

BACTERIOTHERAPY: A NOVEL THERAPEUTIC APPROACH

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ABSTRACT

The alteration in the homeostasis of gut microbiota and harmful bacteria is associated with various kinds of diseases. Many gastrointestinal disorders such as inflammatory bowel diseases, irritable bowel syndrome, colorectal cancer and systemic diseases such as diabetes, obesity and atherosclerosis are due to dysbiosis in human gut. Many recent clinical studies revealed that probiotic is effective in treatment of various ailments by defending against colonization by opportunistic pathogens, production of antimicrobial substances and immunomodulation. Studies have shown that prebiotics and probiotics or the combinations of two i.e. synbiotics can restore the aberrant gut and can improve the health of gut. There is rapid growth and demand of dairy-based probiotics food in the market due to its awareness among the consumers. Bacteriotherapy has good hold future as novel therapy as consumers are looking for safe, cost effective and no adverse side effects therapeutic approach.

Keywords: Probiotics, Prebiotics, Gut microbiome, Synbiotics, Immunomodulation

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INTRODUCTION

Human beings are in a dynamic relationship with the microbes which resides in various parts of the body. The average human being harbour as many as 10^{14} bacterial cells, a number that is 10 times greater than the number of human cells present in our bodies. An imbalance between the protective and harmful bacteria leads to various diseases. Diseases caused by the dysbiosis in the gut can be reversed by the used of probiotic. Earlier researchers were mainly focussed on the pathogenic bacteria which cause various ailments and there were few researchers on the non-pathogenic bacteria which play a significant role in combating various diseases. But interest in bacteriotherapy which is the used of probiotics, prebiotics or synbiotics has increased over the past few decades as people are aware about its positive health impact on the host.

The interest in bacteriotherapy also stems from the growing awareness among the consumers regarding the safety aspects of the use of chemical drugs. There is rising demand for functional food incorporating probiotic worldwide.

The history of probiotics began with the history of man. Cheese and fermented milk were well known to the Greeks and Romans, who recommended their consumption, especially for children and convalescents [1]. The existence of the concept of probiotics took place around 1900 when Nobel Prize-winning Eli Metchnikoff at the Pasteur Institute suggested that "the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes"[2]. Tissier, who is a French paediatrician, recommended the administration of bifidobacteria to infants suffering from diarrhoea, claiming that bifidobacteria supersede the putrefactive bacteria that cause the disease [3].

Gut microbiome

The collection of microorganisms (friendly bacteria) which live in peaceful coexistence with their hosts has been referred to as the microbiota, microflora or normal flora [4-6]. The diversity of microbial numbers is determined by both intrinsic and extrinsic factors. Intrinsic factor includes gastrointestinal tract (GIT) sections, and the extrinsic factor includes diet, stress, drugs, etc. The human body is inhabited by different types of microbes. Microbes flourish on our skin and in the genitourinary, gastrointestinal and respiratory tracts [7, 8, 6, 9]. Among all the sites, GIT is the most densely populated area with the colon alone harbouring over 10^{10}

10^{12} colony forming units per gram, 70% of all microbes in the human body [10]. The GIT of a human being is inhabited by different types of intestinal microbiota which interact with the host dynamically in order to maintain a state of equilibrium. The intestinal microbiota contributed many important functions for its host which includes maturation of the gut, nutrition of the host, resistance to pathogens and the maintenance of host health [11]. The microbial ecosystem of gut influences the overall well-being of the host. Among intestinal bacterial species, lactic acid bacteria and bifidobacteria are considered particularly important for their health-promoting benefits such as the prevention of gut colonization by pathogens [12]. The disturbances of homeostasis of the beneficial microbes and pathobiont lead to dysbiosis-associated diseases.

Probiotics

In recent year, there is a momentum of change among the people with regards to the use of natural therapy without any adverse effects for the various ailments. The global market of probiotic ingredients, supplements, and the food was worth \$14.9 billion in 2007 and it was expected to reach 15.9 billion in 2008, and 19.6 billion in 2013, representing a compound annual growth rate of 4.3 % [13]. The data indicates that people are more inclined to use natural based food supplement as a potential therapy.

The word "probiotic" comes from Greek language "pro bios" which means "for life" opposed to "antibiotics" which means "against life" currently used to name bacteria associated with beneficial effects for humans and animals. The term was first used in 1965 in contrast to the word antibiotic and defined as "substances secreted by one microorganism, which stimulates the growth of another" [14]. Parker in 1974 defines probiotic as "substances and organisms which contribute to intestinal microbial balance" [15]. The definition of probiotics was broadened by Havenaar and Huis in't Veld in 1992 including mono or mixed culture of live microorganisms which applied for animal and man [16, 17].

According to the expert panel of Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [18]. The mechanisms of action of probiotics on the GIT of human host are not fully understood, but different approaches are made to know the underlying mechanism of the probiotics on the host. Some of the mechanism of colonization resistance or competitive exclusion is often used to explain the mode of action of probiotics. The mechanisms by which probiotics exert

biological effects are still poorly understood, but the non-specific terms such as colonization resistance or competitive exclusion are often used to explain their mode of action [19]. Colonization resistance or competitive exclusion describes a phenomenon whereby the indigenous anaerobic flora limits the concentration of potentially pathogenic (mostly aerobic) flora in the digestive tract [20]. The probiotic effects of probiotics may be expressed by three main mechanisms of action [21].

(i) Suppression of pathogenic microorganisms in the intestinal tract by the production of antibacterial substances, competition for nutrients and competition for adhesion receptors on the gut epithelium. Probiotic *Lactobacillus salivarius* subspecies *salivarius* UCC118 produces a 2-peptide bacteriocin, ABP-118 which inhibits several pathogens including *Enterococcus*, *Bacillus*, *Listeria*, *Staphylococcus*, and *Salmonella* species [22].

(ii) Alteration of microbial metabolism in the intestinal tract by increasing the activity of useful enzymes, e.g. β -galactosidase in the alleviation of lactose maldigestion and decreasing the activity of some colonic enzymes such as nitroreductase and azo reductase known to have carcinogenic effects. (iii) Immunomodulation of the host defence which includes the innate as well as the acquired immune system. Probiotics can alter mucosal immunity considerably as they are able to affect many host cell types involved in the local and systemic immune responses including epithelial cells, dendritic cells (DCs), T cells, regulatory T (T_{reg}) cells, monocytes/macrophages, immunoglobulin A (IgA)-producing B cells, natural killer cell and by induction of T-cell apoptosis [23].

Beneficial health effects of probiotics

There is growing scientific evidence to support the concept that the maintenance of healthy gut microflora may provide protection against gastrointestinal disorders including gastrointestinal infections, inflammatory bowel diseases, and even cancer [24, 25]. A number of health-related effects of probiotics have been suggested and are partially established. Probiotics are used to improve the health of the host in various ways such as treatment of lactose intolerance, treatment of allergy, restoration of normal intestinal microflora during the treatment of antibiotic therapy, prevention of antibiotic-associated diarrhoea, rotavirus diarrhoea and the bacterial diarrhoea, treatment of allergy, treatment of irritable bowel syndrome and prevention of cancer. Probiotics are used to improve the health of the host in various ways (fig. 1).

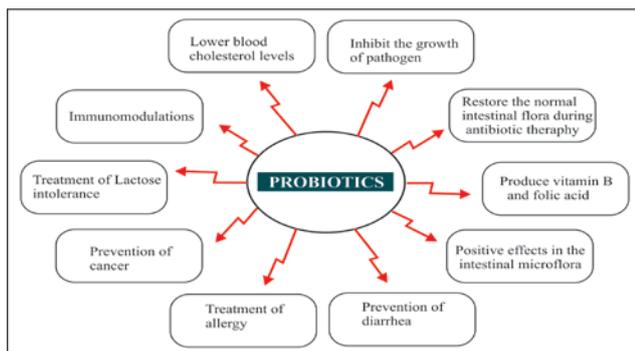


Fig. 1: Beneficial effects of probiotics on human health modified from Gibson and Roberfroid [26]

Lactose intolerance

The person who suffers from lactose intolerance is not able to metabolise lactose which is due to a congenital deficiency of the enzyme β -galactosidase and the symptoms include abdominal pain, bloating, flatulence, cramping, and diarrhoea. There are evidence from several studies that the addition of certain starter cultures to milk products allows the lactose intolerant people to consume those products without the usual rise of breath hydrogen or associated symptoms [27, 28].

Rotavirus diarrhoea

Diarrhoea is a major worldwide health problem which is responsible for the death of several million people annually especially in the developing countries. A few probiotic strains have been reported to be effective in the treatment of rotavirus diarrhoea. The best-studied strain, *Lactobacillus rhamnosus* strain GG (ATCC 53013) reduces the duration of diarrhoea to about half in children with rotavirus diarrhoea in randomised double-blinded and placebo controlled studies [25, 29].

Antibiotic-associated diarrhoea

Antibiotic therapy is well known to destroy the normal bacterial population of the digestive tract, which allows harmful bacteria to colonize and irritate the host gut and cause antibiotic-associated diarrhea [30]. Antibiotic therapy causes diarrhoea which is due to the suppression of normal microflora present in the intestine. Clinical studies indicate that antibiotic-associated diarrhoea has been prevented by *Lactobacillus* GG strain in a yoghurt from using a dose of two cups of yoghurt daily with about 10^7 cfu/ml in healthy volunteers or as a freeze-dried product using doses varying from 10^9 to 10^{10} cfu/day in infants receiving antibiotics [31-33].

Acute diarrhoea

Acute diarrhoea in children is caused by the infection of rotaviruses. In a study, children from one month to 36 mo of age showed that the usage of probiotic lactic acid bacteria and/or bifidobacteria on a daily basis, not depending on whether these organisms were part of fermented milk, capsule or oral rehydration solution, decreases the duration of diarrhea for 30-40 % [34]. The probiotic strains of bacteria *Lactobacillus rhamnosus* GG, *Lactobacillus reuteri*, *Lactobacillus delbrueckii* subsp. *Bulgaricus*, *Bifidobacterium lactis*, and *Streptococcus thermophilus* have not only therapeutic effect but also have a preventive effect on the manifestation of acute diarrhea in children [35].

Allergy

Allergy can be defined as the disordered function of the immune defence system. The prevalence of allergic diseases has increased over the last 3 to 4 decades. The research on the role of probiotic reducing/preventing the allergy is limited. However, they may exert a beneficial effect by improving mucosal barrier function and microbial stimulation immune system [36]. Probiotic bacteria are important in down regulating inflammation associated with hypersensitivity reactions in patients with atopic eczema and food allergy [37, 38].

Cholesterol reduction

Cholesterol is an important basic building block for body tissues, however, elevated blood cholesterol is an important risk factor for coronary heart diseases. WHO has predicted that cardiovascular diseases will remain the leading causes of death, affecting approximately 23.6 million people around the world by 2030. Drugs which effectively reduce cholesterol levels are available for the treatment of high cholesterol however they are expensive and are known to have severe side effects [39]. The serum cholesterol levels of Maasai warriors in Africa are low, and it was noticed that large amounts of *Lactobacillus* fermented milk are regularly consumed [40, 41]. Since then there has been much interest on the investigation of LAB on hypercholesteremia.

Immunomodulation

One of the promising effects of probiotic is immunomodulation. However, the exact mechanism of immunomodulation in the host is not clearly known. Several researchers have studied on the effects of probiotics on immune system stimulation. Probiotics affect the immune system in different ways which include producing cytokines, stimulating macrophages, increasing secretory IgA concentrations [42, 28, 43]. Consumption of yogurt or lactic acid bacteria (e. g., *L. casei*, *L. rhamnosus* GG, and other strains) also modulates the production of several cytokines, which have diverse roles in regulating immune functions [44].

Prevention of cancer

There are many *in vivo* and *in vitro* studies which pointed out the beneficial effects of probiotics on suppression of cancer. In *in-vitro*

experiments, it was found that LAB in dairy products can reduce mutagenicity of some known mutagens such as 1, 2-dimethylhydrazine [25]. Seow *et al.* investigated the high growth

inhibition in human bladder cancer cell lines using *Lactobacillus* and compared with the effect of *Mycobacterium bovis* (BCG) on human bladder cell lines [45].

Probiotic strain	Clinical effects	References
<i>Lactobacillus rhamnosus</i> GG(ATCC 53013)	Lowering faecal enzyme activities, reduction of antibiotic-associated diarrhoea in children treatment and prevention of rotavirus and acute diarrhoea in children, treatment of relapsing <i>Clostridium difficile</i> diarrhoea, immune response modulation, and alleviation of atopic symptoms in children.	[31, 37, 46-49]
<i>Bifidobacterium lactis</i> Bb-12	Prevention of traveller's diarrhoea, treatment of viral diarrhoea including rotavirus diarrhoea, modulation of intestinal flora improvement of constipation, modulation of immune response, alleviation of atopic dermatitis symptoms in children.	[50-57]
<i>Lactobacillus casei</i> Shirota	Modulation of intestinal flora, lowering faecal enzyme activities, positive effects on superficial bladder cancer and cervical cancer, no influence on the immune system of healthy subjects.	[58-62]
<i>Lactobacillus johnsonii</i> (acidophilus) Lj-1 (La1)	Modulation of intestinal flora, immune enhancement, adjuvant in <i>Helicobacter pylori</i> treatment.	[54, 56, 63-65]
<i>Lactobacillus plantarum</i> DSM9843	Modulation of intestinal flora, increase in faecal short-chain fatty acid content	[66, 67]
<i>Bifidobacterium longum</i> SBT 2928	Inhibited adhesion of enterotoxigenic <i>Escherichia coli</i> strain Pb176 which expresses colonization factor adhesion II, to the ganglioside GM1 molecule <i>in vitro</i> .	[68]
<i>Bifidobacterium animalis</i> ssp. <i>lactis</i> HN019	Immunostimulatory properties in children in day care settings and in adults and has inhibitory activity against pathogens (<i>Escherichia coli</i> O157:H7, <i>Salmonella Staphylococcus aureus</i> and rotavirus	[69, 70]
<i>B. animalis</i> ssp. <i>lactis</i> DN-173010	Reduced colonic transit time, possibly providing relief for intestinal discomfort (e. g., bloating and constipation)	[71, 72]

Clinical efficacy of some of the probiotics

Prebiotics

Prebiotics are defined as the dietary fibre which selectively promotes the growth or activity of probiotics in the gut and thus improve the health of the host.

The term probiotics was coined by Gibson and Roberfroid [26]. Even though the concept of prebiotic is recent; it has attracted researchers for its beneficial health effects to the host. However not all dietary fibres are prebiotics, and certain criteria need to be established before classifying dietary carbohydrate as prebiotics. Some of the criteria of being a prebiotics are (i) resistance to gastric acidity, to hydrolysis by mammalian enzymes and to gastrointestinal absorption; (ii) fermentation by intestinal microflora and (iii) selective stimulation of the growth and/or activity of those intestinal bacteria that contribute to health and well-being [73]. Prebiotics were classified based on a set of common criteria, inulin, fructo oligosaccharides (FOS), galacto oligosaccharides (GOS), lactulose and polydextrose are recognized as the established prebiotics whereas isomalto oligosaccharides (IMO), xylo-oligosaccharides (XOS) and lactitol are categorized as emerging probiotic [74]. Dietary fibre mainly oligosaccharides and polysaccharides fermented in the colon may act as prebiotics [27, 75]. The importance of prebiotics as an enhancer of the growth and performance of probiotic bacteria has been documented in humans [76, 27]. A desirable property for prebiotics is the ability to act in the most distal region of the colon, which is known to be the site of origin of several chronic diseases including colon cancer and ulcerative colitis [77]. The consumption of probiotics can modulate immune parameters in GALT, secondary lymphoid tissues and peripheral circulation [78]. Necrotizing enterocolitis (NEC) is a serious intestinal disease in infant and the main cause of morbidity and mortality in preterm babies. Prebiotic supplemented formula increase stool colony counts of bifidobacteria and lactobacilli in preterm neonates without adversely affecting weight gain [79].

Synbiotics

The synbiotics concept was first introduced, along with prebiotics, "mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, thus improving host welfare" [26]. In a study carried out in children with ulcerative colitis, synbiotic therapy (*B longum* R0175+inulin) when provided in addition to conventional treatment found to be a

safe and effective strategy [80]. When FOS, SOS or insulin combined with *Lactobacillus acidophilus*, *Bifidobacterium lactis*, or *Lactobacillus casei* were given to mice, it was found that a SOS-or FOS-containing diet appeared to stimulate the growth of *L. acidophilus* and sustain its highest level. FOS and dietary inulin treatment exerted the same effects on *B. lactis*. SOS, FOS or inulin improved the survival and retention time of *L. casei* [81]. Human milk is considered to be the "gold standard" of nutrition of infant for the first six months. Human breast milk contains human milk oligosaccharide (HMO), which is a complex glycan and it is found in high concentration in human breast milk. HMO is a bifidogenic factor which specifically stimulates the growth of bifidobacteria. Human breast milk is considered as nature gifted synbiotic for the infant.

CONCLUSION

With the increased in changing lifestyle and pollution, man are prone to various diseases and antibiotics has become less effective due to increased cases of antibiotic resistance of bacterial pathogen. The solution for the existing problem lies in searching for the alternative therapy which should be effective and should not have any side effects. Probiotics, prebiotics or the combination of both, i.e., synbiotics is the most promising solution for these existing problems as it is cost effective and safe. Over the past few decades, there is an increasing interest in the field of bacteriotherapy. The interests in this field also stems from the growing universal awareness among the consumers regarding the safety aspects related with chemical drugs. Probiotics being endowed with a large number of beneficial attributes offer tremendous opportunities for their extensive application in almost all segments including food, pharma, etc. Therefore, identification and characterization of the probiotic strains is very important as probiotics function are strain specific. Probiotics should be screened with controlled clinical trials and technological properties such as strain stability and viability in products.

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CONFLICTS OF INTERESTS

The authors do not have any conflicts of interest regarding competing financial and/or personal relationships.

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