of live microorganisms which valuably influences the host creature by enhancing the microbial balance in the intestines. Subsequently, Moriarty [3] recommended that the meaning

of probiotics be reached out to microbial water added substances. Administering probiotics in water has been appeared to enhance water quality by decreasing the centralizations of nitrogen and phosphorus [4]. Probiotics directed in water or eating routine likewise may hinder the development of pathogenic microorganisms, contribute stomach related proteins to expand feed usage, give other development advancing components, and restoring the immune response of the living beings.

Hill et al. [5] updated the probiotics as live microorganisms when given in adequate amount confer health benefits.

2.1 Probiotics: selection criteria

For the living organisms to be termed as probiotics the given criteria should be fulfilled:

1. It must get separated from the same species from its expected host.

2. It ought to have an clear favorable impact on its host.

3. It should not produce any symptoms of disease.

4. It ought to have the capacity to survive the conditions gastrointestinal tract and can easily be passed through it.

5. During storage, greater number of practical microscopic organisms must have the capacity to endure extended lengths of time [6, 7].

2.2 Probiotics: mechanism of action

Mechanisms for the advantages of probiotics are not completely understood. However, generally it includes [8].

• Adherence and colonization of the gut.

• Suppression of development or epithelial authoritative/intrusion by pathogenic microscopic organisms and generation of antimicrobial substances.

• Improvement of intestinal hindrance work.

• Controlled exchange of dietary antigens.

• Stimulation of host mucosal and systematic immunity [9].

2.3 Standardization of probiotics

In Europe health claims of probiotics are assessed by the "European Food Safety Authority" (EFSA). For the selection guidance of probiotics European Commission published probiotics health claims [10] this meant that probiotics are not considered as nutrition claim but it is a health claim. It is further clarified that this general health claim is not mention for any body functions because the health claim of probiotics and strain specific. Consequently, probiotics term is banned with regard to advertising and labelling as food supplement in Europe.

In USA probiotic companies face legal encounters. In the research of probiotics, there are also FDA's restrictions for human trials. The study with regard to human trial must be filed as "Investigative New Drug," which appears redundant and costly obligation.

In Canada, the government has also issued regulatory documents for the use probiotics in food. Canada has a list of 17 probiotic species that can make nonspecific health claims, but no strain-specific probiotic claims have been approved [11].

In 2012, the Chinese Food and Drug Administration (CFDA) issued "Requirements on and a Guide to the Naming of Health Foods" and this year, provision for health food registration and filing was released. Currently, there are 27 health food categories based on health effects recognized in China, including enhancing immunity, the maintenance and relief of constipation, and modulating the intestinal bacteria. The health claim of any probiotics are governed an approval process involving technical review of safety, scientific substantiation of the health effect (based on animal and/or human studies), quality control, quantitative assessment and assessment of the product formulation [12].

2.4 Commonly used probiotics

A portion of the widely utilized probiotic microorganisms are *Lactobacillus rhamnosus, Lactobacillus reuteri, bifidobacteria* and certain strains of *Lactobacillus casei*, *Lactobacillus acidophilus*-groups, *Bacillus coagulans, Escherichia coli* strain Nissle 1917, certain enterococci, particularly *Enterococcus faecium (SF68)*, and the yeast *Saccharomyces boulardii*. Bacterial spore formers, for the most part of the class Bacillus influence the scene. The given probiotics are being used in variety of food products such as fermented milks either independently or in mixes. With the new research endeavors new genera and strains of probiotics are being developed.

Probiotic can either include a single stain or a blend of at least two strains. For example, VSL3 is a probiotic that comprises of blend of eight different strains. The effects of probiotics are strain specific and cannot be summed up. A one strain may display diverse advantages when utilized exclusively and in mix. The benefits arising from the use of probiotics vary according to patient group. Constrained studies showed the adequacy of use of multiple probiotic strains [13].

FAO and WHO have mutually introduced advanced rules with a specific end goal to establish the systematic outlook for a successful Probiotic assessment probiotics in nourishments to support the health cases and other advantages. The standard rules on probiotics developed by FAO/WHO could be used worldwide for assessing probiotics in nourishment that can lead to the supporting of health claims. According to the rules following activities must be fulfilled:

1. Identification of strain.

2. Functional portrayal of the strains and safety properties.

3. Proving the medical advantages in human examinations.

4. Labeling of the adequacy claims and the shelf life of the substance must be honest and should not be deceiving.

2.5 Therapeutic potential of probiotics

The probiotics have most imperative and recorded helpful impacts that incorporate the prevention from various diseases including diarrhea, increasing the

effectiveness of antibacterial activity, reduction in the symptoms of constipation, changes in the conjugation of bile and salts. Moreover, they are also involved in nutrient formation and in enhancing their utilization by the body; a few probiotics are thought to have anti-oxidant action as entire cells or lysates. Probiotics have additionally exhibited their intrinsic impacts in reducing manifestations of allergy, cancer AIDS, various infections of respiratory system and urinary tract. There are different random reports on their advantageous impacts on autism, fatigue, type 2 diabetes, aging, fatigue, obesity and osteoporosis [14].

2.5.1 Diarrhea

The Diarrhea is defined by World health Organization as three to four watery stools in the period of 24 hours. Over the period of more than 20 years several examinations have been done on the microorganisms by several in vitro studies, animal models and some other suitable clinical investigations have confirmed the role of probiotics in reducing the severity of various type of diarrhea [15] *Saccharomyces boulardii* is the only yeast probiotic that is used for treating the diarrhea.

2.5.1.1 Acute infantile diarrhea

Rotavirus is the major cause of acute infantile diarrhea and is the most con-centrated gastrointestinal condition and it requires quick oral rehydration as an essential treatment. Along with the oral rehydration therapy Probiotics are thought to have beneficial effects. Although further data is needed to confirm the investigations however 10 billion CFU is the minimum potent dose in kids within the 48 hours [16]. A trial was conducted on *C. difficile*-related colitis and results showed that *S. boulardii* reduce the onset of infection in the patients who had more than one consecutive *C. defficile* disease. *S. boulardii* is yeast which produces prote-ases that inhibit the *C. defficile* toxins and also blocks its receptors in the intestines. The yeast *S. boulardii* discharges a protease that separates *C. difficile* poisons and hinders the poison intestinal receptors. The Probiotic additionally found to activate particular antitoxin A which is an immunoglobulin that attacks the factor involved in increasing the risk of diarrhea [17, 18].

2.5.1.2 Antibiotic associated diarrhea

Disruption of the natural micro flora is due to the use of anti-microbial medica-tions, i.e., antibiotics frequently prompts diarrhea. The principle component by which antimicrobials cause loose bowels is through weakened protection against pathogens because of disruption of gut micro flora and as a result there is a modi-fication in the digestion of sugars bile acids and unsaturated fatty acids resulting modifications in the digestion of sugars, short-chain unsaturated fats, and bile acids [19]. Probiotics are more powerful in reducing the onset of diarrhea as a result of use of antibiotic medications and these includes strains of *L. acidophilus*, yeast *S. boulardii, L. delbrueckii, L. rhamnosus GG, L. fermentum,* etc. [18]. However, further studies are needed to confirm the beneficial role of probiotics in various diseases, which probiotic is more potent and their recommended dosages [20].

2.5.1.3 Traveler's diarrhea

It has been assessed that around 20–60% of the people traveling around the world are affected by this type of diarrhea. It especially influences individuals those

who travel from the developed or industrialized areas to the backward or remote areas, specifically traveling between tropical regions and other non-tropical areas. The major cases that are recognized are due to the microscopic organisms which accounts for approximately 60–85% of the cases and most causative bacteria for causing this is *E. coli* and *Compylobacter jejuni* and some other species of *Salmonella* and *Shigella* disease is also caused by parasites and viruses which accounts for around 10% and 5% toward causing the infection [21]. It was observed that the bacteria that was effective against Bacterial diarrhea is *S. boulardii* whereas the activity of Lactobacillus *GG* was observed to be more potent against diarrhea caused by the virus and the idiopathic diarrhea [22–24]. Bacterial Strains that are thought to have preventive effect on the traveler's diarrhea include the *Bifidobacteria, Lactobacilli, and Streptococci* and *Enterococci* [18].

2.5.2 Inflammatory bowel disorder (IBD)

IBD is a long term degenerative disease which involves the severe irritation of G.I.T tract that leads of watery and bloody diarrhea along with the abdominal pain IBD affects the small intestines and a portion of large intestines that is colon and it includes three different conditions which are Crohn's disease, ulcerative colitis (UC) and pouchitis. The other factors that are associated with the onset of disease include the genetic factors, ecological factors, oxidative stress and the weakened immune system [25].

Crohn's disease and ulcerative colitis are long term diseases of the gastro-intestinal tracts that are associated with the inflammation of the immune and likely are because there is absence of balance of the natural immunity with the surroundings and the with the advancement of the culture [26]. Intake of probiotic bacteria can possibly balance out the immunological boundary in the gut mucosa by diminishing the production of nearby proinflammatory cytokines [27–29]. *Bifidobacterium infantis* 35,624 helps in elevating the symptoms of abdominal pain, bloating, passage of gas, straining, bowel dysfunction and incomplete evacuation in the patients of IBD. It has been observed through recent studies that Probiotic supplementation is very helpful in elevating the symptoms of IBD and in some cases even the medical therapy can be replaced by probiotic supplementation. However it is not recommended for the Crohn's disease as the data does not support the use of probiotics. Therefore Probiotics have shown promising role in reducing the symptoms of ulcerative colitis but not in the case of Crohn's disease [30].

2.5.3 Ulcerative colitis (UC)

Ulcerative colitis like Irritable bowel disorder fundamentally influences the inner layer of the rectum and that of large intestines. Long-term ulcerative colitis can lead to the development of colon cancer. Use of different probiotics including *S. boulardii, L. casei, Bifidobacterium bifidum* has demonstrated beneficial results in terms of this disease [31]. A Study was conducted and the results demonstrated that the addition of probiotics bacterial strains of *L. acidophilus, B. bifidum*, and *B. breve* in the fermented milks was helpful in mitigating the diseases in patients [32].

2.5.4 Crohn's disease (CD)

It is a type of Irritable bowel syndrome which ordinarily influences the digestive system; however it may affect any organ from the mouth to the end of the end of digestive tract. Crohn's disease leads to the development of the ulcers and irritation that influences the capacity of the body to process food, assimilate nutrients and excretion of unwanted products in a healthful form. The Bacterial strains that

are involved in causing Crohn's disease include *Salmonella, Clostridium difficile, Campylobacter jejuni, Mycoplasma* and *Adenovirus*, There are various reports that influence the efficacy of various probiotics, e.g., *S. boulardii, L. GG, VSL3, E. coli* Nissel 1917, in combating the issues of CD in human beings [33].

The role of probiotics in therapeutic utilization of Crohn's disease is because of its competition with the groups of bacteria, maintaining the immune system and also destroying the various pathogenic microorganisms. Irritable bowel syndrome can additionally be prevented by using the probiotics which help in maintaining the defensive functions of the mucosa of intestines [34].

2.5.5 Pouchitis

Pouchitis is third kind of Irritable bowel syndrome where there is inflammation of ileal pouch particularly after the procedures such as colectomy and the other include anastomosis of ileal pouch. Various Studies were done and it was observed that the blend of probiotic VSL3 plays a significant role in the reduction of this disease [35].

The strains of powerful probiotic stimulate definite IL-10 and IL-4 mucosal cytokine. Also Probiotics may impact the cell to cell association of mucosa and integrity of the cells through upgrading activity of intestinal mucosa through the regulation of phosphorylation and cytoskeletal activity of tight junctional protein and furthermore through the production of enzymes having anti-oxidant nature. For example, catalase and superoxide dismutase hence remediating the symptoms of IBD [36].

Ileal pouch-anal anastomosis (IPAA) is the favored option to proctocolectomy with perpetual ileostomy in ulcerative colitis and the inherited disease in which adenomatous polyps are formed in the epithelial of large intestines. The most as often as possible noticed chronic problem of IPAA is potentially perpetual inflammation of the ileal supply which is either acute or chronic and is called pouchitis. Gionchetti et al. in a randomized, controlled treatment containing placebo preliminary in which VSL3 was regulated to 40 patients quickly after carefully surgical formation of ileal anal pouches, accomplished abeyance of 90% in the group treated with VSL3 versus 60% in the placebo treatment group following a year [37]. These examinations have confirmed the role of VSL3 in the administration of pouchitis [38].

According to the Rome II Crieteria irritable bowel syndrome is defined as syndrome that lasts for greater than 3 months with the implications of either diarrhea or constipation and also accompanied by the abdominal pain. Many studies on the role of probiotics in preventing Irritable bowel syndrome have been conducted [39–43]. Out of five surveys conducted three trials showed a decrease trend in the symptoms of Irritable Bowel Syndrome. As there is a beneficial role of the probiotics but the reduction in the symptoms of disease without the clinical manifestations has not yet confirmed. However, beneficial role of probiotics in terms of clinical improvement can be attained in reducing the symptoms related to the diarrhea. Many new studies are needed to be conducted on variety of bacterial strains to draw any conclusion regarding their beneficial role [44].

2.5.6 Lactose intolerance

It is a type of disorder that comprises of intolerance to the digestion of carbohydrate lactose that is present mainly in dairy products having prevalence of around 7–20% in Caucasians, 90–100% in Asians and from around 50–85% in Africans [45]. Lactose intolerance can be solved by using the probiotics that alleviate

the symptoms by reducing the transit time through gastro-intestinal tract [46]. Probiotics such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus* undergo the process of fermentation and as a result produces an enzyme lactase that helps in the breakdown of lactose into its subunit glucose and galactose. Thus, it is suggested that the intake of probiotics can help in the digestion of lactose inside the gut lumen and reducing the symptoms of lactose intolerance in both children and adults [47].

2.5.7 Colon cancer

The intake of probiotics and prebiotics has several antagonistic effects on mutagens [48, 49]. The link between the colon cancer and diets that are high in fats and oils and low in fiber and some other western diets lead to the alteration in the bacterial enzymes in the feces. The process through which the probiotic bacteria such as lactobacilli reduce the onset of colon cancer may include various modifications on the metabolic pathways of the intestinal microflora, inhibiting and binding of the cancer causing agents, physicochemical alterations in binding to colon, formation of compounds cancer causing agents and several other mutagens. Improving the immune system and physiology of the host. There is a remarkable evidence on the basis of various animal models and controlled studies [50, 51]. However, there is not enough data or studies to confirm the role of probiotics for protection against the onset of colon cancer.

The process through which the probiotics exhibit tumor suppressing activity includes the modification in the immune functions that are related with the various immune responses, Regulating cell differentiation and apoptosis, inhibiting the formation of the enzymes produced from the pathogenic bacteria *E. coli* and *C. perferinges* that includes the ureases different reductases specifically nitroreductases and oxidoreductases, β-glucuronidase and choloylglycine hydrolase. Enzymes such as ureases and beta glucosidases are involved in the conversion of pro carcinogens to the active carcinogens. In a study probiotic namely *Propionibacterium freudenreichii* produced Short chain fatty acids in the culture media and caused the cell death of gastric and colon cancer cells in humans [52, 53].

2.5.8 Constipation

It is common problem among the older population. Several controlled studies showed that in constipation either the bowel movements are increased or there is decrease in the transit time through the gastro-intestinal tract, for this purpose probiotics are utilized in the treatment of constipation [54, 55]. The most frequently used laxative is lactulose which is also a probiotics is not affected by the disaccharidases of the humans and the microflora in the colon, *bifidobacterium* utilizes it as a substrate that breakdown it into the smaller subunits leading to the creation of the osmotic effect. However, larger controlled studies are needed in order to confirm the use of prebiotics and probiotics apart from lactulose [56, 57].

2.5.9 Immunomodulation

Probiotics are involved in the modulation of the immune responses, are anti-inflammatory in nature and also have effect on humoral and cell regulated immunity. Probiotics are thought to involve in secretion of the factors that are responsible for regulating the immunity. For example the factors secreted from the probiotic strain *L. reuteri* limit the gene expression that is dependent on NF-Kb which results in the decrease in cell growth and greater protein kinases activated by nitrogen which leads to the induction of apoptosis [58].

Probiotics are present in greater quantity in fermented milks. During milk fermentation, *L. helveticus* is able to produce factors that are involved in enhancing the expression of calcineurin which is involved in the formation of mast and goblet cells in the gastrointestinal tract of mouse [59]. The intake of probiotic strain VSL3 is involved in down regulation of such immune responses by decreasing the secretion of IL-8, regardless of the presence of the bacterial pathogen *Salmonella dublin* [60].

2.5.10 Helicobacter pylori

The most common and worst bacterial infection found in human is the *Helicobacter pylori*. It causes a number of diseases like peptic ulcers, chronic gastritis, lymphoma, gastric adenocarcinoma and number of other diseases not related to gastrointestinal tract. Probiotics prevent the *H. pylori* infection by enhancing the immunoregulation functions that works as antagonistic to infection caused by *H. pylori* [61–63]. Many studies reported that *H. pylori* growth and gastritis infection can be minimized but the causative organism cannot be completely removed. It is noted that prebiotics plays their role for the suppression of *H. pylori* as well as they also enhance the efficacy of eradication therapy by stopping the side effects caused by antibiotics thus improving compliance [64]. *saccharomyces boulardii* is found helpful in reducing the symptoms of *Helicobacter pylori*.

2.5.11 Liver disease

2.5.11.1 Hepatic encephalopathy

By changing the microbiota count in the gut either by using probiotics or fermentable fiber has been related for the treatment of hepatic encephalopathy in experimental studies [65, 66]. This therapy works as it lowers the blood ammonia level due to inhabitation of bacteria that is acid resistant as well as not producing urease Further work on this approach is still under work [67].

2.5.11.2 Nonalcoholic fatty liver disease

Nonalcoholic steatohepatitis is a disease in which patients are not edict of alcohol but showing the systems of liver biopsy. Experiments on model rats suffering from fatty liver disease have been indicated that intestinal microbiota, bacterial endotoxin and tumor necrosis factor α modulate liver damage caused by alcohol. There is a perception that intestinal microbiota produces endogenous signals, which shows a pathologic role in nonalcoholic fatty liver disease, suggests a role for novel probiotic therapy in this not so uncommon condition [68, 69]. Recent studies have showed that nonalcoholic fatty liver disease is also thought to be linked with the dysbiosis of gut microbiota. By restoring and maintaining the gut microbiota, nonalcoholic fatty liver disease can be controlled. However extensive studies on the strains of gut microbiota and tests on various probiotics may be helpful in determining the use of specific probiotic for this disease [70].

2.5.12 Probiotics in infants health

Human milk defends infants from contagious diseases by several mechanisms. Human milk has various components that modulate the intestinal microbiota and *bifidobacteria*. These bacteria are the basic and significant constituent of the infants that are fed with human milk [71, 72]. Human milk can also minimize the incidence and harmful effects of inhabiting pathogens [73, 74]. Consequently,

it has been observed that a balanced increased in bifidobacterial concentration and reduction in enteric bacterial count and luminal factors of host may show a positive role in defending premature offspring and infants from diarrheal disease. Alteration of the intestinal microbiota by improving the prevalence of nonpathogenic bacteria is an effective method to attain a therapeutic treatment against enteric pathogens [75].

2.5.13 Urogenital infections and HIV

Various urinary tract infections like yeast vaginitis, Bacterial vaginosis and frequent urinary tract infections are major problems faced by the patients. In premenopausal women normal microflora consist of lactobacilli that protect host from infections but various patho-physiologic causes can results infection due to unstable microflora. A very few studies that reports the importance of probiotics for urogenital health [76, 77]. The awesome practices have reported that *L. acidophilus* has positive effect in urogenital health. Promising studies showed evidence that probiotics are also important in preventing recurrent urinary tract infection in women [78]. The women suffering from human immunodeficiency virus infection, prevention of bacterial vaginosis is very important. Previous literature indicates that women that have improper balance of lactobacilli (vaginosis) are in a great risk of human immunodeficiency syndrome [79, 80]. Therefore an adequate level of lactobacilli and preventing bacterial vaginosis is a best method to reduce the risk of getting human immunodeficiency virus infection, gonorrhea and trichomoniasis. One of the recent study shown that a probiotic strain (Lactobacillus reuteri RC-14) specific for human vagina can give strong protection from virus that can reduce the transmission of sexual immunodeficiency virus infection. Still, further work is required to approve these results before the extensive use of probiotics for the treatment of these diseases can be suggested [81].

2.5.14 Breast cancer

Several studies have been conducted on animal and human models to check the effect of probiotics in breast cancer. Several in vitro studies have shown that probiotics are involved in reducing the tumor size and stopped the growth of tumour. In Human studies it was observed that the use of probiotic *Lactobacillus casei shirota* prevented the onset of breast cancer. Also the use of fermented dairy products such as yogurt and fermented milk was inversely linked with the onset of breast cancer. Probiotics can be used for both preventive and treatment purposes of breast cancer. However more studies are needed in order to confirm the safety and efficacy of probiotics in breast cancer [82].

2.5.15 Type 2 diabetes mellitus

Experiments have shown that Type 2 Diabetes Mellitus can be result of dysbiosis of gut microbiota. This microbiota is involved in the permeability of gut mucosa and immune system of host which are linked to the type 2 diabetes mellitus. Different type of probiotic strains either single or multiple can be used for intervening type 2 diabetes mellitus. Probiotics that are mainly used to treat the symptoms of type 2 diabetes are *Lactococcus*, *Bifidobacterium* and *Lactobacillus*. After the oral probiotic administration can be helpful in modulating the immune function, gut microbiota and balancing the energy metabolism. Therefore it was suggested that probiotics are involved in reducing the incidence, delaying and reversing the complications of Type 2 diabetes [83].

3. Meat and dairy products as a functional agent

3.1 Dairy Products

Authors have considered different traditional dairy products such as yogurt, milk and cheese as a functional products as these are rich source of various vitamins and minerals and other constituents that can be used as functional agents [84]. Such as conjugated linoleic acid (CLA) and Selenium, that play an important role in lowering the serum cholesterol level thus preventing the atherosclerosis and other cardiovascular diseases also having antioxidant functions in the body [85].

Yogurt is a traditional dairy product that was prescribed by physicians in Middle East as a treatment of gastrointestinal diseases [86]. Yogurt is manufactured using *Streptococcus thermophilus and Lactobacillus bulgaricus* which both work in synergism. Health benefits of yogurt can be enhanced by the addition of probiotic bacteria in yogurt such as *Bifidobacterium, Lactobacillus acidophilus* and *L. rhamnosus* [87]. Yogurt has been used a food source consisting of probiotic organism throughout the world.

A wide variety of fermented milks have also been developed and various health benefits were linked with the consumption of fermented milks such as lowering of cholesterol. They also contain the important probiotics strains that are involved in improving the health [88].

Probiotic cheeses are available in soft as well as hard varieties. Various Probiotic Strains such as *Bifidobacterium and L. rhamnosus* are readily used in such cheeses and these cheeses can be beneficial source of Probiotics [89].

3.2 Meat products

Meat products are good source of protein and fat along with water and other vitamins which are highly bioavailable. Meat products can be made highly beneficial for health either by incorporating any ingredient in it that is considered healthy or eliminating or reducing any component that can be considered harmful [90].

Fatty acid and cholesterol level of meat can be modified by selecting different breeds, modifying the feed patterns, addition of some feed additives and several interventions regarding animal metabolism [91].

Similarly addition of olive oil to variety of meat products was thought to be associated with the high biological value and decrease onset of breast cancer and heart diseases [92].

Soy protein can also be added into the meat products. In 1999, US FDA stated that any product low in saturated fats and cholesterol containing 25 g of soy proteins may be helpful in preventing the cardiovascular diseases [93].

Lipid oxidation is the major issue in meat products thus instead of using synthetic antioxidants that pose toxicity and other harmful effects can be replaced by the antioxidants from the plat source such as rosemary, tea, etc. Green tea is associated with reducing the level of Cholesterol in body as well as increasing the portion of High density Lipoproteins and decreased the oxidation of lipoproteins [90, 94, 95].

Addition of salt for the preservation purpose can lead to high percentage of salt intake and can lead to the development of hypertension. Several substitutes were assessed and one of the best substitute was the addition of calcium ascorbate. This substitute causes higher acidification due to the fermentation by lactic acid bacteria. Replacement of salt with Calcium ascorbate helped in reducing the sodium chloride level as well enriching the meat with nutrition [96].

4. Prebiotics

Prebiotics are the food that is not digested in the human gut but food ingredients boost the growth and activity of bacteria that are good for our intestine, consequently they balance the intestine of an organism [97]. The most commonly used bacteria that are good for our health is amplified by prebiotics comprise of genus *Bifidobacterium* and *Lactobacillus,* which control the growth of harmful bacteria. Most commonly used prebiotics are mannan-oligosaccharides, galactoglucomannans, lactose, inulin and oligofructose. Short chain carbohydrates used as prebiotics consist of three to ten sugar units derived from the cell wall of yeast and plants. Prebiotics functionality are not alter by any food processing treatment and require little or no regulatory requirements, hence making their consumption more easy as compare to chemical treatment.

4.1 Mode of action of prebiotics

Important modes of action of prebiotics are:

- These are non-digestible carbohydrates that are not digested by the upper gastrointestinal tract and travel in the ileum and colon where fermented by the resident microbes.

- Prebiotics do cause intestinal homeostasis.

- Host surface receptors are coated by prebiotics.

- They produce bateriocins.

- Favorable bacteria produce short chain fatty acids with the help of non-digestible carbohydrates.

- Short-chain fatty acids are the energy source of epithelial cells.

- They regulate metabolic function and modulate immune system [98].

4.2 Targets for prebiotics: gut microbiota and intestinal health

Fermentation of resistant starches and dietary fiber caused by bacteria in colon release short chain fatty acid metabolites (SCFA). SCFA are well known for gut health as well as their part in provision of supplementary energy to the host in fatness is in debate. On contrary, 10% of entire energy in humans is provided by SCFA. According to energy yield hypothesis, dysbioticmicrobiomes have an amplified capacity to remove energy from the food, so that the bacterial conversion of non-digestible carbohydrates and dietary fiber to SCFAs could give additional energy to the host and result fatness of individual over time. Likewise, G-protein coupled receptors (GPR) can sense SCFAs that tie them with lipid and glucose breakdown. SCFAs activate the two major proteins, GPR41 and GPR43 which are expressed on adipocytes and enter endocrine L cells. Peptide YY is released by stimulation of intestinal GPR41 which enhances gut passage time and increase satiety. Inflammation lessens by activation of intestinal GPR43 as well as it simulates glucagon-like peptide (GLP), a hormone which contributes in regulation of insulin secretion. Enteroendocrine L cells express GLP-1 as well as secrete the gut-trophic

hormone GLP-2. Chief stimulus for GLP-2 secretion is nutrient consumption. SCFAs maintain the release of GLP-2 which is important in regulation of the gut fence system and decreases lipopolysaccharide translocation [99].

4.3 Healthy diet for probiotic

4.3.1 Dietary fiber

Diet is an element on which unequal antagonism exists between the microflora of gut and specific group of bacteria get their nutrition. It also serve as direct medium for the gut microflora since it may act as a direct substrate for the microbiota through its indigestible constituents and some by-products of digestion. Among all the nutritional constituents, dietary fibers are vital as they cannot be absorbed in the upper part of the digestive system; although they can be fermented in the lower part of the gut by the intestinal microbiota [100].

4.3.2 Prebiotics

Prebiotics are a subcategory of nutritional fibers that is not affected by the gastric acidity and the enzymes present in the digestive system of mammals which is very crucial for our health [101]. The main distinguishing benefit of prebiotics is their stimulation in growth of intestinal bacteria connected with fitness and comfort [102].

4.3.3 Complex carbohydrate

Some complex carbohydrates include resistant starch and plant cell-wall polysaccharides are not digested by the gut microflora. Polysaccharides comprising of cellulose, hemicelluloses (xylan, xyloglucan, mannan, β-glucan) and pectin. These all complex carbohydrates have positive simulative effect on microbial populations in the gut [103]. A number of bacteria present in human gut that possess genes programming with carbohydrate-active enzymes (CAZymes) in their genomes. These bacteria can digest such compound carbohydrates.

4.3.4 Prebiotic carbohydrates

Prebiotics definition is fulfilled by resistant starch [101]. In the colon prebiotic type of fermentation is provided by it and has lots of metabolic advantages, for instance it increase the bile salt production and laxation, lowers the danger of gastrointestinal tract cancers, and lowers the after meal glucose stimulus and blood lipid levels [104, 105]. Farther more it assists in growing of epithelial cells and propagation by increased butyrate concentration via its fermentation by the gut microbiota [106].

4.3.5 Algal (AGAL) polysaccharides

AGAL polysaccharide provides with dietary fiber, e.g., alginates, agars and carrageenan from seaweeds, consumed in food industry as thickeners and stabilizers or emulsifiers [107]. Many health benefits provided by dietary fibers due to its gel-forming ability and other physicochemical properties, including ability to ferment by the alimentary canal microbiota [108]. These benefits include controlled appetite, type 2 diabetes and obesity by increasing satiation, refining of gut barrier working and lessening the ad worse effects of luminal components.

Gut bacteria cause fermentation of compound carbohydrates that has beneficial affect for the host. This activity makes complex carbohydrate as potential prebiotics. Proteolytic activity produce potential harmful product and the fermentation of complex carbohydrates reduce risk caused by these harmful metabolites [109]. Furthermore, some useful metabolites with anti-cancer and anti-inflammatory activities, like phenolic substances and short chain fatty acids are also produced by the microbial fermentation of complex carbohydrates in the gut [110].

Chemically, inulin-type fructans are a linear polydisperse carbohydrate material consisting mainly, if not exclusively, of β-(2–1)-fructosyl-fructose glycosidic bond linkages [81]. Fructans are proposed to be classified as "functional fiber" according to recent concepts drawn from physiological effects on human individuals [111].

Guar galactomannan was enzymatically hydrolyzed to obtain partially hydrolyzed guar gum which can be utilized as prebiotic source. Partially hydrolyzed guar can be considered as potential prebiotic compound that may further stimulate the growth of potentially probiotic bacteria or native gut microflora [112].

4.3.6 Herbal prebiotics

Some herbs are used for the treatment of inflammatory immune diseases that also have prebiotic effect in the host. These herbs include *Ocimum sanctum*, *Piper nigrum* and *Zingiber officinale*. *Zingiber officinale and Ocimum sanctum* exhibited greater prebiotic activity, with higher growth of *Lactobacillus* and *Bifidobacterium* as compare to most commonly used prebiotic, fructo-oligosaccharide (FOS). However, *Piper nigrum* have similar prebiotic activity as that of most commonly used prebiotic FOS. These herbs used to regulate gut microbiota which ultimately prevent systemic swelling and related disorders [113].

4.3.7 Fractionated lotus seed resistance starch

Fractionated lotus seed resistance starch (LRS3) was fractionated and tested for its structural properties and prebiotic activities. LRS3–20% showed higher prebiotic activity against these bacteria *Bifidobacterium adolescentis* and *Lactobacillus acidophilus as* compared to LRS3–30% and high concentration of amylose maize starch [114].

4.3.8 Cereal grain fraction

Most commonly used cereal grains to develop new classes of prebiotic are corn, rice, wheat, barley and oats which also have high economic value [115].

4.3.9 Burdock root

It is rich source of fructo-oligosaccharides and phenolic compound but its use in food products are limited. It is widely used in bakery product for consumers that search for functional foods of health benefits. Burdock roots are effective alternative for functional foods with health benefits [116].

4.3.10 Lentil

Lentil food are rich source of prebiotic carbohydrates which have raffinose family oligosaccharides, fructooligosaccharides, sugar alcohols and resistance starch.

4.4 Therapeutic potential of prebiotics

4.4.1 Prebiotic impact on intestinal micro flora

Dietary fibers which are part of prebiotic play positive impact on intestinal micro flora [117]. In cholesterol metabolism prebiotic fibers play a very unique action specifically on fermentation of products and modulation of micro flora. Some research show both probiotics and prebiotics (in which non digestible food ingredients are more beneficial for host which stimulate the growth of colonic bacteria) to suppress tumor and preneoplastic lesions in the colons of animal treated with carcinogenic chemicals [118]. The presence of beneficial bacteria in gut of infants fed with human breast milk is supported by the metabolism of the complex mixture of oligo saccharides present in the milk. More mature gut microbiota is found in the formula milk. In adults formulation infants is used to achieve a type of intestinal microbiota. Improper gut microbiota (dysbiosis) can cause autoimmune disorders, infections and allergic reactions in old age [59].

4.4.2 Colon cancer and prebiotics

Aberrant crypt foci (ACF) is the first detectable abnormal growth of cells appears as lesions in the large intestine. To identify ACF lesions chromoscopic colonoscopy of high magnification is used, generally it appear as colonic mucosa. They comprised of crypts that are raised on upper side of normal mucosa. It consists of extremely condensed epithelia and has changed luminal openings visibly confined from the normal neighboring crypts. The development of the ACF to polyp, adenoma and adenocarcinoma equivalents buildup of numerous genetic and biochemical modifications. A little amount of ACF can be a cause of colon cancer. Presently, it is unidentified that which crypts are responsible for the development of tumor. Though, various researches support the idea of development of colon cancer from ACF [119]. Perrin et al. [120] reported that some fibers which stimulate the formation of stable butyrate-producing colonic ecosystem. This type of colonic environment diminished the rate of ACF. Therefore it is clear that colonic ecosystem that produces stable butyrate decreases the risks of developing colon cancer.

4.4.3 Prebiotics modulation of immune function

Immunological functions are modulated by our diet and affect resistance of host by various ways. Along with essential food components non-essential nutrients like non-digested carbohydrates also play an important role in controlling the immunological responses, particularly lymphoid tissues of the gut. Bodera [121] reviewed the previous literature by various scientist on the effects of prebiotics in enhancing the immunity of host and observed that there is sufficient proof indicating prebiotics such as inulin helps in the modulation of immunological functions. He suggests to take prebiotics for the modulation of immunological parameters in gut associated lymphoid tissues, secondary lymphoid tissues and peripheral circulation. First line defense is provided by the innate immune system and prevent the entry of contagious agents or eradicating entering pathogens. It encompasses physical fences like skin and mucous membranes, blood cells and tissue, such as phagocytes, natural killer cells and soluble mediators, like supplement proteins and cytokines.

4.4.4 Prebiotics as dietary modulators of gut microbiota in obesity

It is suggested that gut microbiota plays an important role in obesity and obesity-associated comorbidities that gut microbiota could be a possible goal for fatness

involvements. It is assumed that microbial dysbiosis is associated with fatness, it is concluded that restoring the potential symbiosis between the gut microbiota and host holds great potential. Prebiotics are consumed so that gut microbiota can be modified. Prebiotics are not capable to be digested by host enzymes although they move in the large intestine and are particularly fermented in the gastrointestinal tract where they help in growth of bacteria particularly *Bifidobacterium* and *Lactobacillus* that have been related with health benefits [122].

4.4.5 Prebiotics assist in the management of cognition

Worldwide, Schizophrenia is the 25 leading cause of disabilities that decrease the life about 10–20 years. Inappropriate treatment like pharmacotherapy in the administration of cognitive defects and weight gain are recognized a significant contributors of these diseases; therefore there is a need to develop such treatments that alleviate one, or both, of these diseases would be very helpful. By using gut microbiome from dietary source like prebiotics for the treatment of these diseases may be one such intervention. Moreover, prebiotics also effect breakdown, and in case of obesity they rise the concentration of anorexigenic gut hormones such as peptide tyrosine tyrosine, glucagon-like peptide 1 and leptin and decrease the amount of orexigenic hormones such as ghrelin [123].

4.4.6 Prebiotics for the improvement of enzyme activity and phosphate uptake

Prebiotics improve the activity of enzymes and phosphate uptake in the intestine. One of study was conducted to recognize the impact of prebiotics supplementation in infant formula on enzyme activity and phosphate uptake in the small intestine of Sprague Dawley (SD) rats. Forty-eight fifteen days old SD rats of similar weight were divided into three groups randomly: A was selected as control group in which SD rats were fed with standard infant formula with no prebiotics treatment. Rats in groups B, C were nourished standard infant formula supplemented with oligosaccharides, and the standard infant formula supplemented with polysaccharides, correspondingly. The results were checked at 28 days. It was observed that as compared to group A, group B and C showed the following: (1) In SD rats group C showed greater activities of sucrose and lactase in the small intestine; (2) In SD rats of group C comparative expressions of lactase gene in the anterior and posterior segments of the small intestine were increased by 1.68 and 2.26, and the *Mgam* gene relative expression in the posterior segment of the small intestine was improved by 0.99 in SD rats of group C; (3) the relative expressions of *Na/Pi-IIb* gene in the anterior and posterior segments of the small intestine were increased by 1.85 and 2.28 in SD rats of group C. These results specify that the supplementation of prebiotics in infant formula can stimulate enzyme movement in the small intestine by improving the relative expression of enzyme gene and *Na/Pi-IIb* gene and by reducing the intestinal injury. The polysaccharides improved to be healthier than that of oligosaccharides [124].

4.4.7 Prebiotics and the bioavailability of minerals and trace elements

Prebiotic carbohydrates have positive influence in the absorption of minerals and trace elements. There exist a promising evidence about the stimulatory impact of prebiotic carbohydrates (non-digestible oligosaccharides and lactulose) on the bioavailability of minerals and trace elements. However, most of the studies is on the rats because, the studies on the human is limited. It has been hypothesized that prebiotics create strong osmotic effect that reduce the pH and stimulate the exchange of

protons and increase level of calbindin and butyrate. Colon surface area also enlarged that is a good source to increase the minerals and trace elements absorption [125].

4.4.8 Prebiotics in diabetes and cardiovascular diseases

Prediabetes is linked with the long term inflammation that is associated with greater risk of developing type 2 diabetes and cardiovascular diseases. Increased concentration of lipopolysaccharides is linked with dysbiosis of the natural microbiota that is involved in the development of type 2 diabetes and cardiovascular diseases. Prebiotics involved in the selective functioning of natural micro biota such as inulin decreases the concentration of endotoxin, reduces permeability of the intestines and limit the metabolic dysfunction in rodents. The impact of prebiotics on the cardiovascular functions in patients at the risk of type 2 diabetes is still unknown. The prebiotic supplementation along with inulin could be used as preventive strategy for limiting the risk of cardiovascular diseases in the patients at risk of type 2 diabetes. This strategy can affect the clinical practices by setting and accepting dietary recommendation of prebiotics by medical and scientific community [126].

4.4.9 Prebiotics and renal profile

It is well documented that prebiotics, probiotics and the symbiotic supplementation had resulted in the improvement of renal function. Firouzi and Haghighatdoost [127] studied renal profile of a meta-analysis of a clinical trial. They pointed out that level of glomerular filtration rate was reduced whereas the concentration of creatinine was increased in the intervention group in comparison to the placebo group and the results were non-significant. The combined effect on the blood urea nitrogen demonstrated that the level declined as compared to the placebo group. Whereas the levels of uric acid increased in intervention group in comparison with the placebo group. The supplementation of prebiotics, probiotics and symbiotic should be limited among those in which renal function is compromised until well designed and large scale randomized controlled trials prove the efficiency and safety of prebiotics, probiotics and symbiotic supplementation for having improved effects on the renal function.

4.4.10 Prebiotics in controlling cancer

A study was conducted to check the impact of prebiotics intake consisting of xylo-oligosaccharides, fructo-oligosaccharides, resistant dextrin and polydextrose on immunity and the structure of gut microbiota in perioperative colorectal cancer patients.

A double blind, randomized clinical trial was conducted that consisted of 140 perioperative colorectal cancer patients involving 50 females and 90 males having age between 40 and 75 years. Patients were divided on random basis into two groups consisting of intervention group that received 30 g/day of prebiotic supplementation for the period of 7 days and control group that did not receive supplementation. The immunological and nutritional indices of both groups were evaluated before and after operation and were compared with their baseline levels. Also the fecal samples of random 40 patients from the both groups were taken to examine the gut microbiota. There was significant reduction in the abundance of intestinal microbiata from pre to post operation in the control group. Intake of prebiotics is recommended to improve immunological indices in patient having colorectal cancer for 7 days before operation. Prebiotics enhanced the concentration of four commensal microorganism containing opportunistic pathogens in patients having colorectal cancer. Surgical stress decreased the population of intestinal microbiota

but increased the population of commensal microbiota and opportunistic microorganisms. *For further research Bacteroides* is considered as a relevant bacterial species on the mechanism of prebiotics [128].

4.4.11 Anxiolytic effect of prebiotics

Long term treatment of prebiotics particularly combination of fructooligosaccharides and galactooligosaccharides have antidepressant effects and also involved in combating the issues related to anxiety. The effect of Galactooligosaccharide (GOS) and the combination of fructooligosaccharides and galactooligosaccharides is involved in reducing the level of corticosterone level in the body by mainly limiting its release. Prebiotics are involved in modifying the expression of specific genes related to the concentration of short chain fatty acids in the hypothalamus and hippocampus. Administration of Prebiotics resulted in the increased level of acetate and propionate and reduction in the concentration of isobutyrate that have significant correlation with the positive effects on behavior. Moreover, the administration fructooligosaccharides and galactooligosaccharides reduced the level of corticosterone and proinflammatory cytokines resulted from the chronic stress as well as normalizing the anxiety and depression like behavior along with mitigating the impact of stress on the microbiota [129].

5. Conclusion

Probiotics and prebiotics are functional foods that have significant biotechnological benefits with wonderful potential for invention. Though probiotics and prebiotics are likely to be used in treating many communicable and non-communicable disorders but it is very hard to draw conclusions from a number of researches that indicating the therapeutic potential of probiotics and prebiotics because scientists used different strains and prescriptions for various disorders. Further research using reliable regulations will permit more decisive results on the effects of probiotics and prebiotics for treatment of various diseases. Although various products with probiotic, prebiotics and symbiotic are usually available in food and supplement formats, in near future these products will target to specific disease and will be available as drug.

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Chapter 3

Gut-Brain Axis: Probiotic, *Bacillus subtilis,* Prevents Aggression via the Modification of the Central Serotonergic System

Heng-Wei Cheng, Sha Jiang and Jiaying Hu

Abstract

Intestinal bacteria release various neuroactive compounds directly or indirectly regulating brain function to modulate host health and behavior through the gut-brain axis. Probiotics have been used as dietary supplements to target gut microbiota (microbiome) for prevention or therapeutic treatment of various diseases including mental disorders. In our study, chickens were used as an animal model to assess, if dietary supplementation of probiotic, *Bacillus subtilis*, reduces aggressive behaviors following social challenge. Chickens of an aggressive line were housed in single-hen cages. At 24 weeks of age, the hens were paired with similar body weight to identify the dominance rank (day 0). The subordinate and dominant of each pair were fed a regular layer diet or the diet mixed with 250 ppm probiotics for 2 weeks, then the second behavior test was performed between the same pair (day 14). The display of aggressive behaviors in the regular diet-fed chickens was not affected between the levels at day 0 and day 14, while the frequency of threat and aggressive pecking were reduced in the probiotic-fed chickens compared to the levels at day 0. These results suggest dietary probiotic, *Bacillus subtilis*, could be a suitable strategy for increasing hosts' mental health.

Keywords: probiotics, *Bacillus subtilis*, aggression, serotonergic system, gut-brain axis

1. Introduction

Human emotional susceptibility in an inter-group is associated with individual differences in the functions of the hormonal and/or neurochemical systems in response to internal and external stimulations. Aggression, as one of emotional disorders, has been commonly defined as feeling of anger or antipathy of an organism during social interaction, leading to hostile of violent behavior or attitude, provoking attack or confront toward another individual [1]. Aggression affects millions of people's health and welfare around the world annually, resulting in significant social destruction and economic costs.

Intestinal bacteria, as a virtual endocrine organ, release various neuroactive compounds directly or indirectly regulating brain function, including the serotonergic system, to regulate host health and behavior through the gut-brain

axis [2–10]. Intestinal bacteria, as well as *Bacillus subtilis*, used as probiotics are involved in tryptophan metabolism [11–14]. Tryptophan metabolites have roles in protecting intestinal mucosa from inflammation and regulating gut immune homeostasis [15–18]. Tryptophan, a precursor of 5-HT, directly affects brain 5-HT synthesis as that tryptophan can pass the brain-blood barrier [19, 20]. The brain serotonergic system plays a critical role in regulating behaviors, especially aggression [21, 22]. Concentrations of 5-HT and its metabolites, as well as the density of 5-HT receptors, have been used as major indicators of aggressive behaviors in humans and experimental animals [23–26].

Probiotics (also called psychobiotics or bio-friendly agents), defined "as a source of live (viable) naturally occurring microorganisms (direct-fed microbials, DFMs)", have been used as dietary supplements to target gut microbiota (microbiome) for a novel promising therapeutic approach of various diseases including social stress-induced mental disorders in humans and various experimental animals [27–36]. Different probiotic strains, for example, have been investigated as functional food or therapeutic treatment of various diseases, including *Bifidobacterium bifidum* [37–39]; *Bifidobacterium bifidum* (BGN4) and *Bifidobacterium longum* (BORI) [40, 41]; *Bifidobacterium pseudocatenulatum* [42]; *Lactobacillus helveticus* [31, 32]; *Lactobacillus plantarum* [43]; *Lactobacillus paracasei* (KW3110, [44]); *Lactobacillus rhamnosus* [45]; and *Clostridium butyricum* [46]. The results collected from these studies indicate that the effects of probiotics on physiological homeostasis, immunity, stress resistance, and related health status are affected by multiple factors, including the probiotic species, its concentration, and duration as well as the host's age and health status [47, 48].

Bacillus subtilis, as one of the three most common species of probiotic products in the United States [49], has been widely used as functional feed supplements, such as in a number of dairy and non-dairy fermented foods, for improving human health and well-being [50–52]. Similarly, *Bacillus subtilis*, as growth promoters, has been demonstrated to improve chickens' growth performance [53–55]; regulate intestinal microstructure [56] and digestive enzymes [57, 58]; synthesize and release antimicrobial and antibiotic compounds [15, 59–63]; increase immunity [57, 64] and neurochemical activities including serotonin [8, 65, 66] as well as affect animal behavior [67, 68] following various stressors. In addition, *Bacillus subtilis* can overproduce L-tryptophan [11, 69], consequently increase 5-HT in the hypothalamus [70], function as an antidepressant and anti-anxiety agent [71, 72], and eliminate nervous tension in mice [73]. In the current study, chickens were used as an animal model to assess if dietary supplementation of probiotic, *Bacillus subtilis*, reduces aggressive behaviors following social challenge.

2. Gut microbiota and the gut-brain axis

Gut microbiota is a collective name of ten of trillions of microorganisms living in our intestine, including more than 35,000 different species of known bacteria [74]. Microbiome refers to the collective genomes of more than 3 million genes of the microorganisms in a particular environment. Gut microbiota, like a virtual endocrine organ, reacts to various internal and external stimuli [5, 75–78]. Consequently, gut microbiota influences and regulates hosts' health and mood statue including aggression by integrating metabolic, immune, endocrine, and neural reactions through the bidirectional communication of the gut-brain axis (**Figure 1**) [7, 33, 79–83]. Gut microbiota in hosts, for example, regulates brain neurotransmitters [84–87] such as serotonin (5-HT) through releasing its precursor, tryptophan, an essential amino acid [75, 88–90]. Serotonin is a key neurotransmitter within the brain, contributing to the development of the central nervous system (CNS)

[91–94] and social behavior [95, 96], affecting mental health [97–100] and providing therapeutic strategy for treating or preventing stress reaction and related neuropsychiatric disorders [94, 97].

2.1 Social stress and the hypothalamic-pituitary-adrenal axis

The social world is filled with different types of interactions, such that social challenges (fight-or-flight) and social environmental changes (group instability), as stressors, can cause highly potent stress [101–103]. Some management practices used in the intensive farm animal production systems, for example, may cause stress in animals. Farm animals are often housed in large groups at high densities, and during the animals' lifetimes, they are repeatedly transported to new environments and mixed with unfamiliar individuals. Based on the social network theory, animal group disruption can lead to social stress and related aggression [104] when they are unable to cope with these management practices and/or to reset their dominance rank (a form of animal social structure hierarchy) [105, 106].

Aggression has been commonly defined as feeling of anger or antipathy during social interactions, leading to hostile or destructive behaviors or attitudes, provoking physical or verbal attack or confront toward another individual [1]. Aggressive encounters among conspecifics for obtaining or maintaining a socially dominant position or rank cause a negative or unstable social environment to activate the hypothalamic-pituitary-adrenal (HPA) axis which is known as the key stress response system [107–109]. Cortisol (or corticosterone, CORT, in rodents and birds), as one of the final hormones released from the adrenal cortex, has multifunctional functions in both normal and abnormal states, regulating behavioral styles, metabolic patterns, and endocrine and immune functions, and ensuring an adequate coping strategy and well-being [110–112]. In mammals, hypercortisolism in response to novel environment exposures has been evidenced to be of adrenal origin [113, 114]. The value of circulating cortisol (or CORT) has been used as a criterion of stress response in humans and various animals [115–117]. Corticotrophin-releasing factor facilitates aggression [118, 119], and related elevation of CORT (or cortisol) also affects neuroendocrine functions through binding to their receptors [120, 121], causing neuron loss in the hippocampus [122], dysfunction of the serotonergic system [123], and inhibition of immunity (increased pro-inflammatory cytokines as neuroinflammatory response) within stress-sensitive brain regions [124]. These changes finally lead to the development of psychological disorders such as aggression [109, 125]. Similar to that the HPA hyperactivity generates aggressive behavior, exogenous glucocorticoid treatments increase exacerbated aggressiveness in both humans and experimental animals [123, 126]. Taken together, emerging
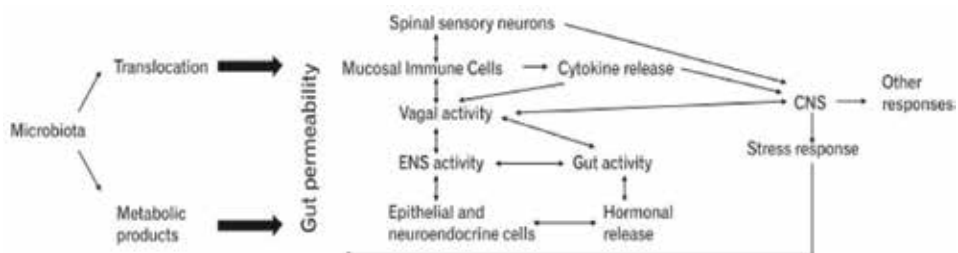

Figure 1.
The microbiota-host interaction occurs at the level of the gastrointestinal mucosa via local neural, endocrine, and immune activities, influencing brain neurotransmitter expression, physiological homeostasis, and immunity (modified from [79]).

evidence have indicated that the changes of the programming of HPA stress reactivity [127] cause long-term effects on the host physiological homeostasis and neurobehavioral functions [128].

2.2 Microbiota, stress-associated aggression

Aggression within a group is to establish a dominance hierarchy when the animals are first brought together in a common environment [129–131]. From an evolutionary perspective, aggression is adaptive behaviors that are related to an individual's survival, growth, and reproductive success within a group [132, 133]. However, aggression with long-term impact is often destructive and maladaptive in today's society, affecting millions of people's health and well-being around the world annually, resulting in significant social destruction and economic costs [134–136].

Intestinal microbiota plays an important role in regulation of social behavior, emotional expression, and mental health within the animal kingdom [3, 137, 138]. They are essential players in stabilizing homeostasis of the GI tract in response to both acute and chronic stress via the microbiota-gut-brain axis [16, 139]. Normally, intestinal microbiota provides protection for animals by competing for attachment sites and nutrients with pathogens as well as production of antimicrobial peptides and neuroactive compounds [140, 141]. The gut microbiota is also a key pathway to modulate brain processing the integrated information received from the peripheral nerve systems (the vagus nerve, enteric nerve, and autonomic nervous system), hormone signaling, the immune system, and microbial metabolites (short-chain fatty acids) [5, 137]. Under social challenges, stress and related oxidative damage cause anatomical and functional disorders of the GI tract by: (1) disrupting the commensal bacterial populations and colonization, thus reducing beneficial bacteria and increasing pathogens; (2) increasing pathogen survivability and innovating capability; (3) disrupting absorption of nutrients and minerals, including calcium; (4) disrupting microbial neuroendocrine functions; (5) disrupting the gut epithelial barrier, thereby increasing intestinal permeability causing the gut to leak certain bacteria (leaky gut), resulting in metabolic disorder; (6) damaging epithelial cells, thus producing free radicals and reducing antioxidant efficacy; and (7) interrupting intestinal integrity, thereby leading to intestinal inflammation [142–144]. These changes in gut microenvironment affect brain functions, resulting in exacerbated HPA axis activity, increased chronic inflammation, and/or disrupted neurotransmitter balance, leading to emotional damage [139, 145] and mental disorders [137, 138]. Sudo et al. [146] reported a correlation between the changes of gut microbiota and the function of the HPA axis. Germ-free (GF, antibiotic-treated microbiota-deficient or raised without any exposure to microorganisms) rats show exaggerated HPA responses to psychological stress [75, 147] with significantly higher levels of both ACTH and CORT in response to restraint stress compared to control rats [146]. In GF mice, gut microbiota also modulates the 5-HT synthesis and release at both the brain and peripheral levels directly and indirectly via the microbiota-host interactions [148]. Reduced 5-HT activity (a 5-HT deficiency) has been associated with personality traits (interspecific social behavior), such as impulsivity and aggression, and deteriorated stress coping capability (increased stress response) in humans and various animals [149, 150] including chickens [24, 151–154].

3. Serotonin and aggression

Serotonin, as an ancient chemical, is a key neurotransmitter. It plays a critical role in shaping social responses by regulating both basic (proactive) behaviors

(such as feeding, drinking, and sexuality) and reactive behaviors (fearfulness, anxiety, and cognition) including aggressive behaviors [155, 156] and mood disorders [157, 158]. Abnormalities of blood and brain levels of 5-HT, 5-HIAA (its metabolite, 5-hydroxyindoleacetic acid), tryptophan, and its receptors have been used as major indicators or targets in the diagnoses and treatments of psychiatric and compulsive disorders in humans and various experimental animals [62, 63, 159, 160].

In the CNS, 5-HT functions to inhibit aggression, thereby controlling domestic behaviors [161–163]. The 5-HT deficiency theory of aggression is driven from the negative correlation between the changes of the CNS 5-HT and aggressiveness in humans [164, 165], non-human primates [166], rodents [25, 167], and chickens [24, 168]. Aggressive animals have low levels of 5-HT in the brain, including in the hypothalamus [169–171]. Experimental increase of 5-HT and/or 5-HIAA in the brain, such as in the lateral hypothalamus and amygdala, blocks or retracts killing behavior in rodents [172, 173]. Hypothalamic injection of a 5-HT1a agonist inhibits aggression in male hamsters [174]. Depletion of brain 5-HT in TPH2 mutant mice marks aggression and lowers habituation in novel environments [149]. In addition, 5-HT1BR knockout mice show increased aggression and impulsivity [175, 176]. The implication of 5-HT successfully relieves the depression syndromes in humans [177] and reduces aggressive behaviors in primates and rodents [178, 179].

In the peripheral system, however, pathophysiological roles of 5-HT in behavioral and motivational regulations are unclear. Reduced, elevated, and unchanged blood 5-HT concentrations have all been reported in association with behavioral dysfunctions, including aggressiveness [180, 181]. The conflicting data from different investigations could be related to the differences in species of animals, behavioral evaluations, and/or stressors used as well as duration and frequency of stressors presented.

4. Chicken as an animal model for social stress and related aggression

Human emotional susceptibility in an inter-group is associated with individual differences in the functions of the hormonal and neurochemical systems in response to internal and external stimulations [182, 183]. Various social and biological factors are associated with the development and expression of aggressive behaviors, including environmental, genetic, cognitive, hormonal, and neurotransmitter circumstances [184]. Aggression and associated mental illness are an emerging public health problem [29]. Animal models are critical for investigating the potential biological processes involved in human aggression and mental disorders.

Although there are dissimilarities between humans and chickens, as indicated, the neural circuitry for aggression and social behavior appear to be evolutionarily conserved across the vertebrates [185]; chickens have been used as an animal model in various clinical and psychopharmacological studies, such as anxiety, depression, and aggression [186, 187]. A commercial hen can have more than 300 offspring with similar genetic characteristics during her lifetime, and chicks can be hatched without maternal condition effects. Previous studies have reported that birds' brain possess a core "social behavioral network" which is humongous to the social behavioral network of mammals [188]. There are evidences that the central nuclei involved in moodiness in avian, at least in part, are morphofunctional homologous to the mammalian nuclei [189], such as the hypothalamus [190], nucleus taeniae (homolog to the amygdala of mammals, [191, 192]), and Raphe nucleus [193]. These nuclei exert similar cognitive abilities and consciousness [194] with capability of plasticity in response to environmental stimulations [195]. In addition, there are similar distributions of neurotransmitter receptors, including serotonergic receptors

Behavior	Description
Feather pecking	One bird pecking at feathers of another bird, can be gentle (nibbling or gentle pecking in which feathers are not removed or pulled) or severe (vigorous pecking to feathers in which feathers are often pulled, broken, or removed)
Threat	One bird standing with its neck erect and hackle feathers raised in front of another bird
Aggressive pecking	Forceful downward pecks directed at the head or neck of other birds
Threat kick	One bird forcefully extending one or both legs such that the foot strikes another bird

Table 1.
Behavioral ethogram.

between birds and mammals [196, 197]. Particularly, it has been evidenced that the development and function of avian HPA axis in response to stimuli are analogous to those of mammals [198, 199] and there are similar distributions of cortisol-like molecules in the same organs from both birds and mammals [200]. Birds' CORT biosynthesis and metabolism can be stimulated in the same way as that of mammals [201–203]. During breeding, functional integrations among behavior, physiology, morphology, and environment in chickens may create suites of various traits that are simultaneously acted upon each selection program [204]. For example, selection based on individual production capability (phenotypic characteristics) increases animals' competition and aggression [205, 206] such as potentially injurious feather pecking and cannibalism. For instance, through more than 20 years of selection, egg production had been increased significantly in a former commercial Dekalb XL strain, whereas mortality due to aggression and cannibalism in non-beak trimmed hens had also increased about 10-fold [132, 152].

Results from previous studies conducted in our laboratory have found that chicken strain differs in social reactions through a diversely genetic selection program. The DXL hens have distinct stress levels in attempting to adapt to their social environments [207, 208] and exhibit different levels of aggressiveness [168]. In addition, neurotransmitters, 5-HT and dopamine (DA), and the hypothalamic-pituitary-adrenal (HPA) axis are regulated differently between chicken strains [168, 207]. There results suggest that injurious behaviors and related social sensitivity of chickens, similar to that in mammals, are regulated via the serotonergic system and the HPA axis [123, 209], and mechanisms underlying aggression in laying hens may be analogous to those of humans and rodents [210]. Similarly in humans and rodents, stress-induced destruction of intestinal bacteria disturbs the bilateral connection of the microbiota-gut-brain axis in chickens, affecting physiological homeostasis and behavioral exhibition [211]. In one of our studies, the role of probiotic, *Bacillus subtilis*, on aggression in DXL line was examined. In the study, one-day-old female chicks were reared in single-bird cages up to 24 weeks. At 24 weeks of age, the hens with similar body weight were paired for the first aggression test (pre-treatment, day 0) in a novel floor pen [212]. Behaviors were video-taped for 2 h immediately after releasing the two hens simultaneously into the floor pen to determine the dominant individual per pair. Following the behavioral test, the subordinate hens were continuously fed the regular diet, while the dominant hens were fed the diet mixed with 250 ppm probiotic (1.0×10^6 cfu/g of feed) for 2 weeks. The probiotic contains three proprietary strains of *B. subtilis.* After the treatment (day 14), the second aggression test was conducted within the same pair of hens. The video recording was analyzed for frequency of feather pecking, threat, aggressive pecking, and threat kick by the routine lab procedures (**Table 1**) [154, 213].

5. Probiotic, *Bacillus subtilis*, and aggression

As social animals, chickens show fear, depression, and/or anxiety in novel environments and show aggression toward ones for establishing social dominance rank in unfamiliar social groups [214, 215], which is similar to rodents used in human psychopharmacological studies [216, 217]. The paired social ranking-associated behavioral test used in this study has been routinely performed in chicken behavioral analysis [154, 213, 218]. The rationale and cellular mechanisms of the test is similar to the resident-intruder test which is a standardized method used in rodents for detecting social stress-induced aggression and violence [219, 220].

5.1 Probiotics and *Bacillus* spp.

A healthy intestinal microbiota community is important for maintaining physiological and behavioral homeostasis as that the gut microbiota regulates appetite, local and systemic immunity, stress responses of the HPA and sympathetic-medullary-adrenal (SMA) axes, and circadian rhythms [5, 221]. The new strategies of psychotherapy aimed at restoring the normal gut microbiota and intestinal homeostasis have been developed for the prevention and/or reduction of stress-induced abnormal behaviors and mental disorders.

Probiotics are commensal bacteria ("direct-fed microbials", DFM) that offer potential health beneficial bio-physiological effects to the host's stress response (acute, chronic, or both). Probiotics aid animals in adapting to their environments and protect against pathogens by: (1) altering the microbiota profile with beneficial bacteria to prevent the growth of pathogens and to compete with enteric pathogens for the limited availability of nutrient and attachment sites; (2) producing bacteriocins (such as bacteriostatic and bactericidal substances) and short-chain fatty acids against pathogens to regulate the activity of intestinal digestive enzymes and energy homeostasis and to increase mineral solubility; (3) reducing oxidative stress, inflammation, and acinar cell injury; (4) modulating host immune and inflammatory responses and restoring the intestinal barrier integrity which prevents pathogens from crossing the mucosal epithelium; (5) stimulating the endocrine system and attenuating stress-induced disorders of the HPA and/or SMA axes via the gut-brain axis; and (6) inducing epithelial heat shock proteins to protect cells from oxidative damage; and (7) synthesis and secretion of neurotransmitter such as 5-HT and tryptophan [16, 17, 222–226]. In both human and rodent studies, probiotics reduce cognitive dysfunction, decrease the stress response and related oxidative damage by lowing plasma CORT and ACTH levels, restore hippocampal 5-HT levels, and normalize immunity with low plasma levels of TNF-α (tumor necrosis factor-α), a proinflammatory cytokine, but high levels of IL-10 (Interleukin-10), an anti-inflammatory cytokine [67, 227–230]. It has been stated in nonhuman primate models that the composition of the gut microbiota has potential effects on hosts' aggressive behaviors and anxiety symptoms [127], which is similar to the findings reported in humans [137, 231–233]. In rodent studies, GF animals with exaggerated HPA responses to social stress can be normalized by certain probiotics [147]. In addition, probiotics have successfully attenuated anxiety and depressive behaviors in rat offspring separated from their mother [234–236] and the obsessive-compulsive-like behaviors in house mice [28, 237]. These results support the psychobiotics theory [238] and provide a new insight into the possible use of probiotics to improve a host's cognitive function in humans [9, 40, 41, 239–243].

A proposed strategy for improving human health is dietary supplement with probiotic microorganisms including Bacillus species [52, 244]. *Bacillus subtilis* is spore-forming bacteria. They have heat stability and low pH-resistance (the

gastric barrier), and tolerate multiple environmental stressors [245, 246]. Several Bacillus spp. such as *B. coagulans* and *B. subtilis* have been used as probiotics in both humans [247–249] and animals [246] including poultry [65, 67, 250, 251]. Several mechanisms of action of *Bacillus* spp. have been proposed: the improvement of hosts' growth, survival, and health status via their anti-inflammatory functions through immunomodulation and cytoprotection [252, 253]. And, they have been used as antibiotic growth promoters alternatives with health-promoting benefits by naturally synthesizing proteins, enzymes, antimicrobial peptides, vitamins, gut flora modulation to promote beneficial microbiota along the GI tract and to correct and repair immunological and gut morphological alterations [244, 246, 250, 254, 255]. In addition, numerous studies have shown that probiotic-induced changes in the composition of gut microbiota lead to alterations of neuroendocrine functions. For example, in response stimulations, *B. subtilis* alleviate oxidative stress, provoke a specific biological response, and improve mood status of hosts via the gut-brain axis [52, 147, 256].

5.2 *Bacillus subtilis*-based probiotic and social challenge-induced aggression

Aggression, in nature, is associated with competition (natural selection) for survival and reproduction [22, 257, 258] (please also see above for the detailed description). However, in artificial production environments, such as in the poultry industry, aggression causes increased social stress and feather and body damage, in some instances these injuries leading ultimately to cannibalism. Cannibalism is a major concern related to non-beak trimmed bird deaths in current housing environments [259, 260]. Beak trimming (BT) is a routine procedure practiced in the US egg industry for reducing social stress by preventing and/or inhibiting feather pecking and cannibalism. However, BT is criticized for causing tissue damage and pain (acute, chronic, or both), negatively affecting the welfare of billions of chickens annually [261, 262]. Considerable concern from the public has led to a growing movement against procedures causing pain and suffering in farm animals. In response to growing pressures, housing environments of laying hens have been modified and/or various dietary supplementations have been provided in attempts to prevent social stress and stress-associated injurious behaviors. For instance, modifications include reducing light intensity, changing the nutritive value or taste of diets [263–265], providing straw or grain [266, 267] or pelleted diets [263], housing hens in floor-pens [268], and developing enriched cages [269, 270]. However, these methods have had limited success and provide no guarantee of preventing these injurious behaviors. Therefore, an obvious solution is to develop a welfare-friendly alternative to BT that minimizes social stress, thereby preventing feather pecking and cannibalism. The hypothesis was tested in this study: probiotics could be an alternative to beak trimming in chickens for reducing feather pecking and aggression via regulating the gut-brain axis.

Based on the 5-HT deficiency theory of aggression, social challenge-induced changes in 5-HT concentration were examined in this study. In the current study, the data showed that prior to the treatment (day 0), plasma 5-HT levels were higher (26% increase) in the dominant hens than that of subordinate hens but were not statistically significant (P = 0.24. Dominant$_{5\text{-HT}}$ = 17.46 ng/ml, Subordinate$_{5\text{-HT}}$ = 13.87 ng/ml). This finding is in agreement with the results reported previously [152, 168]. In those studies, higher plasma levels of 5-HT were detected in hens from mean bad bird (MBB) strain, a high aggressive strain selected for both low productivity and low longevity resulting from injurious pecking and cannibalism, compared to hens from kind gentle bird (KGB), a low aggressive strain selected for both high productivity and high longevity. In

addition, Bolhuis et al. [271] proposed that blood 5-HT activity is correlated with the development of severe feather pecking in laying hens. A similar correlation between blood 5-HT levels and aggressiveness has also been detected in humans and various other animals, that is, a lower blood 5-HT level was associated with less aggressive individuals in humans [180, 272] and canine [273], while an elevated blood 5-HT level was determined in patients with aggressive behaviors [274] and in aggressive teleost fish [275].

Post-treatment (day 14), plasma 5-HT levels were reduced toward the levels of controls (subordinates) in the probiotic fed dominant hens ($P = 0.02$) compared to their related levels prior to treatment (day 0) (**Figure 2**). There were no treatment effects on plasma 5-HT concentrations in subordinate hens fed a regular diet ($P = 0.88$). Although the reasons of the reduction of plasma 5-HT concentrations in probiotic fed hens are still unclear but could be similar to those proposed in probiotic-treated patients with intestinal inflammatory disorders such as irritable bowel syndrome (IBS) and IBS experimental animals. Probiotics reduce IBS-associated abdominal pain and abnormal bowel habits [276, 277] through regulation of both the central and peripheral serotonergic systems via the microbiota-gut-brain axis [278, 279] and gut epithelial enterochromaffin cells [19]. In the peripheral system, probiotics reduce or inhibit IBS-associated serotonergic system abnormalities, that is, great hypersensitivity and spontaneous release of 5-HT [280, 281]. Serotonin reuptake transporter (SERT or 5-HTT) has functions in inactivating 5-HT. Down-regulation of SERT receptors has been found in the intestinal mucosa of IBS patient whose symptoms are similar to those found in the SERT knockout mice [282]. Serotonin 5-HT3 receptor antagonist also has shown efficacy in treating IBS patients [278, 283]. In addition, Wikoff et al. [284] reported that conventional mice had lower concentrations of 5-HT compared to GF mice. GF mice also had an exaggerated stress response [146] with anxiety-like behaviors [285]. These abnormal behaviors in GF mice can be inhibited or reduced by feeding probiotics [286] or transplanting fecal samples of conventional mice [287]. The current and previous

Figure 2.
Plasma serotonin (5-HT) levels at day 0 (pre-treatment) and day 14 (post-treatment) in probiotic fed dominant hens and regular diet fed subordinate hens. Compared to subordinate hens, plasma 5-HT concentrations were higher in dominant hens at day 0 but without statistical difference ($P = 0.24$); the difference disappeared at day 14. Compared to the levels at day 0, blood concentrations of 5-HT were reduced in probiotic fed dominant hens at day 14 ($P = 0.02$) but were not in regular diet fed subordinate hens ($P > 0.05$). [a,b] between the concentrations at day 0 and day 14, least square means lacking common superscripts differ ($P < 0.05$).

results indicate that normal health gut microbiota plays an important role in regulating social stress and stress-associated behaviors.

Whether the changes of blood 5-HT levels in probiotic fed dominant hens represent a similar change of 5-HT concentrations in the brain is unclear, as 5-HT cannot pass the brain-blood barrier and is regulated differently between brain neurons and peripheral tissues [288]. The plasma 5-HT is synthesized mainly by the EC cells of the gut and stored in the platelets [289]. However, it has been proposed that platelet 5-HT uptake is a peripheral marker of brain 5-HT [273]. Dietary probiotic, *Lactobacillus plantarum strain* PS128, increases the levels of 5-HT as well as dopamine in the striatum, which is correlated with the improvement of anxiety-like behaviors in GF mice [290]. Similar results have been received from our current studies. In one study, chickens (broilers) were fed *Bacillus subtilis* from day one. At day 43, *Bacillus subtilis* fed chickens had higher levels of 5-HT in the raphe nuclei and lower levels of norepinephrine and DA in the hypothalamus than controls [291]. Probiotic fed chickens also had improved bone traits (bone mineral density, bone mineral content, and robusticity index). Under heat stress (32°C), *Bacillus subtilis* fed chickens had lower heat stress-related behaviors and inflammatory response and reduced IL-6 levels in the hypothalamus compared to controls [67]. Further studies, however, are needed to examine if there are correlations between the regulations of peripheral 5-HT and CNS 5-HT in probiotic fed hens.

The gut commensal microflora may have an indirect effect on 5-HT synthesis by regulating tryptophan metabolism. The degradation of tryptophan, a precursor of 5-HT, is mainly through the kynurenine pathway which regulates over 95% of tryptophan in the peripheral system and is functionally mediated by gut microbiota and probiotics [147, 292]. In the present study, the tryptophan level was not significantly affected by dietary probiotic (P = 0.35), but the initial levels of tryptophan in dominant hens were approximately 28% higher than the subdominant hens (P = 0.21) (**Figure 3**). The pattern of changes in blood concentrations of tryptophan in probiotic fed dominant hens was correlated with the changes of peripheral concentrations of 5-HT, indicating that probiotic may directly or indirectly regulate 5-HT synthesis in the peripheral system.

In this study, behavioral changes in dominant hens were correlated with the changes of blood 5-HT concentrations following fed probiotic. In the probiotic fed dominant hens, the levels of threat kick were reduced (**Figure 4a**, P = 0.04), and the frequency of aggressive pecking tended to be lower (**Figure 4b**, P = 0.053). The levels of feather pecking in dominant hens were reduced compared to their initial levels at day 0 but without statistic significant (**Figure 4c**, 58%, P > 0.05). There was no change in injurious behaviors in the regular diet fed subordinate hens between day 0 and day 14 (**Figure 4a–d**). The same or similar cellular mechanisms proposed in humans and rodents may be applied to the changes in the probiotic fed dominant hens. In humans and rodents, probiotics directly manipulate commensal bacteria releasing neuroactive factors, such as 5-HT and norepinephrine [8], and indirectly affect the neurotransmitter metabolisms, such as tryptophan as well as cytokines, through the microbiota-gut-brain axis [5, 40, 41, 279, 293–297]. In addition, the effects of probiotics, including *B. sublitis*, on behavioral exhibitions have been conducted on GF mice. Bercik et al. [287] reported that anxiety behaviors can be induced in less anxious phenotypic mice by colonization of the gut bacteria from anxiety-like phenotypic mice (FMT, fecal microbiota transplant). Probiotics also have therapeutic effects on neurodevelopmental disorders [28, 95, 298–301], for example, reduced anxiety-like behaviors by providing *L. helveticus*, *Mycobacterium vaccae*, and/or *Bifidobacterium* strains [231, 287, 302, 303], and alleviated autism-related stereotypic behaviors by treating with *Bacteroides fragilis* [304] and behavioral dysfunction with *Lactobacillus reuteri* [301, 305].
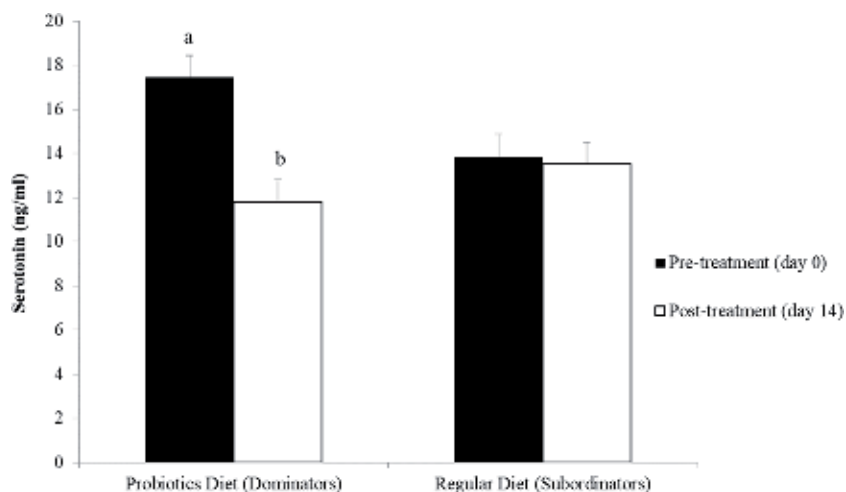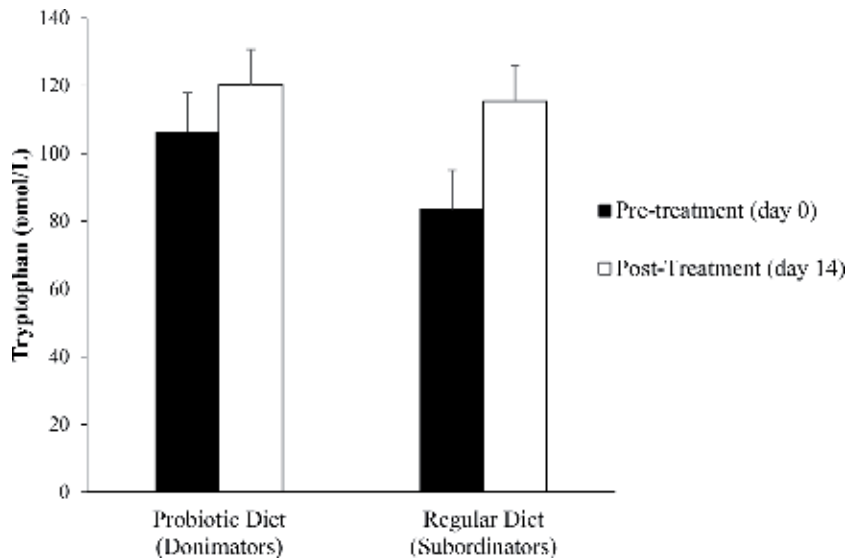
Figure 3.
Plasma tryptophan levels at day 0 (pre-treatment) and day 14 (post-treatment) in probiotic fed hens and regular diet fed hens. Prior to treatment, dominant hens had higher tryptophan concentrations compared to subordinates but the difference did not reach statistical difference (P = 0.21). There were no treatment effects on tryptophan concentrations in both probiotic fed hens and regular diet fed hens (P > 0.05, respectively).
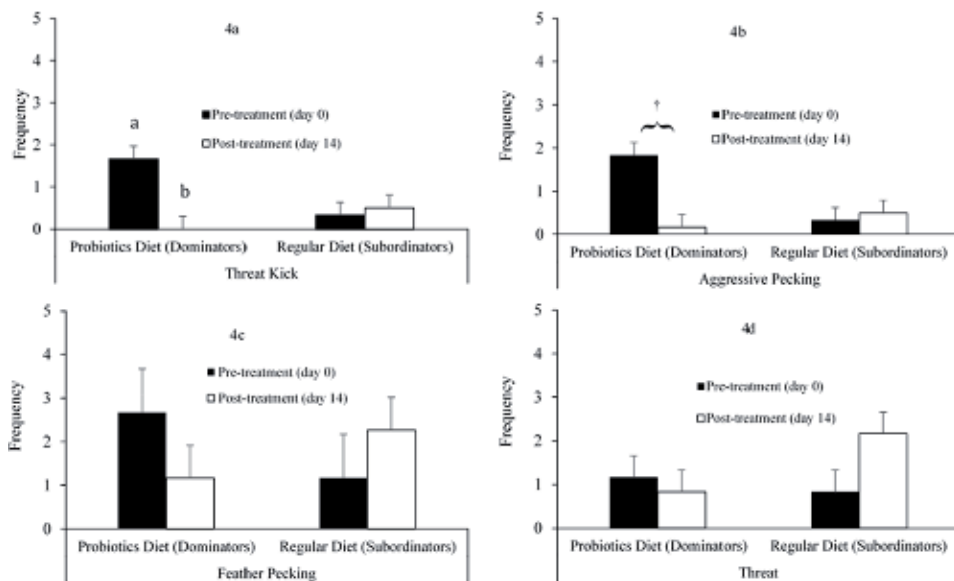
Figure 4.
Frequency of aggressive behaviors at day 0 (pre-treatment) and day 14 (post-treatment) in probiotic fed hens and regular diet fed hens followed the paired social test. The exhibitions of aggressive behaviors in the regular diet fed subordinates were not affected by treatment (P > 0.05, respectively), while the frequency of threat kick (P = 0.04) was reduced, aggressive pecking (P = 0.053) was tend to be lower, and feather packing was declined (60%, P = 0.33) in probiotic fed dominates post-treatment. Notes: the treatment effects on the measured behaviors were reversed between dominants and subordinates during the 2nd social rank test. [a,b] between the frequency at day 0 and day 14, least square means lacking common superscripts differ (P < 0.05); and [†] a trend difference (0.05 ≤ P < 0.10).

In the current study, body weight gain and hen-day egg production were not affected in the probiotic fed hens compared with the regular diet fed control hens (Control = 2.83%, Probiotics = 2.2%, *P* = 0.76; Control = 73.6%, Probiotics = 87.5%,

P = 0.18, respectively). Previous studies have reported the beneficial effects of dietary supplementation of probiotics on daily weight gain, finished body weight, and feed conversion rate in broiler chickens [306, 307], turkeys [308], and swine [309]. Several studies also reported that probiotic diets improved egg production in hens [310]. The underlying mechanisms of these effects may be related to the beneficial bacterial growth in the gastrointestinal tract to facilitate the fermentation process which improves the digestion and utilization of nutrients in animals [311]. The beneficial effects on growth performance, however, are affected by the bacterial strains, preparation process, dosage, animal's age, and genetic type [312, 313]. In the current study, the probiotic was provided for 2 weeks only, which may not be sufficient to functionally improve both growth and production performance.

6. Conclusions

Our data suggest that dietary inclusion of probiotics has positive effects on reducing agonistic behaviors in laying hens through modification of the serotonergic system without negative effects on growth and production performance. The data indicate that dietary probiotic supplementation could be a useful management tool for preventing aggressive behaviors in laying hens. In addition, the current chicken strain could be a useful model to investigate mechanisms underlying the potentially probiotic therapy for preventing and reducing emotional susceptibility associated with psychiatric disorders such as depression and anxiety in humans.

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Oral Health by Using Probiotic Products

Razzagh Mahmoudi, Sara Moosazad and Katayoon Aghaei

Abstract

One of the most prevalent and important health problems in the world is periodontal and plaque-related diseases that antibiotic drugs are used with their side effects as their treatment. With increasing resistance to antibiotics and a desire from the general public for "natural" therapies, there is a need to minimize antibiotic use and develop new treatments for oral diseases without antimicrobial agents. Probiotics are viable microorganisms which provide a health benefit to the host when administered in adequate amounts; studies show that probiotics have the potential to modify the oral microbiota and decrease the colony-forming unit (CFU) counts of the oral pathogens being investigated to prevent or treat oral disease, such as dental caries and the periodontal diseases. In addition, the identification of specific strains with probiotic activity is required for any oral infectious disease, in order to determine the exact dose, the time of treatment, and the ideal vehicle.

Keywords: probiotics, periodontal diseases, dental caries, oral health

1. Introduction

As we know tooth decay and oral health is one of the most common worries in the world [1], and with widespread public concern about the use of industrial drugs to reduce dental caries, natural base treatments are highly welcomed, but the question is: Can probiotics be used as a therapeutic agent? A few years before 1908, the theory is drawn up according to this theory; the Bulgarian people have a longer life; the reason of longer life was Bulgarian people used more fermented products. These products have increased the health of their digestive system [2]. This theory won the Nobel Prize in Physiology and Medicine (1908) [3]. Since then, many publications that have referred to this theory said using probiotic products can be factor in improving the health of the digestive system, reproductive organs, and oral hygiene [4, 5]. Oral holes are environments with homeostasis conditions, which affect the nutritional conditions and individual health conditions and conditions of the environment [3, 4, 6]. Probiotics, as nonpathogenic microorganisms, can have beneficial health benefits at low levels in food. They have the ability to change this homeostasis environment [3, 7]. Probiotic bacteria consist of two main groups of *Lactobacillus* and *Bifidobacterium*. The *Lactobacillus*, as a member of oral microbial flora, can play an important role in the microcosm balance of the oral cavity. Less information is available about the useful role of *Bifidobacterium* in oral cavity health [8, 9].

2. Oral health

The mouth has a varied and heterogeneous microbial community. Oral health can be a state of being free from chronic mouth and tooth pain, such as oral and throat cancer, tooth loss, periodontal disease, and the other diseases that affect this tissue. The etiology of periodontal diseases and caries shows the prevalence of these both having microbial components. Host microbiota has important role in the individual's health. The theory from Nobel laureate Ilya Metchnikoff says "we fight microbe with microbe" and has attracted numerous followers [10].

3. Probiotics background

As we know, the presence of microorganisms in the body always had been a concern of a large group of scientists. Because the health of the oral and intestinal organs is depends on the balance of these microorganisms and their functioning [10]. For many years, scientists and doctors have been using antibiotics to improve the function of the oral cavity, lymph system, and intestinal organs and control the demographic of these microorganisms. However, the widespread use of antibiotics not only caused a lot of problems in the human body but also led to microorganisms exhibiting resistance and even in some cases caused new species in microorganisms. All these problems forced scientists and researchers to find new methods for controlling microorganisms in the body organs.

4. Probiotic bacteria

As it is mentioned in the introduction, probiotic bacteria consist of two main groups of *Lactobacillus* and *Bifidobacterium*. The best growth environments for these two groups are anaerobic or minimal oxygen conditions, for example, in saliva, vagina, vegetable juices, and dairy products. Probiotic bacteria were used in a variety of ways over time. For example, and prevent food corruption, Today, these bacteria are also used in industrial food products such as milk, wine and vegetables [11]. The name probiotic comes from the Greek word *probosis*, which means for life. This word was first used in 1965 by Lily and Stool to describe the secreted material by microorganisms to stimulate the growth of other microorganisms. In 1974, Parker introduced the word to describe microorganisms controlling the intestinal microbial population [12–14]. Today, probiotic bacteria refer to viable microorganisms that control the microbial population of the gut. They have lots of positive effects on the health of the host such as reduced constipation, decreased blood cholesterol, and improved lactose tolerance and calcium intake [14].

5. Probiotic foods are functional foods

Diets play an important role in health. These diets can play a leading role in reducing disease. Today, with increasing awareness of people about the beneficial effects of probiotic bacteria, consumption of functional foods is increasing. But what are these foods? Functional foods are such food that promotes health [14]. A wide range of foods are included, but the most important ones are oligosaccharides and probiotic foods [15].

What are the main criteria for selecting probiotic food as functional? Functional food must have three items:

1. They have a different effect from a nutritional point.

2. They reduce the risk of pathological illnesses.

3. Consuming them benefits the community and the consumer [15].

Probiotic foods not only have the main characteristics that are mentioned but also have more benefits. These features include:

A. Useful effects on the host

B. High shelf life in the product

C. Ability to survive the intestines

D. Ability to produce antimicrobial agents

E. Stabilization of intestinal flora

F. Nonpathogenicity and non-toxicity [16]

6. Which foods have the best tissue for the transfer of probiotic bacteria to the human digestive system?

New food products are converted into probiotic foods by adding probiotic bacteria. These foods include a variety of different types such as cheese, ice cream, milk-based dessert, baby milk, and mayonnaise. In making these foods, the main thing is the texture of these foods. It can be said that among all foods, dairy products are known as the best option for the transmission of probiotic bacteria [17–20].

7. What are the main mechanism probiotics in the body?

Probiotics can function through several mechanisms. Probiotic bacteria through colonization in the intestinal environment, connect to each other, using the food in the body before it is taken by pathogenic microorganisms so by creating competition, it eliminates pathogens. Also, probiotics, unlike many harmful bacteria, are capable of producing acid to survive in the regulation of the local and systemic immune system [21].

8. Probiotics and dental caries

Dental caries is one of the most common oral diseases, even though it is preventable. There are different reasons for dental caries such as such as maternal characteristics, environment, child's individual factors and epigenetics, and sugar and sugar-rich diet [10]. A usual feature for caries-promoting bacteria is that they are acidogenic and aciduric. One of the most caries-promoting bacteria is *Streptococcus mutans*. For this reason lactobacilli and bifidobacteria are excellent acid producers, since we can use probiotics several times a day and they are safe for infants [22].

9. Probiotics and periodontal disease

Periodontal diseases include gingivitis and periodontitis. They are manifested by bleeding on probing, swelling, color alterations, pain, and tooth mobility in

advanced stages. Scaling and root planning and deep pocket debridement are usual treatments [10]. Periodontal pathogens could be regulated by means of antagonistic interactions. According to the results of studies probiotics have been shown to reduce the number of the most frequently isolated pathogens such as *Bacteroides* sp., *Actinomyces* sp., *Staphylococcus intermedius*, and *Candida albicans* at optimal concentrations of 10^8 cfu/ml [23].

10. Probiotics and oral candida infections

Another role of a probiotic is to restrain the chronic candida infections of the soft tissues in the mouth. Those that are isolated from oral cavity include *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*. Many in vitro studies have shown that the strains of lactobacilli have inhibitory activity against oral candida, but the true value of probiotics in controlling oral candida is still an open question [10].

11. Probiotics and halitosis

One of the most common problems that people in the world suffer from is halitosis. This phrase is used in 1921 to describe the unpleasant odor of the mouth [23]. This complication has many causes such as bacterial coating of the tongue, systemic disorders, and different types of food [24]. At all levels, all physical and chemical treatments alone have not been able to completely control this pathology. All physical and chemical treatments alone have not been able to fully control this problem. Today, scientists believe that the use of probiotics as supportive therapies will play a role in controlling this problem. But how can these bacteria help?

1. Probiotics can act as antibiotics and inhibit gastrointestinal diseases, which can reduce the odor associated with these diseases.

2. Probiotics can reduce bacteria in terms of immunity.

These two methods are the main methods for reducing odor in the mouth by probiotics [25].

12. Mechanism of probiotics in the oral cavity

The ability to adhere to and colonize surfaces in the oral cavity is an essential requirement for an oral probiotic [9]. There are four main mechanisms for the effect of probiotic bacteria in the mouth. Probiotics compete with pathogens to stick to the mucosa and tooth and prevent the sticking of pathogenic bacteria [26]. Probiotic produce peroxide and bacteriocin as antibacterial agents against oral pathogens, so they remove from oral area [27]. The presence of probiotics can change the oral conditions such as reducing pH that can prevent the growth of bacteria producing tooth decay or change the protein structure of salivary gland [28]. Probiotics can have beneficial effects on dental health by stimulating non-specific immunity and regulating cellular and humoral immune responses [29].

13. Probiotic strain in oral cavity

According to the researches and their results, *Lactobacilli* and *Bifidobacterium* could play an important role in the microecological balance in the oral cavity. *B. bifidum*, *B. longum*, and *B. infantis* are the probiotic species of *Bifidobacterium*. *Bifidobacterium* is seen in deep caries in the oral cavity and plays an important role in the development of caries, but it has been shown that its probiotic species can reduce caries in people who do not have active caries. Acidophilus are the probiotic species of *Lactobacillus*. According to the researches, *Lactobacillus* is more associated with decayed dentin. It is at the point of development of the decayed lesion and is at the onset of the process that caries do not have a role. Consumption of products of probiotics does not predict the cause of decay growth. Firstly, the *Lactobacillus* rarely causes cavity formation. Secondly, all *Lactobacillus* species do not induce caries, and thirdly when carriers of dairy *Lactobacillus* are present, they can have a neutralizing effect on acid production by bacteria [30].

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Chapter 5

Metabolite Multiprobiotic Formulas for Microbial Health

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Abstract

On example of *Lactobacillus* and *Bifidobacterium* strains, approaches in creation of human multistrain probiotic metabolite mixtures for different goals were proposed. Human probiotic lectin systems (LS) (mucosal, others) reveal functions needed for organism. Advanced features of such systems include capability to recognize synthetic polymeric polyvalent glycoconjugates (GC)—imitators of natural ones (modified polysaccharides, glycoantigens of medical significance). Probiotic lectin systems function as imitators of multipro-/sym-/synbiotics in their resulting actions. They serve as carriers of the biotope glycoconjugate décor including glycoprebiotics, glycometabiotics, glycodrugs, and agents supporting décor organization and resistance. Probiotic lectin systems represent new perspective system agents to improve the health of mucosal microbiocenoses (MB) organized as communicative bodies to be corrected according to the principle "there is body—there are diseases." They act as metabolomebiotics according to the principle "the network in the network." They deepen biotope resistance allowing quicker return to balance. They support prophylactic and therapeutic procedures directed to prolong aging and improve quality of life. Multistrain metabolite constructions can predict perspective cellular formulas of multipro-/synbiotics for prophylaxis, supporting and accompanying therapy. Approaches developed are universal. They are useful in the study of any Gram-positive and eukaryotic (yeasts and yeast-like fungi) mono- and mixed cultures. The methodological principles proposed and described are of value for extended fields of clinical microbiology and medical bio-/nanotechnology.

Keywords: multistrain probiotics, probiotic lectins, glycoconjugates, microbiocenoses, antimicrobials

1. Introduction

Pro-/sym-/synbiotics are important objects of human microecology and medical biotechnology [1–4]. Microbes and human communicate each other by the way of recognition and binding glycoconjugates (GC) of varying pattern complexity by proteins (mainly adhesions and lectins) [5–9]. Lectin systems (LS) of symbiotic/probiotic microorganisms (LSSM) recognizing GC represent new multifunctional factors [8–12]. LSSM are relatedly useful for human protein-/peptide-containing compounds and their complexes recognizing GC. LSSM reveal features of imitators of probiotics; members of new class of bacteriocin-like destructors of biofilms of yeast-like fungal and Gram-positive pathogens; systems cofunctioning together

with enzymes of all known classes; and agents possessing antipathogenic synergism of different LS in antimicrobial combinations (between LS of *Lactobacillus* species and *Bifidobacterium* species, between genera *Lactobacillus* and *Bifidobacterium*, between LS of probiotic bacteria and lectins from medical plants, between LSSM and antibiotics) (see **Table 2**) [8, 9, 13, 14].

LS reveal significantly higher multifunctionality (antimicrobial, cytokine-like, others) and adaptive ability in surroundings in comparison to any component of LS. Applied prospects of LSSM in microbial associations of biotopes in the human body are of promised interest. LSSM and their reactive GC support balanced functioning in organism in respect to evolutionary created mucosal organ-like infrastructures of mutual interest for human and biotope microbiocenoses (MB) [12].

The purpose of the review is to evaluate our approaches in creation of probiotic metabolite compositions influencing and improving health of human biotope microbiocenoses. The data presented will be of interest for investigators in the fields of both medical microbiology and laboratory and industrial medical biotechnology.

2. Methods

Lactobacillus and *Bifidobacterium* strains were from the collection of microorganisms of the normal microflora of *G. N. Gabrichevsky* Institute for Epidemiology and Microbiology, and probiotics *Bifidin* and *Acilact* were products of our institute. Bacteria were grown in media containing casein hydrolysate and yeast autolysate. LSSM were extracted from the protein fractions 27–220 kD using isoelectrofocusing (IEF) in a plate of polyacrylamide gel (PAG) in a gradient of pH 4–8. Identification of proteins was performed by electroblotting on a hydrophobic membrane and staining with SYPRO blot stain (Bio-Rad Lab.). Nonstained proteins were evaluated by other spectrophotometric methods. The distribution of LSSM among proteins was determined by treatment of blots with biotinylated GC (GC-b) containing multiple residues of sugar(s) linked to the polyacrylamide (PA) linear core (like in external phenotypes of mucins and mucin-type glycans) (www.lectinity.com;

1.	α/β-D-GalNAc-PA-b (animal mucins, T antigens)*
2.	β-D-GalNAc-PA-b (animal mucins)
3.	β-D-GlcNAc-PA-b (insect chitins and chitosans)
4.	β-D-Gal-PA-b (plant or animal galactans)
5.	β-D-Gal-3-sulfate-PA-b (acidic animal galactans)
6.	α-D-Man-PA-b (yeast mannans)
7.	α-D-Man-6P-PA-b (yeast phosphomannans)
8.	α-L-Fuc-PA-b (fucans from brown algae)
9.	α-L-Rha-PA-b (Gram-negative rhamnans)
10.	MDP-PA-b (bacterial peptodoglycans)
11.	Adi-PA-b (A-blood group substance GalNAcβ1-3GalβA1-)
12.	Fs-PA-b (Forssman antigen GalNAcβ1-3GalNAcβA1-)
13.	Tαα-PA-b (bacterial antigen Galα1-3GalNAcα1-)

In brackets—natural substances which are imitated

Table 1.
A list of synthetic GC used.

Table 1) followed by final treatment with streptavidin-peroxidase conjugate. The bound peroxidase on the blot was registered in the presence of a chemiluminescent substrate in regime of a real time in the system BioChemi System (UVP, CA). Antimicrobial activities and synergism of LSSM, antibiotics, and phytolectins were tested on solid agar media during the prolonged growth and survival of communicative fungal bodies (CFB) in the presence of disks of antimicrobials. Biosurfactants were tested and calculated using detection of sample drop activity against mineral oil film on water surface (the appearance of transparent circles). Amino acid compositions of samples were established using standard amino acid analyzer column chromatography. Oxidoreductase systems were detected on blots after IEF-PAG, resulting in kinetic protein stain disappearance (decolorization). Hydrolase systems were visually evaluated on blots after IEF-PAG (resulting in hydrolysis, splitting, and partial asymmetrical disappearance of protein bands). *Maillard* reaction products were partially evaluated as browning in culture supernatant according to optical density at 420 nm.

3. Results and discussion

3.1 Symbiotic lectins as system regulators and delivery agents

LSSM function as metabolomebiotics regulating metabolome according to the principle "LSSM network—whole organism metabolome network or interactome" [15]. The network of LSSM is created in the following manner: lectin molecule of determined molecular weight (in the *Laemmli* system) is represented by LS including forms of varying charge and possessing a range of biological and physiological activities; such a minimal LS can be further transformed in a natural manner into extended network of complexes and supramolecular ensembles as a result of directional and sequential cascade binding of carbohydrates and GC. As a result of forming complexes and ensembles, lectin specificity of complexes and ensembles can be modified or changed during further development of recognition cascade network that will change the summary vector of specificity of LS. The latter will result in dynamic qualitative and quantitative changes of the local biotope surrounding. Thus, the final whole resulting network of LSSM (as metabolomebiotics) will regulate the whole metabolome and interactome of organism involving human glycome (carbohydrates and GC: glycoproteins, glycoenzymes, glycolipids, receptors, and others [16]). The metabolome possesses the ability to back direct and reversible effects of LSSM representing a part of hierarchic interactome. Multiple forms of adapted functioning LSSM microbiocenosis in the biotope will depend on the originality developed in a joint process of evolution involving host body local infrastructures for the distribution and disposition of microbiocenoses (organ-type constructions of both host and microbiocenosis interests are possible) [11, 12]. LSSM are ready to realize biologically active GC (as prebiotics, therapeutic agents, and metabiotics) in such symbiotic organs. The network of LSSM functions as noncellular simulators of symbiotics (probiotics) in the direct or indirect (through human higher hierarchic protection systems) predictable manners. For example, of communications between LSSM and own human protection systems, LSSM (as well as phytohemagglutinins from plants of medical significance) and artificial polymeric GC influenced peritoneal macrophage mobility in a coupled manner depending on doses of agents; LSSM were involved in modulation of cytokine production by human blood leukocytes [9, 17].

New useful properties of LSSM can be predicted and verified (cofunctioning to enzymes, adhesion, etc.), based on the fact that LSSM form a functional

superfamily of symbiotic lectins (on example of probiotic lectins and lectins of nitrogen-fixing bacteria). In addition, LSSM are members of the new class of destructors of biofilms of yeast-like and Gram-positive pathogens that simplify antimicrobial choice of components among LSSM. Other possibilities to operate with LSSM include their potential participation in a set of hierarchic pathways of advanced human innate protective systems in biotopes for *cross talks*. Both types of communications allow LSSM to be a successful synergistic assistant against pathogens in biotopes together with other antimicrobials and antimicrobial physical stress factors. As a result, LSSM reveal prolonged (early and late) anti-*Candida* activities as cascade (coupled) actions possibly influencing microecological niches of pathogens within biotope [registered during coculturing in the first 3 days (early events; also involving probiotic-like leader strains of *L. acidophilus* and *L. casei*), 1–2 weeks, or 2–3 months (late events)] [18].

The used GC are characterized with known chemical structures simplifying interpretation and prognostics of results. Such GC reveal potential of metabiotics which may use LSSM as carriers [8, 12, 19]. As highly homogenous, synthetic GC better functionally imitate natural GC targets [bacterial proteoglycans, fungal (phosphor)mannans, others] important for antimicrobial/anti-infectious actions of LSSM. As a result, LSSM are very perspective in delivery and deposition of adequate specific GC which are locally releasing as therapeutics possessing anti-infectious, prebiotic, and/or communicative signal abilities and actions.

In biotopes LSSM participate in continuous (on-duty) support and periodical (biorhythmic) completion and exchange of normal GC décor of cells, tissues, and organs that must provide delaying or stopping further the development of the spot/island/mosaic-landscape-originated and directed/metastasized abnormal processes as well as conserve any negative currently developmental events (initiation of appearance and survival of tumor-like cells as a result of innate intercellular communications involving lectin receptor-coupled cell surface receptor mosaics).

LSSM (natural combinative sets of LSSM-GC complexes) influence all yeast-like fungal phases of life by prolonging the degradation and lysis of pathogenic microbiocenosis massifs or biofilms in human biotopes (on examples of *Candida* species). In respect to CFB functioning as niches, lectins of lactobacilli (LL as preferentially mucins/mucus-recognizing) and lectins of bifidobacteria (BL as preferentially mannan-recognizing) synergistically act against internal and peripheral subniche territories, respectively. The late destructive and lytic events in CFB may also take place due to LSSM cooperative complex action as metabolomebiotics together with hydrolases involved in pathogen destruction network. Synergistic actions between LSSM and other antifungals increase resulting in final (early or late [also of apoptotic origin]) antipathogenic events as shown in **Table 2** [20].

Endogenous LS antimicrobial actions (e.g., intestinal probiotic bifidobacterial and lactobacillar LS against intestinal yeast-like fungi) provide more directed, sensitive, and completed resulting effectiveness against pathogens (the primary absence and further late complete destruction of residual resistant colonies in external and internal regions of CFB of *Candida albicans*) compared to the action of phytolectin mixture from grasses of medical significance in the same conditions. In cases of antibiotic-resistant strains, relatively sensitive to LSSM *C. albicans* strain 547 (less potentially pathogenic compared to the strain 515, see below), the sorbed lactobacillar cationic LS revealed their synergistic ability to "regenerate" original anti-*Candida* effectiveness of antibiotic (on example of nystatin) in the internal region of CFB of yeast-like fungus (appearance of pathogen-free landscape connected to the border of the disk antibiotic). The phenomenon of synergistic reparation of the original ability of the sorbed antibiotic indicates prospects of additional mechanisms of LSSM combinative actions and partially

describes how to increase resulting antifungal effectiveness during prolonged contact to pathogenic CFB at fungal late steps. In the case of more resistant (potentially "more pathogenic") fungal strains (like *C. albicans* strain 515), the sorbed

Types of synergism	Lectins[*]	Distant positions (directions from left to right)	CBF as targets, affinity, and significance
Between PL			
Between identical PL disks	aBL and aBL	p-aBL, c-aBL (p-aBL—c-aBL)	*C. albicans* 991
Intra-genus (*Lactobacillus*)	cLL and aLL aLL and cLL	From p-cLL to CR (p-cLL—p-aLL) From p-aLL to CR (p-aLL—p-cLL)	*C. albicans* 515, 547 *C. albicans* 515, 547
Intra-genus (*Bifidobacterium*)	aLL and aBL	p-aLL and c-aBL (p-aLL—c-aBL)	*C. albicans* strains
Intra-genus (*Bifidobacterium*)	aBL and cBL	[PR aBL] and [CR aLL]	*C. albicans* strains
Inter-genera (*Lactobacillus* and *Bifidobacterium*)	aBL and aLL aLL and aBL	[PR aBL] and [CR aLL] (p-aBL—p-aLL) [PR aLL] and [CR aBL]$_2$[*] (p-aLL—p-aBL)	*C. albicans* strains *S. aureus* strains
Between PL and phytolectins			
Between PL and grass lectins (GL)	aLL and GL cBL and GL	From aLL to CR (p-MGBL—p-aLL) From p-cBL to CR (p-MGBL—p-cBL)	*C. albicans* 515 *C. albicans* 515
Between PL and antimycotics			
Between PL and amphotericin B	aBL	p-Amphotericin B, c-aBL (p-Amphotericin B—c-aBL)	*C. albicans* strains > *C. tropicalis* strains
Between PL and itraconazole	aBL	c-Itraconazole, p-aBL (p-Itraconazole—p-aBL)	*C. albicans* strains
Between PL and ketoconazole	aBL	p-Ketoconazole, c-aBL (p-Ketoconazole—c-aBL)	*C. albicans* strains
Between PL and nystatin	cLL cBL	From p-cLL to CR (p-cLL—c-nystatin) From cBL to CR (p-cBL—c-Nystatin)	*C. albicans* 515, 547 *C. albicans* 515, 547
Between GL and nystatin	GL	From p-MGBL to CR (p-MGBL—c-nystatin)	*C. albicans* 515
Multiple synergism			
Between BL and LL	aBL, aLL	In triangle of CFB landscape: p-aBL + c-aBL + p-aLL (p-aBL—c-aBL; p-aLL—c-aBL)	*C. albicans* strains (Not for *C. tropicalis*)

[*]*Diffusion between disks placed on Sabouraud agar in Petri dishes (disk positions: p, peripheral; c, central; CR, central region, between p-disk and the center; PR, peripheral region, between p-disk and the border of agar). Disks included anionic (a) and cationic and (c) lactobacillar and bifidobacterial LSSM (aLL, cLL, aBL, cBL). MGBL as the mixture of the grass lectins from the mill of plants of medical significance: Potentilla album and Stellaria media. Lectins were used in subhemagglutinating doses (less 1 microgram/ml). Standard panel of disk antimycotics (HiMedia Lab. Pvt. Ltd.) was used. CFB, communicative fungal bodies.*

Table 2.
Antimicrobial synergism of LSSM and antibiotics.

bifidobacterial cationic LS synergistically increased anti-*Candida* action of the grass phytolectin mixture.

Results indicate that LSSM may also participate in temporary separation and conservation of natural infectious biofilms to prevent early visual landscape development of diseases. The latter may be perspective as the assistant factor of improving quality of life (its prolongation, mucus and skin reparations, cosmetic significance, etc.) as it can be expected using symbiotics [21].

On the whole, our experimental approach (observations of antagonistic relationships between LSSM and pathogens in prolonged stress conditions) and the data obtained are supported by the conception describing microecological stress events in organism as the normal but sensor natural reactions [22].

3.2 The choice of strains and their consortia possessing potential for constructing new multistrain pro- and symbiotics

Screening, choice, and selection of new or improved antimicrobial and other useful properties of symbiotic (probiotic) cultural properties of strains and consortia of human MB are important and strategic goals in prophylaxis and therapy of diseases, increasing resistance of organism and the acceleration of patient rehabilitation [1–4, 23]. Among GC investigated, glycans of mucin type (mucosal glycans) reveal special interest [24].

On the basis of own results, we proposed new algorithm of screening adequate probiotic-like microorganisms and their consortia possessing increased directed anti-infectious LS to construct new multipro-/sym-/synbiotics. The algorithm using LSSM (involving complexes to GC) among a panel of key factors in creating multiprobiotics (MP) included (a) the choice of synthetic GC-imitating bacterial proteoglycans and (phospho)mannans of yeast-like fungi; (b) identification of different LSSM (GC-type-dependent) among proteins of cultural fluids of probiotic strain or consortium of strains; (c) comparison of (GC-type)-dependent LS (evaluation of summary LS intensity, length of LS distribution in pH-gradient tested, mosaic asymmetric configuration of distributed forms in LS, major forms as dominating in contribution to antimicrobial actions of LS, minor forms as expressing signal regulators of biorecognition in microbiocenoses, signals of communications to surrounding infrastructures, as well as additional participants of recognition of new types of biomarker GC); (d) identification of unique sets of components of LS significant for typing strains, species, or genera; (e) revealing and choice of combinative sets of potential antimicrobial forms of LS for further control and testing; and (f) control of antimicrobial activities initiated, supported, and/or influenced by LS-containing preparations in other (nondependent) methods.

The data which were useful for constructing any multiprobiotics (*Acilact*-like) is presented in **Table 3**.

In **Table 3** the data needed for constructing formulas of any MP are presented on example of strain component compositions of *Acilact* (the well-known MP which served as a model). Algorithm for constructing formulas includes a few steps:

The first step: For formulas of any MP of category A (formulas as sum of wishful selected superiorities): the choice of all parameters of superiorities of MP (No. 4 as MP in code notes: positions 1.3, 1.4, 1.6, 2.7, 2.8, 2.13, 2.14, 2.15, and 4.3; major ingredient strain contributors are at the second position in the code sequences [from left to right] position in code).

The second step: For formulas of any MP of category B: accounting additional superiorities of MP [No. 4 as MP in code notes; selected minimal positions of parameters for No. 4 in sequence indicate maximal expression of the contrary (by implicity) parameters]. For example, in the case of No. 4 in codes 1.1

No.	Parameters of supernatants, ranging MP and its strains, proposals (P)	Code ranging MP and its strains[*]
1	Status of proteins	
1.0	Content of partially hydrolyzed protein K3III24 > MP > 100 ash > NK1 P: K3III24 as the main source of active or signal oligopeptides	3. 4. 2. 1.
1.1	Acidic proteins pI 4–5 NK1 > 100 ash > K3III24 > MP P: NK1 as the main source of basis cytoagglutinating and adhesive agents coupled to a spectrum of biological activities	1. 2. 3. 4.
1.2	Cationic proteins pI 7–8 NK1 > K3III24 > 100 ash > MP P: Strains and their combinations as sources of lectins and lectin-like agents coupled to exopolymeric substances of compounds	1. 3. 2. 4.
1.3	Oxidase-reductase systems pI 5–5.5 MP > 100 ash > K3III24 > NK1[absence] P: NK1 without extracellular major oxidoreductase systems	4. 2. 3. 1.
1.4	Hydrolase systems MP > K3III24 > 100 ash > NK1 P: NK1 without extracellular major hydrolases (caseinases, peptidases, others)	4. 3. 2. 1.
1.5	Level of aggregation upon storing concentrates K3III24 > 100 ash > NK1 > MP[no aggregation] P: Irreversibility for K3III24 (similar to red cell biofilm storing)	3. 2. 1. 4.
1.6	Ability in membrane ultrafiltration MP > NK1 > 100 ash > K3III24 P: Technological advantage of MP as mixture of strains (mixture of strains is needed)	4. 1. 2. 3.
2	Status of amino acids	
2.0	Production of amino acids NK1 > K3III24 > MP > 100 ash P: Low antagonism between certain strains within MP can be under consideration upon strain choice	1. 3. 4. 2.
2.1	Tyr (sites for serine proteinases, fluorophores) K3III24 > 100 ash > MP > NK1 P: Tyr can serve as criterion of utilization of fluorophores	3. 2. 4. 1.
2.2	Phe (sites for serine proteinases, fluorophores) NK1 > MP > K3III24 > 100 ash P: Support of point 2, revealing mechanism for point 2	1. 4. 3. 2.
2.3	Fluorophores (Trp, Tyr) (excitation at 254 nm) 100 ash > K3III24 > NK1 > MP P: Dominated contribution of Tyr and their derivatives	2. 3. 1. 4.
2.3.1	Fluorophores (excitation at 365 nm) 100 ash > K3III24 > NK1 > > MP P: Degradation/ utilization in MP (fluorophores cannot be used as targets)	2 3 1 4
2.4	Gly (hydrophobic, anti-adhesion action) NK1 > MP > K3III24 > 100 ash P: Gly as natural additive to improve signal activities and communications	1. 4. 3. 2.
2.5	Leu (hydrophobic, site for peptidases) (Parameter is slightly dependent on strain origin) K3III24 > 100 ash > MP, NK1	3. 2. ¼[**]. 4/1.
2.6	Ile (hydrophobic, participation in synthesis of biologically active volatile fatty acids) K3III24 > 100 ash > MP > NK1	3. 2. 4. 1.

No.	Parameters of supernatants, ranging MP and its strains, proposals (P)	Code ranging MP and its strains[*]
2.7	Ala (partially from peptidoglycans, site for exopeptidases) MP > K3III24, 100 ash > NK1 P: Antagonism between strains results in partial cell wall degradation in MP	4. 2/3. 3/2. 1.
2.8	Ser (sites for hydrolase splitting and O-glycosylation) MP > K3III24 > 100 ash > NK1 P: Support of point 1.4	4. 3. 2. 1.
2.9	Thr (site for O-glycosylation) NK1 > 100 ash > MP > K3III24 P: Thr as criterion for evaluation of metabolism of cluster square positions for recognition in proteins (in comparison to point 2.8)	1. 2. 4. 3.
2.10	Lys (from cationic poly/oligopeptides, site for serine proteinases, participation in *Maillard* reaction) MP > 100 ash > K3III24 > NK1 P: Support of point 1.4	4. 2. 3. 1.
2.11	Val (hydrophobic, participation in synthesis of biologically active volatile fatty acids) K3III24 > 100 ash > NK1 > MP P: Mostly important criterion concerning volatile fatty acids producing	3. 2. 1. 4.
2.12	Glu/Gln (also as sites for amidases) NK1 > MP, K3III24 > 100 ash	1. 4. 3. 2.
2.13	Asp/Asn (sites for amidases and N-glycosylation) MP, 100 ash > NK1 > K3III24 P: Partial support of point 1.4	4. 2. 1. 3.
2.14	His (participation in auto-oxidation of protein, high affinity to metal cations, activation of oxidases and heme) MP > NK1 > 100 ash > K3III24 P: Potential for metal chelate affinity chromatography and immobilization (for microassays)	4. 1. 2. 3.
2.15	Arg (from cationic poly[oligo]peptides, destruction during pigments forming in *Maillard* reaction) MP > NK1 > K3III24 > 100 ash P: One of the way of decolorization of MP	4. 1. 3. 2.
2.16	Met (antioxidant) NK1 > MP > K3III24 > 100 ash	1. 4. 3. 2.
2.17	Cys2 disulfide bonds (oxidation of SH-groups into Cys2) K3III24 > NK1 > MP > 100 ash[not observed]	3. 1. 4. 2.
2.18	Pro (Pro-bends in regular structures of proteins) 100 ash > MP > K3III24 > NK1[traces]	2. 4. 3. 1.
3	Status of biosurfactants	
3.0	Associated biosurfactants in complexes >27 kD NK1 > MP > > 100 ash > K3III24 P: NK1 as the main source of movable permeable (detergent like) complex effectors; support point 4.3	1. 4. 2. 3.
3.1	Biosurfactants active against mineral oil K3III24 > MP > 100 ash > NK1	3. 4. 2. 1.
4	Other parameters	
4.1	Emulsifiers 100 ash > NK1 > MP > > K3III24 [absence] P: K3III24 needs combination to any other strain to increase cultural components (>27 kD) to be emulsified	2. 1. 4. 3.

No.	Parameters of supernatants, ranging MP and its strains, proposals (P)	Code ranging MP and its strains[*]
4.2	Pigment products [optical density at 420 nm] K3III24 > NK1 > 100 ash > MP P: K3III24 as the main potential source of neoglycoconjugates of nonenzymatical origin; MP as maximally decolorized product needed cofunctioning strain mixture; partial inverse correlation to point 1.3	3. 1. 2. 4.
4.3	Antimicrobial activity (toward a panel of diagnostic mainly Gram-negative bacteria) MP > NK1 > 100 ash > K3III24 P: Support point 3; potential of K3III24 to other targets (against Gram-positive bacteria and fungi)	4. 1. 2. 3.

[*]1, NK1 (Lactobacillus helveticus NK1); 2, 100 ash (L. helveticus 100 ash); 3, K3III24 (L. casei K3III24);
4, multiprobiotic (MP) on example of variants of Acilact.
[**]Alternative positions.

Table 3.
Strain code ranging for multiprobiotic construction on example of Acilact (the Lactobacillus multistrain probiotic).

(maximal resulting hydrolytic activities in respect to acidic proteins; increased level of antimicrobial peptides), 1.2 (maximal resulting hydrolytic activities in respect to cationic proteins; increased level of antimicrobial peptides including bacteriocin-like), 1.5 (the minimal level of aggregation upon storing concentrates), 2.3 (the minimal level of fluorophores exciting at 254 nm; contribution of Tyr and Trp or their derivatives), 2.3.1 (the minimal level of fluorophores exciting at 365 nm; contribution of Trp); and 4.2 (the minimal level of colored products); the major ingredient strain contributors are accounted as the third position in code.

The third step: The final formulas (formulas of category C) include combinations of formulas A and B. Multifunctionality of parameters analyzed can be extended (as in cases of amino acids [25]).

Extended approach for constructing more adaptive mixtures of lactobacillar and bifidobacterial MP (on the basis of *Acilact* extended by accounting industrial bifidobacterial strains) is presented in **Table 4**.

As expected, taxonomically mixed probiotics (symbiotics) will possess increased survival in biotopes of human organism. The same principles and algorithm (as for formulas of *Acilact* variants described above) can be applied. Combinations of *Acilact* ingredient strains and ingredients of *Bifidin* (*B. longum* spp. *adolescentis* MS-42), *Bioprotectin* (*B. bifidum* No. 1), and other bifidobacterial probiotics produced in Russia are of priority interest (also due to the possibility of their usage as standard models).

Table 4 demonstrates algorithm of further passage from intra-genus cases (*Lactobacillus*) of Gram-positive MP to inter-genus cases of MP as combinations of probiotic lactobacilli and bifidobacteria of the human gut origin.

Selective sets of parameters (points in **Table 4**) indicate principle differences between lactobacilli and bifidobacteria (as blocks of lactobacillar and bifidobacterial strains in 7-mark code). Completing lactobacillar/bifidobacterial synergism is expected and predicted. Selected combinations of strains from both blocks can be used for creation of directed MP. Other more complex cases include unblocked lactobacillar and bifidobacterial sequences within the code (additional prognostic conclusions are possible). Code positions "1" and "5" (both strains produce high levels of cytoagglutinating LS) reveal adjacent/similar behavior that indicates high level of compatibility of the strains NK1 and MS42.

No.	Parameters of concentrate (C), their ranging, proposals (P)	Code ranging strains and MP
1	Antifreeze components >27 kD, pI 4–8 (against any type of crystal forming during IEF-PAG): gall > bif1 > MS42 > NK1 > MP > K3III24, 100 ash P: bifidobacterial C for stabilization of K3III24 and 100 ash	*7. 6. 5.* **1. 4. 3/2***. *2/3.*
2	Formation of organic crystals in the presence of components >27 kD (in conditions of 7M urea, 5% saccharose, 8°C, night, IEF in slab of PAG): pI 4–6: 3III24, 100 ash > MP > NK1 > MS > bif1 > gall (not) pI 6–8: K3III24, 100 ash > MP > NK1 > gall > bif1 > MS42 P: Potential approach to micro- and nanoassembling effectors	**2/3. 3/2. 4. 1.** *5. 6. 7.* **2/3. 3/2. 4. 1.** *7. 6. 5.*
3	Complex protein C > 27 kD: pI 4–6: NK1 > MS42 > K3III24 > gall> 100 ash > bif1 > MP pI 6–8: NK1 > gall > K3III24 > MS42 > 100 ash > MP > bif1 P: Donors of cationic bacteriocin-like associates with exopolymeric compounds	1. 5. 3. 7. 2. 6. 4. 1. 7. 3. 5. 2. 4. 6.
4	Adhesins as colorless transparent not water-soluble drops on polystyrene (number of drops): gall > MS42 > NK1 > bif1 > MP > K3III24 = 100 ash(not) P: The size and numbers of drops indicate level of emulsification of C compared to original supernatant	7. 5. 1. 6. 4. 2/3. 3/2.
5	Associated biosurfactants: bif1 > gall > K3III24 > MP > 100ash > MS42 > NK1 P: C "NK1 + gall" and "NK1 + bif1" as synergistic antimicrobials	6. 7. 3. 4. 2. 5. 1.
6	LSSM as mucin-binding: pI 4–5.5: gall > MP > NK1 > MS42 > bif1 > K3III24, 100 ash pI 5.5–8: MS42 > bif1 > gall > NK1 > MP > 100 ash, K3III24 P: C for delivery into intestinal and urogenital mucosal cavities	*7. 4. 1. 5. 6.* **2/3. 3/2.** *5. 6. 7.* **1. 4. 2/3. 3/2.**
7	LSSM as mannan-binding: pI 4–5.5: 100 ash, K3III24 > NK1 > MS42 > gall > MP > bif1 pI 5.5–8: gall > bif1 > MS42 > NK1 > MP > 100 ash, K3III24 P: Potential against eukaryotic (yeast and yeast-like), prokaryotic (staphylococci), and HIV/HIV-related infections; delivery into cell and cell organelles	2/3. 3/2. 1. 5. 7. 4. 6. *7. 6. 5.* **1. 4. 2/3. 3/2.**

*1, NK1 (L. helveticus NK1); 2, 100 ash (L. helveticus 100 ash); 3, K3III24 (L. casei K3III24); 4, MP (Acilact); 5, MS42 (B. adolescentis MS42); 6, bif1 (B. bifidum No. 1); 7, gall (B. gallinarum GB); C, concentrate (>27 kD) of cultural fluid supernatant; IEF, isoelectrofocusing; PAG, polyacrylamide gel; P, prognostic proposals; *Alternative position. Blocks of lactobacillar (**boldface**) and bifidobacterial (italic font) strains are shown.*

Table 4.
Strain code ranging for multiprobiotic construction on example of new multiprobiotics including bifidobacteria and lactobacilli.

Further constructing other or extended multistrain symbiotics is depended on choice of important parameters of interest (to increase the number of comparable codes used in **Tables 3** and **4**). Important prospects in constructing taxonomically mixed symbiotic formulas are expected on the basis of identified LSSM sets of the strains as counted ingredients of multisymbiotic as well as evaluation of the relative contribution of LSSM types in resulting multifunctional activities of mixed product. For example, the general properties of LS of lactobacilli and bifidobacteria investigated by us are "recognition of mucin-type targets" more or less than "recognition of mannan-type targets" for LL or BL, respectively [8]. As a result, LSSM-dependent synergism (which can be directed and predicted using extended panel of GC for LSSM selection and choice) of new taxonomically mixed symbiotics can be achieved.

Universality of approach proposed in **Table 4** means that the panel of comparative parameters of investigation is unlimited for selection. As advantages of this approach for creation of perspective formulas of multipro-/symbiotics, some properties of future combinative products can be predicted and verified.

Aforementioned codes (**Tables 3** and **4**) extend the potential of using traditional MP. Results open new possibilities for investigation of LSSM types among strains and constructed consortia to develop perspective GC-type-dependent LSSM combinations possessing needed actions toward human interactome. In terms of personalized medicine, individual LSSM applications are of reality. For example, LSSM could be applied as "a functional tissue biotope" or mucosal organ-specific agents and organizers of lectin-coupled reactions and activities [26, 27].

3.3 Anaerobic synergistic preparations containing LSSM for support of human protective systems

Due to high distribution in organism, oxidative stress (as the power destructive factor initiating diseases) needs the constant presence of the power protective antioxidant systems [28, 29]. Some therapeutic proteins regulating cellular consumption of oxygen can be involved into development of tumor and other side pathologies in organism. We isolated system anaerobic (without oxidases initiating of oxygen and peroxide radicals) preparations of acidic/anionic and alkaline/cationic LSSM from cultures of symbiotic (probiotic) industrial strains of human bifidobacteria and lactobacilli as consortia that were successfully applied. Such preparations devoid the ability to induce destructive oxidative stress (crosslinking and inactivation of therapeutic proteins, etc.) in respect to surrounding infrastructures.

The used synthetic GC in our work were characterized with antioxidant properties in respect to LS as carriers of GC (prolongation of chemiluminescence of protective complexes was observed). Similar resulting protection of LSSM was also registered in the presence of neutral and cationic bifidobacterial and lactobacillar cultural exopolymeric compounds (EPC) of nonprotein origin (as observed on the blot after IEF-PAG). Acidic and alkaline anaerobic LS of bifidobacteria and lactobacilli revealed the following general antipathogenic actions: (a) own and overlapped/synergistic; (b) toward communicative bodies of microbial massifs and biofilms of the potentially pathogenic yeast-like fungi and Gram-positive bacteria. All four types of preparations of LSSM used were characterized by own mechanisms of antimicrobial actions in comparison to action of other antimicrobial systems (antibiotics, bacteriocins, phytolectins, subisotype products of isotypes C4B and C4A of the human complement component C4) [30, 31]. LS from human probiotic bacterial cultures revealed the ability to act as cascades in such reactions as initiation/changing or switching recognition of GC of different types (imitators of mannans, mucins, components of bacterial walls, Forssman antigens, Tn, blood group substance A) using the same original pool of lectin forms of taken multistrain probiotic. The presence of cations Ru^{2+} (ingredient of SYPRO involved in photosensibilization) strongly increased discreteness and number of forms of acidic lectins—potential carriers and deliveries of GC. Stability of obtained mosaic asymmetric landscape pictures of the systems LSSM-GC as multistrain probiotic-depending and multistrain probiotic-supporting biotope balance of recognition and reversible retaining/depositing of GC (therapeutics, biomarkers, others) was observed. Combinations of anaerobic LS-containing proteins revealed themselves in respect to yeast-like and Gram-positive pathogenic targets as more selective in the choice of the adequate regional territory of massif of pathogen and limitation of early and late time (depending on localization of targeted region of communicative

body of pathogen) for the mostly effective visible actions of LS, obtaining uniform pure landscapes of LSSM action on massif of *Candida albicans* (the absence or minimization of LSSM-resistant residual fungal colonies in the interacting intestine system "LS of human intestinal bifidobacteria and lactobacilli—human intestinal *C. albicans*"). Antimicrobial activities of LSSM and phytolectins (phytohemagglutinin from the kidney bean) could be realized not only directly but also through the influence (together with synthetic mannans and mucins) in respect to macrophage migration as well as through inducing production of cytokines by stimulated blood lymphocytes (on the example of tumor necrosis factor-α). Results indicate prospects of anaerobic LSSM as assistant ingredients of the possible drug forms.

3.4 Synbiotic minibioreactor using LSSM for screening GC

During the last time, synbiotics and symbiotics (as synergistic sum of probiotics and prebiotics) are of increased investigator interest due to their antimicrobial and other useful reactions [1, 2]. In this respect LSSM represent new class of antipathogenic proteins (possessing extended potential of application) which recognize different GC. LSSM represent multifunctional potential of relatively highly molecular mass polymeric metabolites of cultures of the human microbiota (microbiocenoses) and consortia (also multistrain probiotics) of human indigenous microorganisms. LSSM cofunction together with artificial and biologically active natural GC [32].

According to own results, we proposed suitable laboratory minisystem for screening prebiotic and therapeutical GP using LSSM and sterile heparinized insulin syringes of 1 ml volume. The following results were obtained: (1) LSSM-containing fraction stimulated production of both the whole and adhesive mass of bifidobacteria, (2) LiCl (15 mM and higher) increased dose depending on the number of adhesive colonies, (3) bifidobacterial LSSM (within pI 4–4.5) were characterized with strong affinity to anionic synthetic GC (possessing exposed residues of sulfated galactosides or, in a less extent of affinity, exposed residues of mannose-6-phosphates), and (4) sulfated glycosaminoglycans together with cations Li^+ and LSSM as potential carriers of Li^+ participated in functioning bioreactor imitating synbiotope (multiplication of bifidobacterial colonies and their survival were observed).

Proposed synbiotic system is perspective for screening prebiotic GC (as it is known for prebiotic derivatives of chitin, chitosan, fucoidan, and glycopeptides [33, 34]). **Table 1** includes potential prebiotic sources such as chitin and α-L-fucan which react to LSSM (LSSM may serve carriers of metabiotic GC; the list of GC can be unlimitedly extended).

3.5 Membrane technological prospects of LSSM

Progress in membrane and solid-phased technologies using LS-GC interactions includes the potential of their application in microassays, biochips (membrane bound glycoarrays or lectin arrays), and biosensors [35, 36].

The use of affine pore hydrophobic (uncharged) membranes predictably covered with mosaics of multifunctional sets of LSSM (additional significant purification of LSSM on membranes is reached) allows prolonged storing LSSM without decreasing samples in activities. The following prospects of LSSM-GC combinations may be of practical interest: (a) antifungal covers of prolonged action in combination with antibiotics and physical factors of stress [radiation (ultraviolet and ultrasonic), light (biorhythm "day-night"), temperature, pH, oxygen, season changes (also biorhythmic), others] and (b) chemiluminescent systems cofunctioning in regime of real time for medical and industrial biotechnology and

bionanotechnologies [our results include the following coupled systems: "low acidic LSSM, low acidic oxidoreductases of lactobacilli"; "alkaline bifidobacterial LS, alkaline bifidobacterial exopolymeric compounds"; "neutral lactobacillar/bifidobacterial LS, neutral lactobacillar/bifidobacterial biosurfactants"; "LS, strongly acidic (pI 3–4) serial phyto-oxidoreductases/phyto[glycosyl]oxidases"].

Membrane technologies using separated proteins, oligopeptides, and their complexes (especially) together with intrinsic or exogenic (SYPRO dye) fluorescence registered in live bioimagination are especially sensitive and perspective (protein band discreteness using fluorescence technique was better compared to the chemiluminescence technique). The latter allows identification stabile boundaries of the whole protein massifs for further establishment of LSSM and other biologically and physiologically active components among protein mosaics. Bioluminescence (fluorescent technique in combination with chemiluminescence technique) in optimal (depending on the object and the goal of study) conditions allows express-ranging cultural fluid groups of proteins and LS according to molecular mass (for additional standardization and typing of strains), evaluation of interstrain synergism and contribution of protease and oxidoreductase systems of mono- and multistrain probiotics (symbiotics) and other type consortia, and identification of mosaics of complexes containing fluorochromes in extended interval of pI/pH (complexes and cell wall fragments as carriers of visible energy which is ready for energetic exchange with surrounding infrastructures as well as for monitoring directed supramolecular assembling and their reorganization).

Aforementioned data presented develop other possible important prognostic approaches and proposals. The choice of wished prognostic (LSSM-selected-type and GC-type interactions)-directed events in biotope can be determined and regulated by involving GC types needed (panels of natural and artificial GC used in biotope; extending the list of GC indicated in **Table 1** is possible). The use of artificial polymeric GC with established chemical structures allows receiving adequate, understandable, and reliable results.

According to the multifunctional potential of artificial GC used, resulting events in biotope can be the following ones: antipathogenic actions (the use of GC-imitating pathogenic cell surface structures), prebiotic and symbiotic (synbiotic) actions (the use of GC-imitating prebiotic structures), increasing own human organism protective systems (the use of GC-interacting and GC-regulating macrophages and macrophage-like lymphocytes through their systemic receptor lectins), antitumor actions (potential using antigenic GC together with LSSM as antagonists and synergists in intercellular antitumor and anti-infectious communications [37]), and cytokine lectin/lectin-like cascades initiated/regulated with LSSM potentially influencing redistribution of cytokine network in organism.

3.6 Prospects of LSSM for prophylaxis and therapy of infectious diseases

Innate immunity plays an important role especially when antibody immunity is under development, suppression, or alteration. So the search of new natural anti-infectious agents is of actuality. In contrast to probiotic cells, LSSM (as systems of LL or LB) are not sensitive to the presence of antibiotics and chemotherapeutics as well as do not need special conditions for survival. LSSM function as metabolome-biotics according to the principle "the network in the network"; participation of major lectins in supporting physiologically significant glycodecor of biotope mucosal probiotic compartments, and minor lectins—in signal communications; support of such compartments (compensation of the absence of probiotic cells in MB upon therapy with antibiotics, delivery of fucosylated and galactosylated prebiotics of mutual supporting influence between populations of bifidobacteria and lactobacilli,

cofunctioning to anti-infectious agents (antibiotics, metabiotics, GC) and cells of organism protective systems (macrophages and leukocytes).

We characterize LSSM (acidic, low acidic, cationic: aLL, laLL, cLL, aLB, and cLB) of domestic probiotics which recognize polymeric artificial GC mucin-like analogs of antigens and EPC (fungal and bacterial mannans, bifidobacterial α-L-Fuc-containing, sulfated and phosphorylated).

Antimicrobial directions of LSSM action were established [26, 31, 38].

3.6.1 Anticandidosis activities

- Against intestinal and urogenital *Candida* of epidemiologically significant species of group I (*C. albicans* and *C. tropicalis*: early action of aLB within the first 2 days; delayed action of cLL and LB within 1–2 months; synergism between acidic and cationic LSSM, cLB, and grass lectins, aLSSM/cLSSM, and some antimycotics)

- Against *Candida* of epidemiologically significant species of group II (*C. glabrata*: inhibition of virulent factors such as fungal IgA1 and IgG proteinases, EC 3.4.21.72 and EC 3.4.21., respectively [9])

- Against *Candida* of epidemiologically significant species of group III (*C. krusei*: delivery of potential effector GC on cell surface)

- Interruption of mycosymbiosis parasitism of *Candida—Aspergillus*: the drugs against candidosis as predictably affective against aspergillosis)

Activities of LSSM against staphylococci (on example of patients *S. aureus* isolates): aLL > aLB; aLL and aLB; LSSM activities accompanied with:

- Delayed degradation of microbial massifs

- Partial directed lysis of microbial massif

- Tearing away fragments of massif (by two mechanisms depending on lectin type)

- Synergism between aLL and LB in antistaphylococcal actions

LSSM possess potential of diagnostics and prognostics of biotope diseases [37]. Results support important principle approach that diseases of MB (as in the case of CBF according to the principle "there is the body—there are diseases") reflect own "biotope diseases" in organism [39]. LSSM can be used in phenotyping prognostics and diagnostics of infectious processes in organism; for prophylaxis and therapy of candidoses, staphylococcoses, mixed fungal-bacterial infections, mixed symbiotic mycoses; innate infections; immunodeficiency and infections against the background of immunodeficiency, upon antibiotics-/chemo-/radiotherapy. Such GC effectors as metabiotics, prebiotics, glycoantigens, and drugs of selective directed action are of availability.

It is of importance to consider the participation of LSSM in supporting biotope mucosal MB in conditions of oxidative stress (cofunctioning of laLL and probiotic oxidoreductase systems) [26]. Panel combinations of LB and LL are perspective in early evaluation (before the inflammation) of status of MB of functionally different biotopes of the vaginal tract.

LSSM are similar (in specificities, systemic action) to communicative lectins of intercellular reception in innate immunity.

LSSM act as essential part of human protective supersystem (the supersystem with probiotic type action) [31]. Such supersystems contract disturbing the balance of the body's health processes; keep and maintain the integrity of biotope micro-biocenoses like healthy consortia. There are supersystems with antimicrobial or antifungal action in organism.

3.7 Potential of results for innovations

Results obtained by us in a study of probiotics can be useful as a set of approaches in study of the following aspects of clinical microbiology and medical biotechnology (in brackets—significance and prospects of results for infectology) [40, 41].

3.7.1 Metabolite technologies

- Cultural metabolites 27–200 kD within pI 4–8 (separation of acidic and alkaline protein and nonprotein of taxonomically significant systems); phenotyping of strains in cationic region containing protected sets of proteins Subcytoagglutinating activities: initiatively coupled or non-coupled to lectin and cytoagglutinating activities (cytokine activities; synchronization of cultures for increasing vulnerability of pathogens; typing).

- Functional blotting analysis of cascade cultures in combinative nutrient media (NM) (as in the case of milk followed by transfer into "casein hydrolysate-yeast autolysate" media; monitoring lectins, enzymes, and exopolymeric compounds [EPC])

- Development of strain-supporting NM (comparison of blotted maps of components of cultures in identical conditions)

- The use of α-S-, β/γ-, and kappa-caseinases (increase of effectiveness of NM, accumulation of physiologically active protein and nonprotein metabolites and their complexes)

- Systems of proteinases, EPC depolymerases, and/or peroxidase-like catalases in cultures (revealing strain-dependent producers of enzymes, proteins, and EPC; evaluation of cytolysis populations, oxidative stress, virulent factors, changes, and aging of cultures upon passages and storage)

- Lectin systems: major and minor, building and signal, *cross talk* and *quorum sensing* (standardization of cultures and analysis of communicative networks) *Evaluation of early and prolonged landscape synergism of recognizing components of cultures and antibiotics (individual choice and replacement of combinations of probiotics and antibiotics upon therapy of patient)

- Natural and recombinant systems oriented to recognition of polyvalent glyco-conjugate targets of known structure (standardization of strains; monitoring and revealing signals of carbohydrate metabolism)

- Imitation by cultural metabolites in respect to strain or consortium effectiveness (standardization of strain multifunctionality)

- The use of recognizing metabolites instead of cells or in combinations with cells for support of feeble living and antibiotic-sensitive cultures (also in cases of non-culturable microorganisms).

- Constructing balanced supporting MB of probiotic cell-metabolite consortia (constructing symbiotic consortia, balanced vaccines)

3.7.2 Intercellular and cell-metabolite technologies

- For Gram-positive bacteria (opportunistic and pathogenic)

- Sensor microbial systems of MB (revealing and using key antagonistic systems) Directed assembling of cytokine-cell gradients: reversible functional (1–2 days) or late irreversible conserved and partially inactivated (4–6 weeks), as in cases of biofilms containing erythrocytes—in microassay (evaluation of protection and degradation of human cells in the presence of pathogens)

- Biofilm forming as ranked quantitative and qualitative, early and delayed—in microassay (reactions of mono- and mixed biofilms)

- Species-dependent redistribution of antagonistic MB (prediction of mutual influence between species and strains of probiotics, opportunistic microbes, and antibiotics)

- Leader strains and species within MB (evaluation of temporary MB-destabilizing strains in coexistent antagonistic microbial populations)

- Destabilization and synchronization of MB by recognizing components of cultures for increasing future selective synergistic action of antimicrobials and stress factors (increase of early and late suppression of pathogens) Microecological interniche early and delayed landscape relationships in cases of solid-phased and suspension microbial massifs, for microanalysis (evaluation of opportunistic microbes and MB as communicative bodies opposed to mosaics of antimicrobials)

- Construction of active (involving probiotic leaders) and stabilizing (species-dependent and resistant to antibiotics, probiotic-like supporting MB) consortia (using upon therapy)

- Strain-phenotyping dysbiotic MB using lectin systems (revealing early changes in patient MB in the presence of lectins of MB, for development of new strategies of therapy)

- LS of probiotic consortia (directions of consortium formula actions can be predicted by constructing) as ingredients of functional food to support resistant functioning mucosal organs

4. Conclusion

The data presented consider aspects of constructing probiotic multistrain intra- and inter-genus Gram-positive bacterial metabolites on examples of bifidobacteria and lactobacilli of the human gut origin. Algorithms of creation

of 4- and 7-code MP formulas are presented and argued. It is of importance to consider LSSM and their GC as a new perspective protective basis/scaffold factor influencing and supporting human interactome, involving biotope infrastructures, signaling, and anti-infectious actions as well as cofunctioning together with other protective human systems [17, 42]. Results support the use of pro-/symbiotic LS as assistant-coordinated metabolomic agents; carriers for delivery and releasing GC, metabiotics (including simulators of cell surface patterns of opportunistic microorganisms), prebiotics, therapeutics, and antigens (for cofunctioning to antibodies); and reserves for decoration elements that support stable functioning landscapes of the human cell surfaces and mucosal tissues. Probiotic lectins (interacting with synthetic GC simulators) are important for screening, typing, and selection of useful strains and their consortia supporting organs. The proposed LSSM-containing preparations promise LSSM using a soft adaptive multidirected network synergistically acting together with other protectors (also in conditions of the absence of oxygen [involving oxidative stress and forming of covalently bound by-products] within biotope). Variants of multipro-/sym-/synbiotics constructed can be perspective for prophylaxis for individuals and contingents as well as for supporting treatments of patients (before and postoperative period, during chemotherapy, etc.). Approaches developed are useful in study of interniche relationships of extended set of Gram-positive and fungal microorganisms in mono- and mixed cultures. Procedures can be applied in different fields of clinical microbiology and medical biotechnology.

Conflict of interest

The authors declare the absence of conflict of interest.

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Gut Microbiota and Obesity: Prebiotic and Probiotic Effects

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Abstract

Malnutrition through excessive food consumption is a worldwide pandemic. Changes in lifestyle, diet and physical activity have resulted in an exponential increase in the number of obese people around the world. Multiple factors influence the development of this disease, and recently it has been suggested that gut microbiota (GM) plays an important role in nutrient absorption and energy regulation of individuals, thus affecting their nutritional status. It has been proven that gut microbiota is different in individuals with a normal nutritional status compared to those who are obese. Therefore, to study bacterial populations that make up the microbiota and to understand how prebiotics and probiotics affect the increase of these bacteria has become a promising alternative to treat obesity. This chapter looks at defining the established relationship between probiotics, prebiotics and gut microbiota that develop in obese people and people of normal weight, with the aim of providing future dietary recommendations to treat this medical condition.

Keywords: gut microbiota, prebiotics, probiotics, obesity

1. Introduction

Currently, obesity is defined as a medical condition characterised by an energy imbalance as a consequence of energy intake bigger than energy expenditure [1]. The US Centers for Disease Control and Prevention (CDC) defines it as 'excessive weight that is considered unhealthy for a given height'. It was not until 1997 that the World Health Organization (WHO) described obesity as an excessive accumulation of body fat that can be harmful to people's health [2].

One of the criteria to diagnose obesity is through the calculation of body mass index (BMI); the result, according to the WHO, must be equal to or bigger than 30 kg/m^2 [2].

However, the Asian population including Korea, given their increased risk of developing comorbidities, such as cardiovascular diseases and type 2 diabetes, agreed to an overweight category with a BMI between 23 and 24.9 kg/m^2 and obesity with a BMI > 25 kg/m^2 (**Table 1**) [3].

Worldwide obesity is a public health problem, and despite all the strategies designed and implemented to reverse the situation, these have not been effective. According to the World Health Organization, globally, in 2016, more than

	WHO (BMI)	Asia-Pacific (BMI)
Underweight	<18.5	<18.5
Normal	18.5–24.9	18.5–22.9
Overweight	25–29.9	23–24.9
Obese	≥30	≥25

Table 1.
Comparison of the World Health Organization (WHO) and Asia-Pacific body mass index (BMI) classifications in COPD patients [3].

1900 million adults were overweight, over 650 million were obese, and 41 million children under the age of 5 were overweight. Fifty-seven percent of the world's population lives in countries where being overweight and obesity cause more deaths than being underweight. Furthermore, each year at least 2.8 million people die worldwide due to obesity or being overweight, irrespective of whether the country is economically developed or not [1, 2].

Various studies have shown that patients with illnesses related to the gastrointestinal tract, such as type 2 diabetes, obesity and cardiovascular diseases, among others, present dysbiosis or an imbalance within the gut microbiota (GM).

A hypothesis of the positive influence of microbiota on fat storage exists, and several studies carried out in mice have proven the role of certain fatty acids in the diet in the prevention of obesity, an effect mediated through changes in the composition of gut microbiota. Studies in humans have also shown a relationship between a decrease in *Bacteroidetes* in obese individuals and a greater proportion of these in the faecal microbiota in subjects who have undergone hypocaloric diets and who have managed to reduce their weight [5, 7].

An important role of the regulation of gut microbiota has been proven as the ingestion in the diet of prebiotics and probiotics that modulate the growth of diverse families of bacteria that could influence the nutritional status of individuals.

2. Obesity as a multifactorial condition

Obesity is a medical condition of multifactorial causes, ranging from the influence of the nearby environment on the acquisition of predisposing habits, such as the consumption of foods high in refined carbohydrates and saturated fats and a sedentary lifestyle. Genetic predispositions are also among the principle causes [5]. On the other hand, socioeconomic development, transport, urban planning, environmental, agricultural, educational, processing, distribution and marketing policies on food influence dietary habits and preferences, as well as people's level of physical activity [5, 6].

Research concludes that obesity occurs with greater prevalence in women, older people (25 years and above), those with a lower school level and those with a low socioeconomic income. Geographic location also has an influence, but it occurs in an opposite way in men and women. There is greater obesity in women living in rural areas, but in men rurality is a protective factor since obesity is more prevalent in those living in urban areas. This is most likely explained by the higher levels of physical activity of men living in rural areas [6]. In terms of lifestyles, obese people have lower levels of physical activity, a bigger consumption of salt and low levels of sleep [7].

Another bibliographical review relates meal times with the prevalence of obesity. This study, which is based on an analysis of the circadian rhythm, a biological function regulated by the anterior hypothalamus, reveals clinical observations

and experiments on animals that relate the times of food intake to metabolism and body weight. It is proposed that eating at times when the body is prepared for rest increases body weight. At rest there are low levels of hormonal activity, a low body temperature and low activity of nutrient absorption which alters energy homeostasis and facilitates the accumulation of energy, increasing body weight [5, 8].

A factor recently studied as a cause of obesity is gut microbiota, defined as the communities of living microorganisms that colonise the intestines. It is known that the gut microbiota contributes significantly to the two main functions of the gastrointestinal tract: nutrition and defence.

3. Human gut microbiota: role in people's health

The human gut is colonised by an enormous quantity of communities of living microorganisms, mainly bacteria that form the GM. As well as participating in processes of digestion and absorption of nutrients, the homeostasis of GM has been linked to beneficial health effects [9].

In the womb, the intestine of the human foetus is sterile, and it is after birth when the newborn acquires its gut microbiota. From birth, several factors influence the composition and initial colonisation of the GM. Natural labour favours the rapid settlement of bacteria that come from the vaginal and faecal microbiota of the mother, associated with a low risk of suffering from diseases and some types of malnutrition through excessive food consumption in the future. On the other hand, children born through caesarean section have a slower colonisation of bacteria, mainly from the hospital environment and healthcare professionals who are in contact with the child. It must be considered that the time taken to establish the GM is a risk factor for the colonisation of *Clostridium*. Despite the fact that GM is established from birth, its colonisation continues to vary, given that there are diverse factors that influence its composition: nutrition, exclusively or predominantly breastfeeding, age, geographic location and intake of supplements and medicines [9, 10].

In the first years of life, when nutrition is acquired through breastfeeding or formula milk, GM is mostly populated by *bifidobacteria*. This is highly adapted to processing oligosaccharides in milk. Later, reaching adulthood, the GM is made up of two main families of bacteria: *Firmicutes* and *Bacteroidetes*. *Firmicutes* are the family found in greater proportion, including more than 200 genders, and the most important are *Mycoplasma*, *Bacillus* and *Clostridium*. Once these are established in adulthood, they remain stable in the individual (**Figure 1**) [11–14].

Various studies support the fact that variations in people's health status are directly related to functions of the GM, highlighting its effect on the immune response, nutrition and metabolism [15].

The immune tissues of the gastrointestinal tract make up the most complex and largest proportion of the body's immune system, which is why alterations of GM are caused by disequilibrium of the immune system, such as the development of allergies and autoimmune diseases [16].

There is a complex interaction between the microbiota, the intestinal epithelium and the gastrointestinal immune system, with many metabolites and microbial components that have a direct influence on the host immunity. The production of metabolites from nutrients or the modification of the metabolites produced by the host has a direct effect on the immune cells and on the integrity and permeability of the intestinal epithelium. The enteric immune system is constantly evaluating and responding to the gut microbiota [17].

Nutritional and metabolic functions of GM involve the synthesis of certain vitamins such as K, B12, biotin, folic acid and pantothenic acid, as well as the
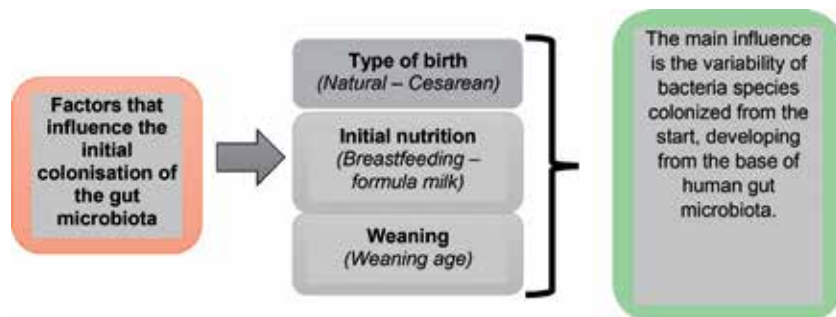
Figure 1.
Factors that influence the initial colonisation of gut microbiota.

fermentation of non-digestible carbohydrates, polysaccharides and oligosaccharides. It makes up an important source of energy for bacterial proliferation and produces short-chain fatty acids that the host can absorb, favouring the recovery and absorption of ions such as calcium, iron and magnesium [18].

As well as the principal functions of GM, recent studies have associated it with the development of obesity and chronic diseases. In order to avoid such conditions, it has been proposed that the role of prebiotics and probiotics in the maintenance of the nutritional status and prevention of diseases should be studied [4].

Undoubtedly, microorganisms present in the guts are fundamental throughout life as they have a direct relationship with health and illness. It is vital to take special care in the factors that make up the initial GM as it is responsible for the general status of future health of individuals.

4. Microbiota and its role in obesity and chronic diseases

As already described, the gut microbiota is made up of a diversity of pathogens which highlights the presence of Gram-negative or Gram-positive bacteria. The equilibrium of this ecosystem provides us great health benefits, in the absorption and digestion of nutrients, as well as influencing immune function [4–18].

In recent years, the prevalence of non-transmittable chronic diseases has increased, which has led different researchers to address the different risk factors related to the appearance of those that have been mentioned and the direct risk between microbiota and diseases [18].

Studies linked to the establishment of microbiota with chronic diseases have described that this stability of original microbiota is affected by diverse factors among which the macro- or micronutrients and dietary patterns are mentioned [18].

When the balance of microbiota is altered by a factor, whether it is the use of antibiotics, stress or diet with an increased consumption of fats, carbohydrates and fibre, it will have an effect on health. It has also been mentioned that abundance or scarcity of food, environmental pollution, chronic stress and food such as dairy products, sugars, coffee, tea and alcohol, among others, affects the intestinal mucous membrane and, consequently, changes the stability and mode of action of the microbiota [19].

All these factors cause an alteration in microbiota where the equilibrium of two large families of bacteria is affected: *Bacteroidetes* and *Firmicutes*. In a study carried out on the composition of the microbiota of obese mice, it was shown that they had increased concentrations of *Firmicutes* by more than 50%, while those of *Bacteroidetes* decreased correlatively [20].

In order to demonstrate that the microbiota changes through time, a study was carried out where old teeth found in skeletons in different periods of history were analysed. It was found that microbial changes are linked to human evolution from the hunter-gatherer period to the industrial revolution, due to the increased consumption of processed foods. These conclusions support the idea that diet and the type of food alter human microbiota [18].

Turnbaugh et al. carried out studies on 'humanised' or sterile mice models to which human faecal matter was transplanted and the microbiota was analysed once they were fed with a Western diet rich in fats and sugars. They found a presence of greater adiposity and a reduced proportion of *Bacteroidetes* than *Firmicutes* in the faecal microbiota of these mice [19].

There is a direct relationship between microbiota and energy uptake and consequently a relationship with the onset of obesity. This has been supported by a study in obese children, which showed a microbiota rich in *Enterobacteriaceae*, low in *Bacteroidetes* and increased in *Firmicutes*. When the children were subjected to diets with low carbohydrate and fat content, the composition of the microbiota changed [18].

Other studies carried out in mice reflect the relationship between energy balance, diet and microbiota. The transplantation of the obese microbiota results in an increase of adiposity in the recipients, which shows that microbiota affects nutrient acquisition, energy storage and consequently the development of obesity [18, 22].

Jumpertz et al. carried out studies on the GM of obese and slim patients. They were given caloric diets, and their stools were analysed by measuring calories ingested and those eliminated in the faeces using a bomb calorimeter. This study concluded that there are changes in the GM where there are increases in energy storage, a decrease in the *Bacteroidetes* and an increase in the *Firmicutes* bacteria. All these changes could explain the variation of the uptake of energy in individuals and therefore a relationship with the predisposition to obtain metabolic disorders [18].

According to the above, the instability of the GM or changes in the original GM affects the metabolism of the whole organism. Recent studies have shown that the increase of bile acids in the intestine when comparing sterile rats with normal rats would show that the GM is not only related with obesity but also with a diverse range of metabolic diseases [21].

When transplanting GM into obese mice, the new term obesogenic microbiota was determined, associated with an increase in hepatic glucose production and promotion of triglyceride deposits. These studies show that there is an increase in TNF pro-inflammatory cytokines which can cause insulin resistance. This can be correlated with the appearance of DM-OB, since its presence has been proven to be directly related to the presence of elevated pro-inflammatory factors [22].

Toll-type receptors are receptors that recognise important patterns within immunity and inflammation processes; they are present in diabetic obese patients with metabolic syndrome. In publications made in the Latin American report, they show the role of GM with regard to the regulation of these diseases, since the mice deficient in Toll-like receptor 5 (TLR5), which recognises microbial patterns, show hyperphagia, become obese and develop characteristics indicative of metabolic syndrome. This is also proven when GM from these mice was transplanted into germ-free mice with the TLR5 gene—the receptor mice developed characteristics similar to the metabolic syndrome. In summary, Larsen et al. demonstrated that GM from diabetic patients produced a significant reduction in *Firmicutes* and *Clostridia* [22, 23].

When administering antibiotics to these types of obese patients, many *Firmicutes* were eliminated, which resulted in improved insulin and glucose intolerance.

The exact mechanisms by which GM contributes to the development of obesity have not been fully elucidated, but it has been suggested that the main ways would

include increased lipoprotein lipase (LPL) activity, increased intestinal permeability and lipogenesis [20]. Faecal microbiota transplantation studies in both healthy individuals and rats to obese receptors have shown the favourable action of the GM of the emitters towards the obese individuals, reducing the levels of glycemia, preventing the expansion of fat and regulating inflammatory processes. Studies also suggest that the reverse mechanism, that is, transplantation of intestinal microbiota from obese to healthy individuals, can transmit the development of obesity, assigning GM as a predisposing factor for obesity [17].

Thanks to many randomised studies that have been carried out, it will be possible to identify the microbiota properties that are contributing to the obesity epidemic, diabetes and metabolic diseases. This will in turn allow scientists to extract information and characterise diseases with the aim to prevent or cure them [21].

5. Role of prebiotics and probiotics in maintaining nutritional status

Probiotics have been defined by the United Nations' Food and Agriculture Organization (FAO) and the WHO as 'living microorganisms that provide their host with health benefits when consumed in appropriate quantities' [4].

Prebiotics were defined by Gibson and colleagues in 2004 as 'ingredients that when selectively fermented, give rise to specific changes in gut microbiota composition and/or activity, which leads to many health benefits with regard to, for example, the wellbeing of individuals' [24].

According to the preceding analysis, it has been recently established that the microbiotics that colonise the human intestine can play an important role in causing obesity and developing other metabolic and immunological illnesses within their host. This statement is based on recent metagenomic studies that have determined that there are differences between obese individuals and those of a normal weight in terms of gut microbiota. These differences are associated with the presence of a microbial population in obese individuals, which has a greater capacity to recuperate dietary energy and/or favour the onset of chronic low-grade inflammation [25].

Some studies conducted on obese individuals in relation to their prebiotic consumption—capable of selectively stimulating a limited number of gut microbiota genres/species which results in health benefits—have demonstrated results that can be categorised by an increasing trend in satiation. This effect has been observed with inulin fibre [24].

The mechanisms implicated in satiation following the consumption of oligofructose and inulin have been associated with intestinal fermentation-induced hydrogen formation, which in turn has been correlated with a postprandial increase in plasma levels of peptides GLP-1 and PYY, which are involved in energy homeostasis through supressing the sensation of hunger (**Figure 2**) [24].

Studies carried out involving the use of probiotics to control body fat accumulation show promising results, but it is also necessary to carry out further, well-designed studies which incorporate randomised, double-blind trials with a placebo control, in order to demonstrate the efficacy of specific probiotic strains when preventing or treating the overweight and obese. In this sense, it is important to highlight that the results obtained in the trials would be specific to the strains analysed, meaning it is not possible to extrapolate the results or action mechanisms to a species or genre as a whole [4, 24].

Consequently, much remains to be done in this field. However, when putting everything in perspective, modulating the composition of gut microbiota using probiotics could be considered as a new avenue through which we can treat the overweight and obese [24].

Figure 2.
Mechanisms implicated in the satiety of obese individuals related to inulin consumption.

On the other hand, prebiotics promote the absorption of minerals such as calcium, magnesium, zinc and iron due to their ability to bind to them. This thereby prevents their absorption into the small intestine to reach the colon, where they are released and subsequently absorbed. Better calcium absorption is linked to prebiotic fermentation caused by gut microbiota, which produces short-chain fatty acids and lowers luminal pH, increasing the bioavailability and passive absorption of calcium through colonocytes. It has also been proven that calcium bioavailability is improved when released through hydrolysis of a compound called 'calcium phytate', which occurs due to action of bacterial phytases present in the beneficial microbiota. It is also improved when calcium becomes more soluble as a result of an increase in the volume of water in the colon, caused by the osmotic effect of prebiotics. This increase in calcium absorption is beneficial for bone health as it increases bone mass and delays the onset of osteoporosis. This is important when considering growth periods, during which peak bone mass is reached in postmenopausal women and in elderly people [25].

Prebiotics stimulate iron absorption into the colon, by increasing the soluble fraction in the cells. Zinc is necessary for the development and maturation of the skeleton, and prebiotics stimulate zinc bioavailability. Therefore, mineral is supplemented through the diet. Furthermore, prebiotics are attributed to another set of properties related to particular systemic disorders. Prebiotic carbohydrates (GOS, FOS, inulin) reduce blood pressure, as well as blood levels of glucose, cholesterol, triglycerides and phospholipids, as well as triglyceride and fatty acid synthesis in the liver, minimising the risk of developing diabetes, obesity and atherosclerosis [25, 26].

6. Influence of probiotics in the development of obesity

The development of obesity is not caused by one specific factor. On the contrary, it is the result of an interaction of genetic, environmental, social, lifestyle factors, etc., which transforms it into a multifactorial disorder, which explains how quickly it has become more prevalent throughout all age groups in developed countries and shows that traditional theories are not adequate to explain the complex phenomenon of gaining body weight [27].

In recent decades and as a result of advances in science, research for 'nontraditional' etiological factors involved in an individual's excessive weight gain has begun [28]. One of these new factors is highlighted in a study on the role of probiotics and their influence on gut microbiota. It should be noted that even now few studies have been carried out on humans to elucidate the effect of these microorganisms on body

weight, and existing studies still show contradictory results; the available literature arises from findings found in animals (rats) [4].

However, several ways in which probiotics may influence adiposity and weight gain have been identified. These range from extracting calories from nutrients, generating specific metabolites, to modulating the behaviour of the brain-intestine axis [27].

These hypotheses arise from research suggesting that obese individuals have a different composition of gut microbiota to subjects of a normal weight. For example, in people with obesity, they have observed a decrease in members of phylum *Bacteroidetes* (*Prevotella* and *Bacteroides* genera) and an increase in members of phylum *Firmicutes* (*Clostridium*, *Enterococcus*, *Lactobacillus*). It has been suggested that the aforementioned microorganisms have a greater capacity to extract energy from the undigestible waste products of nutrients that pass through the large intestine. These alterations are normalised with both phyla, when the subjects lose weight (increase of *Bacteroidetes* and decrease of *Firmicutes*) [29]. It has been observed that obese and inactive people have greater adiposity and insulin resistance but also less diversity and microbial composition (**Figure 3**) [4].

A study conducted at Laval University in Canada evaluated the effect of *Lactobacillus rhamnosus* probiotic (LRP) supplementation on weight reduction, appetite control and eating behaviour in a group of obese men and women. The results showed that, in the group of women, the consumption of this microorganism provides beneficial effects on mood and behaviour linked to dietary intake, compared to the group of men and those who received the placebo. This opens up a series of possibilities for health professionals with regard to the implementation of successful obesity programmes, especially considering that many individuals experience great difficulties when trying to sustain their weight loss. This is mainly due to poor appetite control and not taking the necessary action to lead a healthy lifestyle. Based on the findings of this research, it has been suggested that some probiotic strains could be included as environmental factors with regard to body weight loss and maintenance [27].

Corroborating these findings, a multicentre, randomised, double-blind study conducted on 29 men and 14 women, using the microorganism *Lactobacillus gasseri* and administered through a probiotic yogurt for 12 weeks, demonstrated, in the individuals analysed, a 4.6% decrease in areas of abdominal and subcutaneous fat, as well as a decrease in BMI, waist and hip circumference [4].

Nevertheless, and contrary to these results, several investigations using a similar model maintain that administering yogurt with other species of probiotics such as
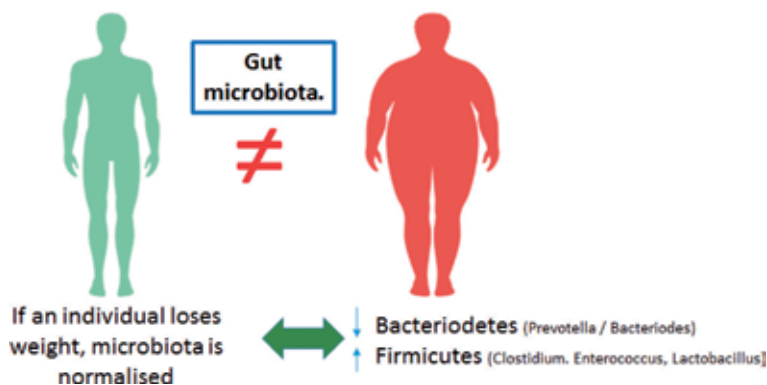

Figure 3.
Gut microbiota imbalance between healthy and obese individuals.

Bacillus lactis Bb 12 and *Lactobacillus acidophilus* La5 has no effect on body weight, BMI and serum lipid levels. This is also the case with *Streptococcus thermophilus* in fermented milk for 8 weeks [4].

As previously mentioned, the impact of consuming probiotics in relation to obesity and a variety of parameters has been widely documented. This is, thanks to studies on rats, used when administering different strains of the *Lactobacillus* and *Bifidobacterium* genera; various hypotheses have emerged from these investigations. More specifically, one of these arose from observing axenic mice (without gut microbiota). Despite consuming 30% more food than conventional animals of the same age and weight, they have 42% less total body fat. However, once the microbiota from conventional mice was transplanted into the digestive tract of the axenic mice, these latter experienced a 57% increase in total body fat. The mechanism associated with this increase is linked to the increased activity of the enzyme lipoprotein lipase, which inhibits the fasting-induced adipocyte factor (FIAF) hormone due to the effect of the gut microbiota. This explains why axenic mice do not show weight gain even when consuming a high-calorie diet. Various studies carried out on rats show that the 'antiobesity' effect is determined by differences between microbial species and strains [20].

There is not much research on the effect of probiotics and their relationship with obesity; however, the findings that are available indicate that the changes caused by gut microbiota could be useful as another strategy in the current quest to combat obesity. However, it is necessary to continue researching in this area, with more emphasis on investigations on humans and populations with a representative number of subjects so that results can be valid and extrapolated.

7. Discussion

Currently, obesity is a major public health problem worldwide. There is no specific cause that determines the development of this disease; on the contrary, it is the result of numerous factors that interact, including genetic, environmental, food, lifestyles, etc. [27]; however, the study of intestinal microbiota and its implication in obesity has aroused curiosity among scientists. The current information obtained comes mainly from the work done in rats and, exceptionally, in humans [17].

Although much of the research indicates that there are differences in the composition of the intestinal microbiota of obese and normal subjects, there is still controversy about it [4].

As indicated in this chapter, the development of these intestinal bacterial communities depends to a large extent on the host and can be modified by exogenous and endogenous influences. Ghosh et al. in 2011 talked about the direct relationship between food patterns and the microbiota composition [18].

In 2013, Devaraj et al. established the existing association in the imbalance of the microbiota where the composition of this is affected producing an increase of the *Firmicutes* genus and a decrease of the *Bacteroidetes*; this instability would affect the metabolism of the human being and as a consequence the appearance of various metabolic diseases among which obesity stands out [21]. In the same way, several publications continue confirming this situation, for example, the study of Jameel Barkat of the year 2018 relates the role of the microbiota in the regulation of energy balance, food absorption and its relationship with the appearance of various diseases: diabetes, metabolic syndrome and obesity, among others [30]. A study was conducted in 36 adults analysing the faecal bacterial composition, finding a decrease in *Firmicutes* and *Clostridia* in diabetic patients compared to the control group. This same research reaffirms that among the factors that alter the stability

and composition of the microbiota are the changes in the diet (high-fat diets) that impact on the relationship between *Firmicutes* and *Bacteroidetes* [31].

In order to demonstrate the aforementioned, it is convenient to point out two investigations carried out in 2015; Kasai et al. [32] conducted a study in Japanese population where they observed that bacterial diversity was significantly higher in obese subjects than in nonobese subjects. In the first group, there was a decrease of the *Bacteroidetes* species and increase of *Firmicutes* [31]. Angelakis et al. showed in a study performed in thin and obese subjects that the *Firmicutes* and *Actinobacteria* genera were the most predominant of the duodenal microbiota; however, in subjects with obesity, a higher proportion of anaerobic and lower proportions of aerobic genus were observed [33].

On the other hand, there are other authors such as Murugesan et al. that indicate in a study carried out in 190 Mexican children between 9 and 11 years old no significant differences between the different bacterial species of microbiota in the different evaluated subjects [34]. The same happens in Hu et al. In 2015, when 134 Korean adolescents from 13 to 16 years old were evaluated, no significant differences were found between the *Bacteroidetes*, *Firmicutes* and *Proteobacteria* populations [35].

It is widely recognised in the scientific community that diets with high-fat content favour the development of resistance to leptin, hyperphagia and therefore obesity. A study points out that the inclusion in the diet of the oligofructose prebiotic prevents the development of resistance to leptin and hyperphagia in rats; from this it is concluded that oligofructose reduces the energy of ingestion, and therefore it is suggested that it has the potential for the obesity treatment [36].

On the other hand, the use of probiotics is associated with multiple health benefits, which are highly endorsed by the scientific community. So far, the data that associate a specific type of microorganisms with human obesity are not conclusive, since they do not say whether it is the microbiota that plays a cause-effect function of obesity or whether it is the intestinal microbiota that is modulated in response to obesogenic diets and other factors related to the pathogenesis of this condition [28–30, 37].

Studies aimed at modulating the gut microbiota to prevent or control the obesity of the host, including the use of probiotics, show positive results [25, 30].

With the reviewed bibliographic evidence, it is crucial to highlight the importance of including the gut microbiota as one of the factors involved in the management of obesity; however, more conclusive information must be analysed about it evaluating what happens even in other specific groups such as the child population.

8. Conclusions

As has been discussed in this chapter, multiple factors influence the development of obesity, including diet, a sedentary lifestyle, selection and an inadequate intake of nutrients such as simple carbohydrates and saturated fats. It has also been observed that obese individuals and those of a normal weight have different compositions of gut microbiota, with some bacterial families more predominate than others, which in turn modulates an individual's energy balance in some way and promotes the development of obesity. It has been established that a dietary supply of prebiotics and probiotics, for which there is scientific evidence demonstrating their benefits, could serve as an additional tool to curb the rising trend of obesity in the population.

However, more studies using randomised, double-blind, controlled trials are necessary to demonstrate the efficacy of prebiotics and probiotics for the prevention or treatment of overweight and obesity.

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Conflict of interest

The work team declares that it has no conflict of interest of any kind.

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One of the most prevalent and important health problems in the world is periodontal and plaque-related diseases for which antibiotic drugs with their associated side effects are used as treatment. With increasing resistance to antibiotics and a desire from the general public for "natural" therapies, there is a need to minimize antibiotic use and develop new treatments for oral diseases without antimicrobial agents. Probiotics are viable microorganisms that provide a health benefit to the host when administered in adequate amounts; studies show that probiotics have the potential to modify the oral microbiota and decrease the colony-forming unit counts of the oral pathogens being investigated to prevent or treat oral diseases, such as dental caries and the periodontal diseases. In addition, the identification of specific strains with probiotic activity is required for any oral infectious disease to determine the exact dose, the time of treatment, and the ideal vehicle.

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