Probiotics: Microbial Therapy for Health Modulation

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ABSTRACT

The human gastrointestinal tract is a complex ecosystem that harbours a rich and diverse microflora. These microbes live in harmony with the host and exert various beneficial effects on human health by their metabolic activities. However, in our modern life style, frequent and indiscriminate use of antibiotics has disturbed this microbial ecosystem, resulting in occurrence of various bowel diseases. Some live microbial food supplements can re-establish this microbial ecosystem. Such a group of microorganisms, which positively influences the intestinal microbiota by stimulating the growth of beneficial bacteria and suppressing the harmful ones, is collectively known as probiotics. These have been consumed in the form of fermented milk products for centuries. However, scientific interest in their use for health maintenance and disease prevention has emerged over the past few years only.

Various scientific evidences show that probiotics help to reduce several disorders including diarrhea, inflammatory bowel diseases, urinary tract infections, hypertension, allergies, and cancer. Besides, probiotics exert several other benefits also to human beings and animals. Important issues in the probiotic therapy include selection of appropriate strain, its viability during storage, gut persistence potential and functional properties. Another category involved in gut health is prebiotics. These are non-digestible food ingredients, which beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of beneficial bacteria in the colon. This review paper highlights the major health benefits of probiotic organisms, mechanisms of their action, criteria of selection, enumeration, and safety of their use for human health.

Keywords: Probiotics, prebiotics, gut microflora, microbial therapy, health modulation, microbial ecosystem, human health, gut health

1. INTRODUCTION

The major three components of our ecosystem, ie, plants (producers), animals (consumers), and microbes (degraders) have very close association among themselves. The interaction of microbes with animals is generally not taken in positive sense because common man considers them as the agents of diseases. But, this is far away from the reality. In an environment, microbes can interact with each other and with animals in different ways that may be beneficial, neutral or detrimental. Various body parts including skin and mucosal surfaces of animals, are colonised by a diverse group of microbes. Most of these microbes live symbiotically with host and cause no harm. However, sometimes, this microbial harmony is disturbed and then some microbes known as pathogens, adversely affect the health.
The relationship between health and diet is well understood as diet provides the energy and nutrients for metabolic activities and growth. There is an age-old quote by Hippocrates, 'Let food be thy medicine and medicine be thy food', which clearly indicates the importance of food for health. However, modern medicine system has ignored the role of food in medicine for long time. Now, efforts are being made to understand the specific physiological role of diet beyond the nutritional effects.

Recently, functional food products are claimed to promote health and wellbeing of animals including human beings. Besides meeting the requirement of energy, proteins, vitamins, and minerals, these food products provide some additional benefits to body that directly promote the state of wellbeing and health, which further help to reduce the disease incidences. Probiotics and prebiotics have occupied the central position among such functional foods.

Probiotics have attracted attention of the nutritional scientists for the last three decades, leading to better understanding of their biological properties. The word probiotic has been derived from Greek, which means for life. The term probiotic is an antonym to antibiotic and was coined by Lilly and Stillwell to describe the substances produced by one protozoan, which stimulate the growth of another. The concept is not new and there are many references of microbial consumption in history. A Persian version of the Old Testament (Genesis 18:8) states 'Abraham owed his longevity to the consumption of sour milk'.

Probiotics have been used for centuries in the form of substances containing Lactobacillus and Bifidobacterium culture without specific knowledge of their active ingredients or how these work. In 76 BC, the Roman historian Plinius recommended the consumption of fermented milk products for treating gastroenteritis. Real scientific understanding of how probiotics work began in 1907 when Metchnikoff introduced intoxication theory in which he stated that major cause of ageing was toxicants formed by intestinal putrefaction and fermentation and further suggested that drinking beverages such as yoghurt containing lactic acid bacteria would prevent ageing. His observations behind this theory were based on the belief that Bulgarian peasants lived very long as they used to consume large quantities of soured milk. Subsequently he isolated Bulgarian bacillus (now Lactobacillus bulgaricus or Lactobacillus delbrueckii) from soured milk for experimental trials. Since then, lactobacilli suddenly attracted world attention.

2. DEFINITION

Probiotics have been defined in several ways, depending upon our understanding of the mechanisms of action of their effects on health and wellbeing of human beings. In 1974, Parker defined probiotics as organisms and substances that contribute to intestinal microbial balance. But this definition does not exclude antibiotics. Subsequently in 1989, Fuller gave a better definition of probiotics as live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance. This definition stressed on viability of culture and animal as host. Later on, Havenaar, et al, elaborated the term as a viable mono- or mixed culture of microorganisms which when applied to animal or man, beneficially affects the host by improving the properties of the indigenous microflora. Salminen, et al, further broadened the term as a live microbial culture or cultured dairy product that has a beneficial effect on the health and nutrition of the host. This definition implies that probiotics necessarily need not to be viable.

Schrezenmeir and de Vrese suggested that the term nutrition might be omitted from the definition because major effects on nutrition also imply effects on health. Further, they suggested that the definition should not be limited to dairy products because other non-dairy products like sauerkraut also contain probiotic microorganisms. Thus, they defined probiotics as a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonisation) in a compartment of the host and by that exert beneficial effects in this host. This definition confines the probiotic concept and is applicable irrespective of the probiotic site of action and route of administration. In 2002, Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food.
defined probiotics as live microorganisms which when administered in adequate amounts confer a health benefit on the host.

3. GUT MICROFLORA

The human intestinal tract is a complex ecosystem, which harbours a diverse and complex bacterial community. Gastrointestinal (GI) tract is a specialized tube extending from the lips to anus and human body is considered merely an outer covering of this tube. Gastrointestinal tract in a healthy adult is about 30' long and is so complex that it is composed of about $10^{14}$ bacterial cells, a number that exceeds the total human body cells by a factor of ten. More than 75 per cent of the wet weight of our fecal output is composed of bacterial cells and each gram of dry weight is thought to contain approximately $10^{11}$ microbes of about 50 different genera belonging to over 500 different species.

The most common microorganisms found in the gut are methanogens, bacteroides, bifidobacteria, Escherichia coli, eubacteria, Klebsiella spp., streptococci, lactobacilli, staphylococci, bacilli, Fusobacterium spp., clostridia, peptococci and peptostreptococci. Moreover, the bacteria in the gastrointestinal tract are not uniformly distributed. Stomach and duodenum harbours only $10^2$–$10^5$ cells of mostly facultative anaerobic bacteria like Lactobacillus spp. and enterobacteriaceae members. The lower distal part of the human gut is populated mostly with anaerobic genera like Bacteroides, Bifidobacterium, Clostridium, Eubacterium, Fusobacterium, Peptostreptococcus, and Ruminococcus.

The indigenous gut microflora interacts with each other and with human host and thus plays important role in the health and wellbeing of the host. The gut lining is primarily nourished by the nutrients produced by these favorable bacteria. Our body is completely dependent upon the activities of these bacteria for the manufacture of key vitamins, the assimilation and distribution of nutrients, and for the suppression of pathogenic bacteria. Probiotic microorganisms fall mainly under two categories. The first include those bacteria that normally inhabit the intestinal tract, and ingestion of these bacteria improves the intestinal microbial balance. The second category comprise those bacteria that do not normally colonise the intestinal tract and the effect on intestinal microbial balance is expected to be less, eg Lactobacillus bulgaricus, Streptococcus thermophilus, etc.

4. HEALTH BENEFITS OF PROBIOTICS

There is a strong health burden on modern society in the form of various allergic and gastrointestinal diseases due to changed food habits and environmental pollution. Significant immunologic and inflammatory activation constantly prevails in the gut but there is lack of stimuli priming for protective mechanisms, which directs the milieu in the gut towards a propensity to inflammatory diseases. Dietary and microbial antigens provide the earliest and the most significant driving force for the development of defence mechanisms in the gut.

In the past decade, there has been a dramatic increase in scientific research advocating the clinical benefits of ingesting specific non-pathogenic organisms. Probiotics have been shown to reinforce the different lines of gut defence, which are immune exclusion, immune elimination, and immune regulation. These are intended to modify the gastrointestinal microflora in such a way that host-favourable microbial activities are stimulated and those adverse to host are suppressed. A large number of microorganisms have been used as probiotics (Table 1). These mainly act in small intestine. Clinical benefits of probiotics are obtained when gut microbial ecology is disturbed by any change in the environment or diet. Several clinical benefits of probiotics have been found by various experimental trials. Some of these are being described here.

4.1 Gastrointestinal Tract Infections

4.1.1 Acute Diarrhea

The best-documented clinical benefit of probiotics is in the treatment of acute diarrhea. A variety of probiotics organisms including Lactobacillus rhamnosus, Lactobacillus reuteri, Saccharomyces boulardii and Enterococcus faecium have been used in oral rehydration for acute diarrhea. Some probiotic bacteria have been found to increase the
level of IgA concentration against rotavirus in children serum.  

4.1.2 Traveler's Diarrhea

This is the most common health problem of travelers to tropical countries affecting 15-56 percent travelers. This is caused by ingestion of pathogens like enterotoxigenic Escherichia coli, Giardia and Entamoeba histolytica through food or water that overcome the protective effects of the normal intestinal flora. Theoretically, traveler's diarrhea can be prevented simply by the principle of cook it, boil it, peel it, or avoid it but practically it a very difficult. Many antibiotics are able to prevent diarrhea for a short period of time but these are associated with other adverse effects. Prophylactic ingestion of probiotics could decrease the risk of disease. However, the reported benefits of prophylactic probiotics like Saccharomyces boulardii, Enterococcus faecium, Lactobacillus rhamnosus for traveler's diarrhea have been found modest.  

4.1.3 Antibiotic-associated Diarrhea

Antibiotic-associated diarrhea or antimicrobial-associated diarrhea is the most common adverse effect of antibiotic therapy. This is supposed to be due to perturbation of the balance of the normal intestinal flora due to which opportunistic microorganisms overgrow and cause diarrhea. Clostridium difficile is the major agent involved in Antibiotic-associated diarrhea and colitis. Recent studies have shown that selected probiotics agents like Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus rhamnosus, Bifidobacterium longum and Enterococcus faecium have potential to prevent Antibiotic-associated diarrhea. Another biotherapeutic agent, non-pathogenic yeast, Saccharomyces boulardii that grows optimally at body temperature has also been used to cure Antibiotic-associated diarrhea.  

4.1.4 Acute Infantile Diarrhea

Acute infantile diarrhea is a persistent problem in developing countries that can be addressed by inexpensive probiotics. Saavedra, et al. evaluated an infant formula supplement having Bifidobacterium bifidum and Streptococcus thermophilus versus the un-supplemented formula in preventing the diarrhea in hospitalised infant population over a period of 17 months. They found that supplemented formula significantly reduces the risk of diarrhea and shedding of rotavirus.

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Table 1. List of important probiotic organisms used for health modulation.
4.2 Urinary Tract Infections

Urinary tract infections is a very common problem in women that usually occurs when bacteria enter the opening of the urethra and multiply in the urinary tract. Escherichia coli is one of the major pathogens responsible for urinary tract infections. Other bacteria that cause urinary tract infections include Proteus, Klebsiella, Citrobacter, Enterobacter, Pseudomonas spp, Staphylococcus saprophyticus, Chlamydia trachomatis, and Mycoplasma hominis.

These pathogens can be replenished with probiotics organisms through vaginal application. The probiotics strains for use in urinary tract infections should be highly adherent and inhibitory to pathogens. Besides, a healthy vaginal tract harbours a high population of lactobacilli. This fact, coupled with the intestinal route of transmission of bacteria to the urogenital tract has led to the theory that oral probiotics may be useful in treatment or prevention of urinary tract infections in women. Probiotic therapy has been shown to be safe and evidences show that this harnesses the potential to make an input in women’s health.

4.3 Bacterial Vaginosis

Vagina is naturally colonised by Lactobacillus bacteria, which acidify the vaginal environment and suppress the growth of other organisms. Sometimes this normal vaginal flora is disturbed due to which some other, less benevolent organisms including Prevotella bivia, Prevotella. disiens, Porphyromonas species, Mobiluncus species, Peptostreptococcus species, Gardnerella vaginalis and Mycoplasma hominis overpopulate and causes bacterial vaginosis.

Complications arising from bacterial vaginosis include increased risk of sexually-transmitted diseases including human immunodeficiency virus (HIV) and elevated risk of pre-term birth. Probiotics have been proposed as an effective and alternative tool to antibiotics for treatment of bacterial vaginosis.

4.4 Inflammatory Bowel Disease

Inflammatory bowel disease results when cells involved in inflammation and immune response enter into Gastrointestinal tract lining. Inflammatory bowel disease mainly covers two diseases - Crohn's disease, which may develop in any part of Gastrointestinal tract and ulcerative colitis that occurs mainly in large intestine. Intestinal microflora plays a key role in the development of the inflammatory bowel disease resulting in the clinical conditions of Crohn's disease and ulcerative colitis. Inflammatory bowel disease is characterised by a chronic dysregulation of the inflammatory response in the gastrointestinal tract where the number of adherent and pathogenic bacteria is high.

Immunomodulators and antibiotics have not been found much effective in a large proportion of patients with inflammatory bowel disease. Therefore, a potential approach to treat or prevent inflammatory bowel disease could be changing the microflora to a more benign nature. Hence, probiotics have enormous potential in treatment of such diseases as these favourably modulate the host intestinal flora. Animal models of colitis have provided the proof of principle that probiotics can prevent and treat the established intestinal inflammation. Floch has reported the success of using Escherichia coli Nissle strain alone and a multiple organism product in treatment of ulcerative colitis and Crohn's disease, respectively.

4.5 Atopic Eczema

The incidences of allergic diseases are increasing day by day all over the world. Probiotics have potential to reduce such instances, especially in atopic eczema. This is an allergic disease in which there is tendency for excess inflammation in the skin and lining of nose and lungs. Atopic eczema is very common in all parts of the world. It can occur at any stage in the life of a human being but is most common in infants. In a study, perinatal administration of the probiotic Lactobacillus rhamnosus strain GG reduced incidence of atopic eczema in at-risk children during the first two years of life. In another study, supplementation of infant formulas with viable lactobacilli helped in the management of atopic eczema and cow's milk allergy. Similarly, Bifidobacterium lactis modified the changes related to allergic inflammation in infants.
4.6 Blood Cholesterol and Hypertension

Cholesterol is the principal body sterol present in all the tissues. This becomes the precursor of all other steroids in the body. Mainly, cholesterol is synthesised in liver, gut, and skin. Its synthesis and absorption occurs in the intestine. Therefore, intestinal microflora could have profound effects on the lipid metabolism. As probiotics modify the intestinal microflora, these may potentially reduce the risk of heart disease by lowering blood cholesterol levels, increasing resistance of low-density lipoprotein (LDL) cholesterol to oxidation, and reducing blood pressure. Numerous studies have demonstrated that bacteria like *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Lactobacillus bulgaricus* lower cholesterol in a significant way when its level is too high.

The negative effects of hypertension on human health are well known. In some cases, probiotics have been found useful in controlling hypertension. The anti-hypertension activities are supposed due to cell wall fragments of probiotic bacteria or production of bioactive peptides from milk.

4.7 Carcinogenesis

Colon cancer is one of the major causes of cancer morbidity and mortality. Probiotics have been found useful in prevention of colon cancer. Clinical studies have shown that consumption of *Lactobacillus casei* delayed the recurrence of bladder tumours. Similarly, dietary administration of *Bifidobacterium longum* in laboratory animals significantly inhibited the incidences of colon adenocarcinomas and colon tumor multiplicity. In another study on animals, *Bifidobacterium longum* inhibited the mammary and liver carcinogenesis, which indicated that anticarcinogenic activity of probiotics may extend beyond the intestinal tract. Femia et al. reported the use of *Lactobacillus rhamnose* and *Bifidobacterium lactis* in protection of rats against azoxymethane induced colon cancer.

5 MECHANISMS OF ACTION OF PROBIOTICS

The mechanisms, by which the probiotics act, are not yet fully understood. A common belief how probiotics work is that ingestion improves the balance of intestinal microflora due to which pathogen growth is restricted. But this concept is a simplistic and probiotics seem to work by multiple mechanisms. Gut microflora plays an important role in the intestine's defence barrier. Many gastrointestinal dysfunctions are based on disturbances or imbalance of intestinal microflora. When microbial balance is disturbed in the intestine, antigen transport is increased, leading to increased allergic sensitivity. There are several factors, which can disturb the delicate balance of microflora in the gastrointestinal tract. Some of these include overgrowth of undesirable microorganisms, poor hygiene, too many sweets, starchy foods or alcoholic beverages, food allergies, frequent use of antibiotics, exposure of radiations, surgical complications, excessive stress, and environmental toxins.

Various beneficial microorganisms produce essential nutrients through fermentation in the intestine. These include vitamins, short-chain fatty acids, (without which cell may be damaged, leading to a loss of function in the gut lining). Probiotics include viable as well as non-viable cell cultures that lead to better human health by various mechanisms. Some of the postulated mechanisms are discussed here.

5.1 Production of Antimicrobial Substances

Antimicrobial compound production is considered as the most important mechanism by which probiotics act. These compounds suppress the growth of potentially harmful microbes that reside in the human intestinal tract. Probiotic bacteria secrete a large number of anti-microbial compounds, which include organic acids (lactic acid and acetic acid), hydrogen peroxide (in aerobic environment), β-hydroxypropionaldehyde and bacteriocins. Bacteriocins are low molecular weight microbial compounds, which restrict the growth of similar or related bacteria. The intestinal growth of all other kinds of non-intestinal pathogens is strongly inhibited by abundant probiotics fermentation in the small bowel because available sugars are easily fermented to lactic acid and/or ethanol. Viral infectivity is also reduced by probiotics due to ethanol or acid-mediated denaturation of viral envelope proteins. Therefore, pathogens do not overpopulate when sufficient number of probiotic
organisms are present. Thus, objective of probiotic therapy is to restore colonisation resistance until the normal microbial flora becomes re-established.

5.2 Competition of Pathogens for Adhesion Receptors

When the number of probiotic bacteria is more in intestine, pathogens have to compete with these beneficial bacteria for nutrient and space. Moreover, pathogens also compete with probiotic strains for adhesion receptors in which they rarely get success. Thus in this race, probiotics microorganisms resist the establishment of pathogens by competition for food and establishment.

5.3 Production of Digestive Enzymes

Probiotics produce certain digestive enzymes or influence enzyme activity in the gastrointestinal tract, which may account for some of their physiologic effects. In a study, probiotics effect was investigated on the development of carbohydrase and peptidase activity in the mucosa at five sites along the small intestine of pigs. Probiotic extended a significant effect on the development of sucrase (EC 3.2.1.48), lactase (EC 3.2.1.23) and tripeptidase (EC 3.4.11.4) activities. When the concentration of lactose cleaving-enzyme, ie β-galactosidase is too small in the brush border membrane of the mucosa of the small intestine, it results in lactose intolerance. Probiotics and fermented milk products help in lactose digestion because active microbial β-galactosidase in bacteria survives gastric passage and is released by bile salts into the small intestine, where it supports lactose digestion. Thus, probiotic organisms help in digestion process by secreting various digestive enzymes that help foods to breakdown.

5.4 Production of Short-chain Fatty Acids

In the colon, short-chain fatty acids (SCFAs), such as acetic acid, propionic acid, and butyric acid are essentially produced by the anaerobic fermentation of carbohydrates, including dietary fibre, resistant starch, and mucus. These short-chain fatty acids exert a variety of physiologic effects. Research studies have suggested that short-chain fatty acids exert a therapeutic effect on some human beings and experimental animal diseases. Relative efficacy of short-chain fatty acids production was found to differ between intestinal microflora, ie, bifidobacteria, lactobacilli, bacteroidaseae, clostridia, and eubacteria.

Out of these bacteria, Clostridium butyricum was having high capability to produce n-butyric acid. Besides, short-chain fatty acids become the preferential fuel for mucosa cells and short-chain fatty acids, such as lactic acid, provide up to 70 per cent of the energy required by intestinal epithelial cells. These make intestinal pH more acidic due to which intestinal pathogens do not overgrow and their population is kept in check. Lower intestinal pH facilitates absorption of minerals such as calcium, magnesium, and zinc. Short-chain fatty acids have been reported to have therapeuetic potential in inflammatory bowel disease, ulcerative diseases, various allergic diseases, and even in cancer.

5.5 Stimulation of Immune System

Probiotic organisms modulate the host immunity functions by enhancing both the cell-mediated and humoral immune systems. It has been well established that germ-free animals have underdeveloped immune system that can be easily restored upon the introduction of even a single bacterial species. Even, microbial imbalance in the gut leads to exacerbated effector response and chronic inflammatory diseases. It has been found that probiotic organisms increase numbers of circulating white blood cells, stimulate phagocytosis, and elevate levels of antigen-specific antibodies.

Experiments with laboratory animals have shown that lactobacilli increase the proportions of CD25+ cells in the lamina propria and decrease the T cell reactivity. Probiotic organising have been reported to modulate the host's immune responses to potentially harmless antigens by prompting down-regulation of hypersensitivity reactions. One study correlated the low levels of lactobacilli in the vaginal tract with increased incidence of HIV-1 in younger women, which proved the importance of maintaining healthy flora to ensure proper immune function.
5.6 Reduction in Food Allergy

Food allergies are caused by the body's sensitivity to certain foods. On the basis of epidemiological studies, it has been hypothesised that stimulation of the immune system by certain microbial products may prevent or treat allergic diseases. Probiotics have been proven effective in food allergy and allergic inflammation. Probiotics promote potentially antiallergenic processes like T-helper1 type immunity, induce oral tolerance, and IgA production. Probiotic bacteria have been reported to promote endogenous barrier mechanisms in patients with atopic dermatitis and food allergy. Kalliomäki et al. found that gut microflora has unique, yet largely unexplored, endogenous immunomodulatory properties indispensable in the fight against the increasing frequency of atopic, and possibly other immunological diseases.

5.7 Anticarcinogenic Activity

Probiotics have been found to inhibit some cancer-causing fecal bacterial enzymes. Lactobacilli do not produce enzymes like nitrate reductase, azoreductase, 7-α-dehydroxylase and tryptofanase, which are involved in the biosynthesis of mutagenous compounds. Besides, they suppress the growth of other intestinal bacteria that convert pre-carcinogens into carcinogens. Consumption of probiotics resulted in decreased activity of β-glucoronidase, nitroreductase, and chloolylglycine hydrolase. Sometimes, probiotic strains bind to mutagenic compounds in intestine and thus reduce the absorption of mutagen from the intestine. Thus, they play important role in preventing the small bowel carcinogenesis.

5.8 Inhibition of Bacterial Translocation

Bacterial translocation and subsequent sepsis is a problem in immuno-compromised patients, where intestinal bacteria pass across the intestinal wall and reach extraintestinal sites. Several facultative anaerobes like Escherichia coli, Klebsiella pneumonia, and Proteus mirabilis can translocate to mesenteric lymph nodes. Probiotics exert inhibitory effects on bacterial translocation. Probiotic bacterium Lactobacillus casei inhibited the bacterial translocation of Escherichia coli in a dose-dependent manner in an in vitro cell culture model.

5.9 Lowering of Serum Cholesterol

Probiotics can improve the health in human beings by lowering the serum cholesterol. This has been hypothesized from the fact that bacteria can remove cholesterol from culture media. Several strains including Lactobacillus acidophilus, Lactobacillus gasseri, Enterococcus faecium, Streptococcus thermophilus have been found to lower serum cholesterol in different studies. Now it has been established that cholesterol is removed from the culture media by precipitation with free bile acids, formed in the culture media due to activity of bacterial enzyme bile salt hydrolase.

6. SELECTION OF PROBIOTIC STRAIN

The selection of health-promoting probiotic agents has been an apparent problem since the time of Metchnikoff, who first proposed the therapeutic use of these bacteria. Commercial products do not always specify the strain of microorganisms they contain. Most of the ingested bacteria are killed in the stomach. Only a small number of strains colonise the human gastrointestinal tract. Such strains are known as implantable strains. Implantation is thought to be the most important criteria of a probiotics strain to harness its full potential.

In the last decade, scientists have arrived on a consensus on some criteria like ecological origin of bacteria, their tolerance to the hostile conditions of stomach and small intestine, and their ability to adhere to intestinal surfaces. Several criteria have been defined that consider a microorganism as a probiotic agent. According to these criteria, a probiotic microorganism should be of human origin, demonstrate non-pathogenic behaviour, should be beneficial to host animal in some way, exhibit resistance to technological processes, prove resistant to gastric acid and bile, adhere to gut epithelial tissues, be able to persist in the gastrointestinal tract, produce anti-microbial substances, modulate immune responses, and should have the ability to influence metabolic activities. Besides all these factors, stability of a probiotic strain is also equally important. Commercially available dried formats of probiotic strains should be stable over a longer period of time at room temperature.
There are several techniques developed for detection and enumeration of probiotic organisms in bio-products. Most of these techniques are based on oxygen tolerance, nutritional requirement, antibiotic susceptibility, and colony morphology. However, phenotypic analysis of bacteria depends upon culture, which makes identification of these organisms a difficult procedure.

Recently, a number of molecular methods have been introduced for detection and enumeration of these bacteria. Systematic bacteriology has undergone a revolution with the advent of 16S ribosomal RNA sequence analyses. The major advantage of these molecular methods is that there is no need of cultivation of microorganisms and the methods are universally applicable. These are helpful, especially for detection of gastrointestinal tract organisms most of which are strict anaerobes.

Most of these methods use sequence analysis of ribosomal DNA (rDNA). 16S rDNA gene from the sample is amplified with polymerase chain reaction (PCR) using primers against universally conserved regions of the gene. The PCR products are directly sequenced and compared to the rDNA database. Plasmid profiling is also done for the characterisation of probiotic strains. However, this is not very much reproducible because of the instability of extra-chromosomal DNA.

Other techniques include restriction enzyme analysis (REA), pulse field gel electrophoresis (PFGE) and random amplified polymorphic DNA (RAPD). In REA, chromosomal DNA is digested with restriction enzymes and fragments are separated on agarose gel and banding pattern is visualised using computer-aided programme. This technique has been used successfully in differentiation of various probiotic bacteria. In PFGE, the orientation of electric field is changed periodically and rare cutting enzymes are used. Therefore, less number of fragments is obtained due to which banding pattern comes very clear. RAPD is a PCR-based technique in which short random primers are used for amplification of randomly-sized DNA fragments.

Microorganisms can also be monitored directly by fluorescent in situ hybridisation (FISH). In this technique, fluorescent probes are prepared by end labelling of rRNA-targeted oligonucleotides with fluorescent dye. Being small in size, these probes penetrate the whole fixed cells and hybridize to intracellular rRNA. In several cases, probiotics organisms are monitored in gastro intestinal tract by tagging the organisms with reporter genes, viz., luciferase gene of Vibrio harveyi and green fluorescent protein (gfp) gene of Aquaria victoria.

7. SAFETY OF PROBIOTICS

Safety of probiotics is of utmost importance as these are the component of food stuffs or drugs. Many of the probiotics strains like Lactobacillus species have been used since ancient times, and until now, the safety of these microbes has not been questioned. Therefore, members of some genera like Lactobacillus and Lactococcus have acquired the status of generally recognised as safe (GRAS).

Generally, microorganisms can be divided into three groups, non-pathogens, opportunistic pathogens, and pathogens. Sometimes, non-pathogenic microbes, which survive within the host, can cause infection under certain circumstances, like in severely immunocompromised hosts. In a few cases, lactic acid bacteria, which are used as probiotics, have been isolated from local or systemic infections, including septicemia, meningitis, and endocarditis. However, such reports are scanty. A Lactobacillus rhamnosus strain indistinguishable from Lactobacillus rhamnosus GG was isolated from a liver abscess from an elderly lady with a history of hypertension and diabetes mellitus. In another case a probiotic Lactobacillus rhamnosus strain was supposed to cause endocarditis in an elderly male. These findings indicate that although probiotic products have been consumed safely, over the years, occasional severe infections may occur in immuno-compromised patients.

Nowadays, new probiotic strains having more beneficial effects are being developed. Therefore, it becomes essential to ensure their safety. Safety aspects include specifications such as origin, non-pathogenicity, toxicity and antibiotic resistance characteristics. The absence of pathogenicity
and infectivity is a requisite of probiotics bacteria. However, it is very difficult to prove the infectivity of probiotic bacteria. The safety of a bacterial strain may be evaluated by considering questions such as whether bacterial invasion of the host leads to infection, whether infection results in severe outcome, and the effect of association of the bacteria with the host. Viable and non-viable cells of *Lactobacillus rhamnose*, *Lactobacillus acidophilus* and *Bifidobacterium lactis* promoted the growth of young Swiss mice ensuring the safety of these strains. Similarly, *Propionibacterium jensenii* survived in vivo gastrointestinal tract of rats with no adverse affect on the animal.

Second important requisite of probiotics is that they should not produce harmful or toxic substances by metabolic activities. In the intestine, ammonia, indole-phenol and amines are produced from the digestion of proteins. Probiotic bacteria have lower deaminase activity involved in the production of ammonia from amino acids and higher ammonia assimilation activity than the other intestinal bacteria. Moreover, probiotics bacteria have been found to lack 7-α-dehydrogenase activity, which is involved in the production of secondary bile acids.

Antibiotic resistance of probiotics organisms is also important. Many probiotics microbes have acquired antibiotic resistance by contact with antibiotics or through transformation. But there is no report of transfer of antibiotic resistance during therapy. Recently, Asahara, et al. suggested that resistance to host innate defence systems should be considered in the safety assessment of probiotic strains.

8. PREBIOTICS

Sometimes, some food components are not completely degraded in the stomach. These are pass to large bowel where specific group of microorganisms utilises these. Such substances that escape digestion in the upper gut, enter the colon and preferentially enhance the growth of non-pathogenic intestinal bacteria, are known as prebiotics. These food gradients are neither hydrolysed nor absorbed in the upper gastrointestinal tract. These become substrate for the beneficial microorganisms and induce the systemic beneficial effects for the health of the host. Several substances including inulin, fructo oligosaccharides (FOS), xylo-oligosaccharides, galacto-oligosaccharides and isomalto-oligosaccharides, lactulose, etc. fall in this category.

Prebiotic research is in its infancy stage and there are scanty reports of clinical trials showing their efficacy. However, preliminary studies are encouraging, which indicate that these possess high potential to influence human health. These have been found useful in alleviation of constipation, treatment of hepatic encephalopathy, improvement of bioavailability of minerals, and production of short-chain fatty acids.

9. CONCLUSION

Probiotics and prebiotics are the microbial drugs that harness the potential of human health modulation. These form a component of our food system since the ancient times in the form of fermented foods. But, over the past few years, these have been commercialised by in vitro culturing. Therefore, probiotics is an old recipe, which is being used now in functional foods. These have been found to give a number of clinical and physiological benefits on human health. The exact mechanisms of their working are not clear, but under the right conditions, these protect the body by suppressing the growth of intestinal and some other mucosal surface pathogens.

Selection of these organisms is critical, which is mainly based upon their origin, gut colonisation potential, and viability under storage conditions. Most of probiotics have been found safe but safety assessment of recently discovered and recombinant probiotic strains is of utmost importance. Thus, probiotics could be the cost effective drugs for the third world countries. Future research for better understanding of the interaction of probiotic strains with gut or vagina by genomics and proteomics approaches is required. Further, unraveling the exact mechanisms of their action, probiotics could be used for specific health benefits.

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* Biodata of *Dr Ajay Kumar Goel, Dr Dev Vrat Kamboj*, and *Dr Lokendra Singh* is available on page 505.