

Review Article

Probiotics: Biotherapeutic agents in the Human Health

Anil Phad*

* Amrutvahini College of Pharmacy, Sangamner, Ahmednagar, India.

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ABSTRACT

Probiotic bacteria have become increasingly popular during the last two decades as a result of the continuously expanding scientific evidence pointing to their beneficial effects on human health. As a result they have been applied as various products with the food industry having been very active in studying and promoting them. Within this market the probiotics have been incorporated in various products, mainly fermented dairy foods. In light of this ongoing trend and despite the strong scientific evidence associating these microorganisms to various health benefits, further research is needed in order to establish them and evaluate their safety as well as their nutritional aspects. The purpose of this paper is to review the current documentation on the concept and the possible beneficial properties of probiotic bacteria in the literature, focusing on those available with health benefits,

Keywords: Probiotics, Lactobacillus, Gastrointestinal disease, Health Benefits

INTRODUCTION

These are the microorganisms contribute to intestinal microbial balance and play a role in maintaining health. The probiotic microorganisms consist mostly of the strains of the genera *Lactobacillus* and *Bifidobacterium*, but strains of *Bacillus*, *Pediococcus* and some yeasts have also been found as suitable candidates. Together they play an important role in the protection of the organism against harmful microorganisms and also strengthen the host's immune system. Probiotics can be found in dairy and non dairy products. They are usually consumed after the antibiotic therapy (for some illnesses), which destroys the microbial flora present in the digestive tract (both the useful and the targeted harmful microbes). Regular consumption of food containing probiotic microorganisms is recommended to establish a positive balance of the

population of useful or beneficial microbes in the intestinal flora.

The global market of probiotic ingredients, supplements and food was worth \$14.9 billion in 2007 and it was reached to 15.9 billion in 2008, and expected to reach 19.6 billion in 2013, representing a compound annual growth rate of 4.3%⁽¹⁾. Now a day probiotics increasing interest in pharmaceutical field for oral and gastrointestinal health. Probiotic bacteria are also available in supplement form. Most supplements contain mixtures of several bacteria. Supplements and foods high in probiotic bacteria may be useful after antibiotic therapy to re-colonize the intestinal track with healthy bacteria.

PROBIOTICS: MEANS

The term probiotic comes from the Greek word 'pro bios' which means 'for life'. The cheese and fermented milk were well known to the Greeks and Romans, who recommended their consumption,

especially for children and convalescents and fermented milk is the main source of probiotics. **Probiotics defined as are live microorganisms that administered inadequate amounts confer a health benefit to the host.** (FAO. Food Agriculture Organization of the United Nations & World Health Organization, 2001)⁽²⁾. The term 'probiotic' was first used by Lilly and Stillwell⁽³⁾ in 1965 to describe the 'substances secreted by one microorganism that stimulate the growth of another'. A powerful evolution of this definition was coined by Parker in 1974⁽⁴⁾, who proposed that probiotics are 'organisms and substances which contribute to intestinal microbial balance'⁽⁵⁾. Salminen *et al.*⁽⁶⁾ defined probiotics as the 'food which contains live bacteria beneficial to health', whereas Marteau *et al.*⁽⁷⁾ defined them as 'microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being'. Charteris *et al.*⁽⁸⁾, for example, defined probiotics as 'microorganisms which, when ingested, may have a positive effect in the prevention and treatment of a specific pathologic condition'. Finally, since probiotics have been found to be effective in the treatment of some gastrointestinal diseases⁽⁷⁾, they can be considered to be therapeutic agents.

To explain practicability Havenaar *et al.*⁽⁹⁾, for example, proposed the following parameters to select a probiotic: safety of probiotics to the host, resistance to gastric acidity and pancreatic secretions, adhesion of probiotics to epithelial cells of host, antimicrobial activity, inhibition of adhesion of pathogenic bacteria, evaluation of resistance to antibiotics, tolerance to food additives and stability in the food matrix. The most commonly used probiotics are the strains of lactic acid bacteria such as *Lactobacillus*, *Bifidobacterium* and *Streptococcus* (*S. thermophilus*); the first two are known to resist gastric acid, bile salts and pancreatic

enzymes, to adhere to colonic mucosa and readily colonize the intestinal tract⁽⁵⁾.

THE HISTORY OF PROBIOTICS

Before 20th century ago, the main functions of gut flora were completely unknown. Elie Metchnikoff (a Russian scientist, Nobel laureate, and professor at the Pasteur Institute in Paris) postulated that lactic acid bacteria (LAB) offered health benefits capable of promoting longevity. He suggested that "intestinal auto-intoxication" and the resultant aging could be suppressed by modifying the gut microbiota and replacing proteolytic microbes such as *Clostridium*—which produce toxic substances including phenols, indoles, and ammonia from the digestion of proteins—with useful microbes. He developed a diet containing probiotics such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which suppress the putrefactive-type fermentations of the intestinal flora and that consumption of these yoghurts played a role in maintaining health, and this fermented milk bacterium is called "Bulgarian bacillus"^(11,12,18). In 1907, he postulated that the bacteria involved in yoghurt fermentation, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, suppress the putrefactive-type fermentations of the intestinal flora and that consumption of these yoghurts played a role in maintaining health. Indeed, he attributed the long life of Bulgarian peasants to their intake of yoghurt containing *Lactobacillus* species⁽¹²⁾. In particular, he reported that the large intestine, useful to mammals in managing rough food composed of bulky vegetables, is useless in humans. Moreover, it is the site of dangerous intestinal putrefaction processes which can be opposed by introducing lactobacilli into the body, displacing toxin-producing bacteria, promoting health, and prolonging life⁽¹³⁾. Tissier's discovery of bifidobacteria in breast-fed infants also played a key role in establishing the concept that specific

bacteria take part in maintaining health. In 1906, Tissier reported clinical benefits from modulating the flora in infants with intestinal infections⁽¹⁴⁾. At the time, many others were sceptical about the concept of bacterial therapy and questioned in particular whether the yoghurt bacteria (*L.bulgaricus*) were able to survive intestinal transit, colonize and convey benefits⁽¹⁵⁾. In the early 1920s, *L. acidophilus* milk was documented to have therapeutic effects, in particular, a settling effect on digestion⁽¹⁶⁾. It was believed that colonization and growth of these microorganisms in the gut were essential for their efficacy, and therefore, the use of intestinal isolates was advocated. In Japan in the early 1930s, Shirota focused his research on selecting the strains of intestinal bacteria that could survive passage through the gut and on the use of such strains to develop fermented milk for distribution in his clinic. His first product containing *L. acidophilus* Shirota (subsequently named *L. casei* Shirota) was the basis for the establishment of the Yakult Honsha company⁽¹⁷⁾.

RATIONALS OF PROBIOTICS

The concept of probiotics combines with synbiotics and prebiotics, probiotics are defined as previously as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. While prebiotics are Nondigestible substances that provide a beneficial physiological effect for the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria and products that contain both probiotics and prebiotics are called synbiotics.

Types of Probiotic Bacteria⁽¹⁰⁾

The most common types of probiotic bacteria are the Lactic acid bacteria. These bacteria are found in yogurt. *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are some common bacteria in different varieties of yogurt. Other bacteria that are used as

probiotics are non-lactic acid bacteria and non-pathogenic yeast.

Microorganisms used as probiotic

Lactobacilli:

Lactobacilli are found primarily in the small intestine and play an important role in the initial stages of digestion and assimilation of food. Lactobacilli provide a number of important benefits that affect the health of the intestinal mucosa as well as the general overall health of the individual.

L. acidophilus: *L.acidophilus* is microaerophilic, and hence can colonize the ends of the small intestine and the colon. It enhances phagocytosis, helps in inhibition of uropathogens in human vagina (especially *C. albicans*), aids in developing natural defenses against intestinal bacteria and viral infections as well as decreasing the duration of diarrhoea in children, reduces faecal putrefactive bacteria and appears to decrease *H. pylori* density in the human stomach.

L. rhamnosus: This probiotic is also one of the normal microfloras of the intestinal and vaginal tract. It is resistant to gastric acidity and is able to grow well in the presence of bile. It transits the intestinal tract relatively rapidly. However, it is capable of adhering to intestinal mucosa, and cells are capable of persistence within the gut.

Bifidobacteria:

While Lactobacilli are resident of the small intestine, Bifidobacteria are abundant in the colon, the lower portion of the intestine and the vaginal tract. The health of the large intestine is dependent upon adequate colonisation of these organisms. Bifidobacteria produce short-chain fatty acids(SCFAs), including acetic, propionic, butyric, lactic and formic acids, with acetic acids being most plentifully produced. Acetic acid is important because GI health exerts a wide range of antimicrobial activity against yeasts, moulds, and bacteria. Thus, a healthy intestinal environment allows for active production

of organic acids and antibiotics, which function as an integral part of our immune system.

- ***B. longum***: this probiotic microorganism reduces erythromycin induced GI distress, ulcerative colitis and display anticarcinogenic activity in the colon. *B. longum* has high affinity for intestinal colonisation, improves intestinal environment, defaecation frequency and faecal characteristics.

- ***B. bifidum***: Abundant in the lower portion of the small intestine and vaginal tract, *B. bifidum* helps manufacture B vitamins and inhibits colonisation of candida. It provides enhanced immune response, inhibits harmful enzymes and lowers pH. It is antibacterial against many pathogens including *E. coli*, Salmonella spp and Shigella spp.

- ***B. infantis***: This probiotic species has been shown to be the main inhabitant of every healthy infant's GI tract. It is also found in small amount in the vaginal tract. *B. infantis* functions synergistically with *B. bifidum*.

- ***B. breve***: It enhances the immune response, provides passive protection against rotavirus-induced diarrhoea, helps in stabilization of intestinal flora, stimulates the immune system, and helps in infection control against *S. aureus* in neonatal ICU.

Saccharomyces:

Saccharomyces belongs to the yeast family. The principal probiotic yeast is *Saccharomyces boulardii*. *Saccharomyces boulardii* is also known as *Saccharomyces cerevisiae* Hansen CBS 5296 and *S. boulardii*. *S. boulardii* is normally nonpathogenic yeast. *S. boulardii* has been used to treat diarrhea associated with antibiotic use.

Streptococcus Thermophilis:

Streptococcus thermophilus is a gram-positive facultative anaerobe. It is a cytochrome-, oxidase- and catalase-negative organism that is nonmotile, non-spore forming and

homofermentative. *Streptococcus thermophilus* is an alpha-hemolytic species of

the *viridans* group. It is also classified as a lactic acid bacterium

(LAB). *Streptococcus thermophilus* is found in milk and milk products. It is a probiotic and used

in the production of yogurt. *Streptococcus salivarius* subspecies *thermophilus* type 1131 is

another probiotic strain.

Enterococcus:

Enterococci are gram-positive, facultative anaerobic cocci of the *Streptococcaceae* family. They are

spherical to ovoid and occur in pairs or short chains. *Enterococci* are catalase-negative, non-spore forming and usually

nonmotile. *Enterococci* are part of the intestinal microflora of humans and animals. *Enterococcus faecium* SF68 is a

probiotic strain that has been used in the management of diarrheal illnesses.

Classification of selected lactobacilli for probiotic use, based on metabolic production of lactic acid from sugars⁽³⁸⁾.

Obligately homofermentative

Lactobacillus delbreuckii group

Lactobacillus acidophilus

Lactobacillus crispatus

Lactobacillus gasseri

Lactobacillus johnsonii

Lactobacillus salivarius group

L salivarius

Facultatively heterofermentative

Lactobacillus casei group

L casei

Lactobacillus paracasei

Lactobacillus rhamnosus

Lactobacillus plantarum group

L plantarum

Obligately heterofermentative

Lactobacillus reuteri group

L reuteri

Lactobacillus fermentum

COMPOSITION

The Probiotics can be bacteria, moulds, and yeast. But most probiotics are bacteria. Among the bacteria, lactic acid

bacteria are more popular^[19] as species used in probiotic preparation. *Lactobacillus bulgaricus* (*L. bulgaricus*), *Lactobacillus plantarum*, *Streptococcus thermophilus* (*S. thermophilus*), *Enterococcus faecium*, *Enterococcus faecalis*, *Bifidobacterium* species, and *Escherichia coli* were listed.

CHARACTERISTICS OF IDEAL PROBIOTICS

In 1989 Fuller⁽¹⁹⁾ listed the following as features of a good probiotic.

- It should be a strain, which is capable of exerting a beneficial effect on the host animal, e.g. increased growth or resistance to disease.
- It should be nonpathogenic and non-toxic.
- It should be present as viable cells, preferably in large numbers.
- It should be capable of surviving and metabolizing in the gut environment e.g. resistance to low pH, organic acids and bile secretion.
- It should be stable under storage and field conditions.
- It should be microbiologically characterized and subjected to randomized clinical trials.
- Principally it has to be of human origin, scientifically demonstrating beneficial physiological effects and proving being safe for human use.
- In addition, it has to be effectively able to adhere to the target tissue⁽²⁰⁾.
- Ability to adhere to the intestinal epithelial lining.
- Human specific organism.
- Stable upon storage.

SAFETY CONSIDERATIONS

In terms of safety of probiotics, a need for standardized assays to determine drug

insensitivity or resistance profiles in lactobacilli and bifidobacteria was recognized. Furthermore, a need for research on potential for transmission of genetic elements to other intestinal/food borne microorganisms was recognized, given that insufficient information is available on situations in which these genetic elements could be mobilized, and it is not known if situations could arise where this could become a clinical problem.

Government regulations differ among countries, and the status of probiotics as a component in food is currently not established on an international basis. Furthermore probiotics come under food and dietary supplements because most are delivered by mouth as foods and, as such, are allowed to make only general health claims. It was recognized there is a need to accurately enumerate the probiotic bacteria in food products to include them on a label and that proper manufacture and handling procedures be employed to ensure maintenance of viability and probiotic activity through processing, handling, and storage of probiotic foods, including powdered milk products. The potential benefits of probiotics were recognized with respect to probiotics in addition to their ability to stimulate indigenous beneficial bacteria in the host. Finally, it was recognized that a proper trace-back system is a necessary prerequisite for surveillance to monitor the health outcomes (and potential side effects) of long-term probiotic administration.

The conclusions of the Expert Consultation were as adequate scientific evidence exists of consuming food containing probiotics for the obtaining of health benefits from. Additional research data are needed to confirm health benefits in humans by applying a systematic approach and following the guidelines for the assessment of probiotics suggested in the report. Good evidence exists that specific strains of probiotics are safe for

human use and able to confer some health benefits on the host, but such benefits cannot be extrapolated to other strains without experimentation. Health benefits include amelioration of gastrointestinal infections, certain bowel disorders, allergy, and urogenital infections. FAO/WHO in London, Ontario, April 30 and May 1, 2002 to generate guidelines and recommend criteria and methodology for the evaluation of probiotics and to identify and define what data need to be available to substantiate

health claims accurately. The report is available on the FAO website⁽²¹⁾:

<ftp://ftp.fao.org/es/esn/food/wgreport2.pdf>.

The minimum requirements needed for probiotic status include the assessment of strain identity, in vitro tests to screen potential probiotics, assessment of safety above all, and in vivo studies for substantiation of effects. It was recognized that it is necessary to know the genus and species of the probiotic strain, and strain identity is important to link a strain to a specific health effect as well as to enable accurate surveillance and epidemiological studies. Probiotic strains should be deposited in an internationally recognized culture collection. In vitro tests are critical to assess the safety of probiotic microbes and are also useful for functional characterization and to gain knowledge of the mechanism of the probiotic effect, as are animal studies.

In recognition of the importance of assuring safety, it was recommended that probiotic strains be characterized at a minimum with the following tests:

- Determination of antibiotic resistance patterns,
- Assessment of certain metabolic activities (d-lactate production, bile salt deconjugation),
- Assessment of side effects in humans,
- Epidemiological surveillance of adverse incidents in consumers,

- Testing for toxin production (if the strain under investigation belongs to a species that is a known mammalian toxin producer), and
- Testing for hemolytic activity if the strain under evaluation belongs to a species with known hemolytic potential. Assessment of lack of infectivity by a probiotic strain in immunocompromised animals would add a measure of confidence in the safety of the probiotic.

It was recommended that the following information be described on the label: genus, species, and strain designation; minimum viable numbers of each probiotic strain at end of shelflife; the suggested serving size, which must deliver the effective dose of probiotics related to the health claim; health claim; and proper storage conditions.

The recommendations as set out in the report⁽²¹⁾ are listed below:

- 1) Adoption of the definition of probiotics as Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.
- 2) Use and adoption of the guidelines in this report should be a prerequisite for calling a bacterial strain “probiotic.”
- 3) Regulatory framework to allow specific health claims on probiotic food labels, in cases where scientific evidence exists, as per the guidelines set forth in this report.
- 4) Promotion of these guidelines at an international level.
- 5) Good manufacturing practices must be applied in the manufacture of probiotic foods with quality assurance, and shelf-life conditions established.

- 6) Further development of methods (in vitro and in vivo) to evaluate the functionality and safety of probiotics.

PRPROBIOTICS AND HUMAN HEALTH.

1) In general health: nutritional effects

A lactic acid bacterium has been shown to increase folic acid content of yogurt, buttermilk and kefir and to increase niacin and riboflavin levels in yogurt, vitamin B12 and vitamin B6 in cheese⁽²²⁾. In addition to nutrient synthesis, probiotics may improve the digestibility of some dietary nutrients such as protein and fat⁽²³⁾. Probiotics can be combined with enzymes that help break down food substances into simpler forms to enhance nutrient digestion. Short-chain fatty acids such as lactic acid, propionic acid and butyric acid produced by lactic acid bacteria may help maintain an appropriate pH and protect against pathological changes in the colonic mucosa. Synthesized nutrients including folic acid, niacin, riboflavin, vitamins B6 & B12 can increase nutrient bioavailability.

2) Probiotics and calcium absorption

Milk is considered to be abundant with calcium apart from other dietary sources. Individuals with lactose intolerance may probably develop osteoporosis due to decreased consumption of milk containing diet. Calcium absorption is favored in acidic PH. So if probiotics are fed to lactose intolerance patients, then milk lactose is hydrolyzed by probiotic strains, favoring calcium absorption⁽²⁴⁾.

3) Lactose intolerance

Few strains of lactic acid bacteria, such as *S. thermophilus*, *L. bulgaricus* and other lactobacilli in fermented milk products, can alleviate symptoms of lactose intolerance by providing bacterial lactase to the intestine and stomach. Because lactose intolerance affects almost 70% of the population worldwide, consumption of these products may be a good way to incorporate dairy products and their accompanying nutrients into the diets of lactose intolerant individuals.

4) Allergy

Probiotics may exert a beneficial effect on allergic reaction by improving mucosal barrier function. In addition, probiotic consumption by young children may beneficially affect immune system development. Probiotics such as *Lactobacillus GG* may be helpful in alleviating some of the symptoms of food allergies such as those associated with milk protein⁽²⁵⁾. Probiotic consumption may thus be a means for primary prevention of allergy in susceptible individuals.

5) Intestinal tract health

A number of studies have found probiotic consumption to be useful in the treatment of many types of diarrhea, including antibiotic-associated diarrhea in adults, travellers' diarrhea, and diarrheal diseases in young children caused by rotaviruses⁽²⁶⁾. The most commonly studied probiotic species in these studies have been *Lactobacillus GG*, *Lactobacillus casei*, *Bifidobacterium bifidum* and *S. thermophilus*. Because diarrhea is a major cause of infant death worldwide and can be incapacitating in adults, the widespread use of probiotics could be an important, non-invasive means to prevent and treat these diseases, particularly in developing countries. Probiotic bacteria have also been shown to preserve intestinal integrity and mediate the effects of inflammatory bowel diseases, irritable bowel syndrome, colitis, and alcoholic liver disease⁽²⁵⁾. The intestinal effects of probiotics are to relieve effects, promote recovery from diarrhea (rotavirus, travelers' and antibiotic induced), produce lactase, alleviate symptoms of lactose intolerance and malabsorption, relieve constipation, treat colitis, and stimulate gastrointestinal immunity⁽²⁷⁾.

6) Immune system

Evidence from in vitro systems, animal models and humans suggests that probiotics can enhance both the specific and nonspecific immune response, possibly by activating macrophages,

increasing levels of cytokines, increasing natural killer cell activity, and/or increasing levels of immunoglobulins⁽²⁸⁾. In spite of limited testing in humans, these results may be particularly important to the elderly, who could benefit from an enhanced immune response. The immune system effects of probiotics are to enhance specific and nonspecific immune response, inhibit pathogen growth and translocation, and reduce chance of infection from common pathogens (Salmonella, Shigella).

7) Cancer

Animal and in vitro studies indicate that probiotic bacteria may reduce colon cancer risk by reducing the incidence and number of tumors. One clinical study showed an increased recurrence-free period in subjects with bladder cancer⁽²⁹⁾. Results, however, are too preliminary to develop specific recommendations on probiotic consumption for preventing cancer in humans.

8) Anti-hypertension

Some preliminary evidence suggests that food products derived from probiotics bacteria could possibly contribute to blood pressure control⁽³⁰⁾. This antihypertensive effect has been documented with studies in spontaneous hypertensive rats⁽³¹⁾. Two tripeptides, valine-proline-proline and isoleucine-proline-proline, isolated from fermentation of a milk-based medium by *Saccharomyces cerevisiae* and *Lactobacillus helveticus* have been identified as the active components. These tripeptides function as angiotensin-I-converting enzyme inhibitors and reduce blood pressure.

9) Probiotics and oral health

Candida infection

Probiotics are used to control *Candida* infection in elderly patient since elderly are more prone to *Candida* infection provoked by chronic diseases, medications, poor oral hygiene, reduced salivary flow and impaired immune response. Bacteria like *Lactococcus lactis*, *Lactobacillus helveticus*, *Lactobacillus*

rhamnosus GG (ATCC53103), *Lactobacillus rhamnosus* LC705 when used in one of the study, showed significant reduction of *Candida* infection⁽³²⁾.

Hypo-salivation and feeling of dry mouth

Evidence suggests that probiotics can also reduce the risk of hypo-salivation and feeling of dry mouth⁽³²⁾.

Dental decay

It should also be noted that as most probiotics are in dairy forms containing high calcium, possibly reducing demineralization of teeth. Probiotics should adhere to dental tissues to establish a cariostatic effect and thus should be a part of the bio-film to fight the cariogenic bacteria.

Periodontal infection

Clinical studies where probiotic species have been investigated specifically from a periodontal disease perspective are sparse. *Lactobacillus reuteri* and *Lactobacillus brevis* are among the species able to affect gingivitis and periodontitis⁽³³⁾. According to Koll-Klais et al⁽³⁴⁾, high levels of *Lactobacillus* in microbiota caused an 82% and 65% inhibition in *Porphyromonas gingivalis* and *Prevotella intermedia* growth, respectively. In one recent study published in 2005, the prevalence of lactobacilli, particularly *Lactobacillus gasseri* and *Lactobacillus fermentum*, in the oral cavity was greater among healthy participants than among patients with chronic periodontitis⁽³⁴⁾.

Probiotics and halitosis⁽³⁵⁾

Regular use of probiotics can help to control halitosis. After taking *Weissella cibaria*, reduced levels of volatile sulfide components produced by *Fusobacterium nucleatum* were observed by Kang et al⁽³⁶⁾. The effect could be due to hydrogen peroxide production by *Weissella cibaria*, causing *Fusobacterium nucleatum* inhibition. In conclusion, probiotics are emerging as a fascinating field in oral medicine.

10) Other health effects of probiotics

Probiotics can also be used to reduce risk of certain cancers (colon, bladder), detoxify carcinogens, suppress tumors, lower serum cholesterol concentrations, reduce blood pressure in hypertensives, treat food allergies, improve urogenital health, and optimize effects of vaccines (e.g. rotavirus vaccine, typhoid fever vaccine).

11) Fertility of soil

Probiotics have been used in agriculture to restore fertility to the soil. Probiotics have

been used in wastewater systems to eliminate the odor. Probiotics have been used in the animal and poultry industry to prevent disease foods.

12) Probiotics in veterinary practice

They are marketed as either pastes or powders and are commonly given to young foals, horses being trailered or in competition. These products are commonly defined as live microbial feed supplements⁽³⁷⁾.

Table 1 Effects of different strains on some gastrointestinal pathologies

Disorder	Strain	Dose	Ref.
Acute infectious diarrhea in children	L. rhamnosus GG	10 ¹⁰ -10 ¹¹ ufc	(38)
	L. reuteri ATCC 55730	10 ¹⁰ -10 ¹¹ ufc×2/d	(38)
	S. cerevisiae (boulardii)	10 ⁹ ufc×3/d	(38)
Treatment of acute infectious diarrhea in adults	Enterococcus faecium LAB SF68	108 ufc×3/d	(38)
Prevention of antibiotic-associated diarrhea	S. cerevisiae (boulardii)	10 ⁹ ufc×2/d	(39)
	L. rhamnosus GG	10 ⁹ ufc×1-2/d	(39)
	B. lactis Bb12 + S. thermophilus Enterococcus faecium LAB SF68	10 ⁹ + 106 ufc/g	(39)
	S. cerevisiae (boulardii)	10 ⁸ ufc×2/d	(40)
Prevention of rotavirus nosocomial infection in children	S. cerevisiae (boulardii)	1g or 3×10 ¹⁰ ufc×1/d	(41)
	L. rhamnosus GG	10 ¹⁰ -10 ¹¹ ufc×2/d	(39)
Adjuvant in therapies for Helicobacter pylori eradication	L. rhamnosus GG	6×10 ⁹ ufc ×2/d	(42)
Reduction irritable bowel syndrome symptoms	B. infantis 35624	10 ⁸ ufc×1/d	(43)
	L. rhamnosus GG	6×10 ⁹ ufc ×2/d	(44)
Remission of ulcerative colitis	E. coli Nissle 1917	5×10 ⁹ ufc ×2/d	(45)

THE MECHANISM OF ACTION OF PROBIOTICS

Some studies demonstrated it may not be necessary to administer the intact probiotic organism to achieve benefit. The secreted proteins and DNA from probiotics (VSL#3) can block inflammation and stop

the death of epithelial cells^(46,47). In another study, DNA from the same probiotic preparation as well as specific *E. coli* strains suppressed experimental colitis in several animal models⁽⁴⁸⁾. Probiotic bacteria can also be genetically modified

for use as carriers for antigen delivery into diseased sites in the intestine⁽⁴⁹⁾.

Further studies are necessary to increase our understanding of how the probiotic agents produce a beneficiary effect on the host as different strains of probiotic bacteria may work by distinctly different mechanisms. It is also important to recognize that *in vitro* effects of a probiotic may display opposite behavior *in vivo*⁽⁵⁰⁾.

The mechanism of action of probiotics can be classified based on specific effects of the bacteria on the microbial milieu, intestinal epithelium, immune response, allergic diseases, distant mucosal sites, and cancer.

❖ ANTIMICROBIAL EFFECTS

• Effect on Microflora

Modification of microflora has long been considered as the mechanism of action of probiotics. Several studies suggest that ingestion of certain lactobacilli and bifidobacteria species decrease the fecal concentrations of clostridia, *Bacteroides*, and *E-coli*, and can increase the endogenous levels of lactobacilli and bifidobacteria, but more importantly affect the metabolic activities of the flora by decreasing the production of carcinogenic substances such as fecal azoreductase, nitroreductase and β -glucuronidase⁽⁵¹⁾. Whether colonization is critical for probiotics to have their effect remains unresolved⁽⁵²⁾. In a infant study by Agarwal, et al, colonization with *Lactobacillus* GG occurred in 21% of infants who weighed less than 1500 grams versus 47% of larger infants. The use of antibiotics interfered with the colonization ability of the probiotic. Therefore, the neonatal response to probiotic preparations is dependent on gestational age, weight, postnatal age and prior antibiotic exposure⁽⁵³⁾.

• Production of Antimicrobial Factors

Probiotics are capable of producing short chain fatty acids, which lower the colonic pH, favoring the growth of less pathogenic organisms⁽⁵⁴⁾. Bacteriocins, which are

antimicrobial proteins elaborated by probiotic organisms, are especially effective against gram-positive organisms⁽⁵⁵⁾. Lactobacilli also produce substances that inactivate viral particles. Soluble substances produced by *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 can inactivate adenovirus and the vesicular stomatitis virus within minutes⁽⁵⁶⁾. *Lactobacillus* GG produces compounds that inhibit the growth of several gram-positive and gram-negative bacteria by producing antimicrobial substances such as lactic acid, hydrogen peroxide, and pyroglutamate^(57,58). In addition, *Lactobacillus acidophilus* strain LA1 produces a non-bacteriocin and non-lactic acid antimicrobial substance against a variety of gram-negative and gram-positive bacteria⁽⁵⁹⁾. Moreover, specific microflora isolated from an infant, were found to be bactericidal against *Salmonella typhimurium*⁽⁶⁰⁾.

• Competition for Adhesion

Binding to intestinal epithelium is one of the determinants in establishing the efficacy of a probiotic⁽⁶¹⁾. Colonization resistance occurs through this binding, competitively inhibiting adhesion of pathogenic bacteria⁽⁶²⁻⁶⁴⁾. For example, *Lactobacillus* GG and *Lactobacillus plantarum* 299V competitively inhibit the attachment of enterohemorrhagic *Escherichia coli* 0157H7 to HT-29 cells⁽⁶⁵⁾. Other lactobacillus strains have been shown to compete with enteropathic *E. coli* for attachment to mucus in pig ileum⁽⁶⁶⁾. *Saccharomyces boulardii* inhibits the attachment of *Entamoeba histolytica* trophozoites to erythrocytes *in vitro*⁽⁶⁷⁾. Furthermore, certain strains of lactobacilli are capable of blocking receptor sites preventing the invasion of pathogens⁽⁶⁸⁾.

• Competition for Nutrients

Probiotics may also compete for nutrients otherwise consumed by pathogenic organisms. For example, consumption of monosaccharides by a probiotic may

reduce the growth of *Clostridium difficile*, which is dependent on monosaccharides for growth⁽⁶⁹⁾.

- **Antitoxin Effect**

Probiotics may also modify toxin receptors through an enzymatic mechanism, which has been seen with *S. boulardii* through its effect on the *C. difficile* toxin A receptor⁽⁷⁰⁾. The effect of *S. boulardii* on *C. difficile* toxins was suspected when investigators observed clinical improvement without a change in the concentration of *C. difficile* in the stools. Similar effects have been postulated for the cholera toxin receptor. In animal studies, gut commensals offer host resistance against pathogens, where the host is able to withstand lethal doses of pathogens such as *Salmonella enteritidis*⁽⁷¹⁾.

Antimicrobial effects of probiotics

- Modify microflora to suppress pathogens
- Secrete antibacterial substances
- Compete with pathogens to prevent their adhesion to the intestine
- Compete for nutrients necessary for pathogen survival
- Antitoxin effect

❖ EFFECTS ON INTESTINAL EPITHELIUM, MUCUS PRODUCTION, AND BARRIER FUNCTION

- **Effects on Barrier Function**

Probiotic bacteria can enhance barrier function by different mechanisms. First, probiotic bacteria such as *Streptococcus thermophilus* and *Lactobacillus acidophilus* enhance activation of tight junction proteins avoiding the development of a leaky intestine⁽⁷²⁾. Second, other probiotic bacteria such as *Lactobacillus rhamnosus* GG can prevent inflammation and programmed cell death of the lining intestinal epithelial cells⁽⁴⁷⁾. Finally, an effect on barrier function with a lactobacillus strain has been demonstrated

by decreased mucosal permeability to mannitol in germfree rats⁽⁷³⁾.

- **Down-regulation of the Secretory and Motility Defenses**

Mucins produced by the host constitute one of the defense mechanisms against pathogens, and *MUC2* and *MUC3* mRNA expression is increased in response to lactobacilli, protecting cells against the adhesion of pathogenic bacterial⁽⁶⁵⁾. Moreover, *Lactobacillus plantarum* 299v was found to decrease the damaging effect of a specific type of *E. coli* on intestinal epithelial cells^(74,75).

- **Trophic Action of Probiotics**

Several studies suggest a trophic intestinal effect secondary to the ingestion of *S. boulardii* by increasing the brush border enzymes in jejunal mucosa⁽⁷⁶⁾. Also, the same probiotic, through its production of polyamines, may enhance intestinal enzyme expression⁽⁷⁷⁾.

Effect of probiotics on the intestinal epithelium

- Promote tight contact between epithelial cells forming a functional barrier
- Reducing the secretory and inflammatory consequences of bacterial infection
- Enhancing the production of defensive molecules such as mucins
- Increase brush border enzyme production.

❖ IMMUNE EFFECTS

- **Probiotics as Vehicles for Immune Modulation**

Probiotic agents such as lactobacilli can be genetically engineered to secrete substances that possess anti-inflammatory effect such as IL-10. When these genetically engineered probiotic agents are injected by the host, anti-inflammatory cytokines can be released locally to the inflamed areas in the gastrointestinal tract⁽⁷⁸⁾.

- **Effect on Molecular Signaling Inside the Cell**

The protective effects of probiotics may be mediated by their own DNA rather than by their metabolites or ability to colonize the colon. Toll-like receptor 9 (LR9) signaling is essential in mediating the anti-inflammatory effect of probiotics, and DNA derived from probiotic bacteria can be sufficient to attenuate experimental colitis⁽⁴⁸⁾. In a human and murine inflammatory model, VSL#3 DNA inhibited IL-8 secretion, reduced p38 mitogen-activated protein kinase activation, delayed nuclear factor kappa B activation, stabilized levels of IkappaB, and inhibited proteasome function⁽⁴⁶⁾. Similarly, *S. boulardii* prevented enterohemorrhagic *Escherichia coli* infection by interfering with the transduction pathways that control tight-junction structure as well as inhibiting NF-kappaB and MAPK signaling pathways leading to the production of IL-8⁽⁷⁹⁾. A study by Petrof, et al demonstrated that probiotics inhibited the pro-inflammatory nuclear factor-kappaB pathway and triggered the expression of cellprotective heat shock proteins in the intestinal cells. Furthermore, the probiotic produced factors that inhibited the breakdown of the heat shock proteins, which would normally occur through intracellular protein destroyers known as proteasomes. Proteasome inhibition was an early event that began almost immediately after exposure of the colonic cells to the probiotic. The resulting inhibition of nuclear factor-kappaB and increased expression of heat shock proteins may account for the anti-inflammatory and cytoprotective effects reported for probiotics and may be a novel mechanism of microbial-epithelial interaction⁽⁸⁰⁾.

- **Effect on Humoral Immunity**

Many studies demonstrate a strong and consistent capability of many probiotic agents in inducing specific antibody response. Viable *L. casei* strain GG stimulate rotavirus specific IgA antibody

responses^(81,82). Moreover, two probiotic strains; *Lactobacillus rhamnosus* GG or *Lactobacillus acidophilus* CRL431, induced an immunologic response towards poliomyelitis vaccine virus by affecting the production of virus neutralizing antibodies⁽⁸³⁾. Also, ingested *B. bifidum* significantly increased the number of immunoglobulin (IgM, IgG, and IgA) secreting cells mesenteric lymph nodes and spleen, in an animal model^(84,85).

- **Effect on Cytokine Release**

The effect of probiotics on cytokine release is a perfect model to highlight the differences in effect between similar probiotic bacteria, as well as differences in effect of the same probiotic bacteria when used at different doses. Not all probiotic strains have similar immune-modulating properties, as a matter of fact, they can exert opposite effects. Different species of lactobacilli exert very different dendritic cell activation patterns and, furthermore, at least one species may be capable of inhibiting activities of other species in the genus. Thus, the T(H)1, T(H)2, T(H)3-response of the dendritic cells in the intestine can be modulated according to composition of gut microflora, including ingested probiotics⁽⁸⁶⁾. Other mechanisms by which probiotics enhance cytokine production can be seen with the administration of VSL#3 in a murine model of colitis. The severity of recurrent colitis was reduced through increasing IL-10 production and increasing CD4(+) T cells bearing surface TGF-beta⁽⁸⁷⁾.

- **Effect on Innate Immunity**

In a clinical trial involving forty-five healthy volunteers, *L. casei* DN114001 consumption increased oxidative burst capacity of monocytes, as well as natural killer cells tumoricidal activity resulting in a positive effect in modulating the innate immune defense in healthy people⁽⁸⁸⁾. Moreover, probiotic bacteria appear to modulate the nonspecific immune response differently in healthy and hypersensitive subjects. This is seen as an immunostimulatory effect in healthy

subjects, and as a down-regulation of immunoinflammatory response in milk-hypersensitive subjects⁽⁸⁹⁾. The probiotic *E. coli* Nissle 1917 bacteria have been shown to stimulate the intestinal innate immune system through up-regulation of antimicrobial peptides such as human beta defensin 2 (hBD-2)⁽⁹⁰⁾. *S. boulardii* was also found to activate complement and the reticuloendothelial system⁽⁹¹⁾.

Immune effects of probiotics

- Probiotics as vehicles to deliver anti-inflammatory molecules to the intestine
- Enhance signaling in host cells to reduce inflammatory response
- Switch in immune response to reduce allergy
- Induce antibody response to reduce infection
- Reduce the production of inflammatory substances

❖ ALLERGIC DISORDERS

Experimental studies suggest that probiotics exert strain-specific anti-allergic effects at the level of the immune cells, the intestinal lumen, as well as the intestinal epithelial cells. Such effects include improved intestinal barrier function, allergic degradation, and down-regulation of immune responses at the local gastrointestinal site as well as distant mucosal sites⁽⁹²⁾. In a double blind, placebo-controlled study, *Lactobacillus* GG was found to alleviate atopic eczema in infants that are IgE-sensitized⁽⁹³⁾. Moreover, lactic acid bacteria can inhibit the secretion of T(H)2 cytokines (IL-4 and IL-5) in a manner dependant on antigen presenting cells, IL-12, and IFN γ . This switch from a T(H)2 to a T(H)1 response is beneficial in allergic patients⁽⁹⁴⁾. In children with atopic dermatitis, lactobacilli may stabilize the intestinal barrier function and decrease gastrointestinal symptoms⁽⁹⁵⁾. Probiotic bacteria reduce CD34+ hemopoietic precursor cells, which are increased in

allergic subjects with systemic allergic inflammation⁽⁹⁶⁾. In infants with cow milk allergy, *Lactobacillus* GG can increase the secretion of IFN- γ ⁽⁹⁷⁾. Specific strains of *Bifidobacterium* and *Lactobacillus* appear to be promising in the treatment and prevention of eczema and dermatitis in infants and children^(98,99). It is interesting to note that in these studies, supplementation with the probiotic did not appear to alter bacterial microflora in the colon. This suggests that these results are related to altered immunity rather than altered colonization⁽¹⁰⁰⁾. For more details on the role of probiotics in allergic disorders, the reader is referred to an excellent review recently published by Kalliomaki⁽¹⁰¹⁾.

❖ EFFECT OF PROBIOTICS ON DISTANT MUCOSAL SITES

Lactobacillus GG has been the most widely studied of probiotic agents. In addition to having been used with varying degrees of success for treating and preventing urinary tract infections, vulvo-vaginal candidiasis, otitis media⁽¹⁰²⁾ and bacterial vaginosis⁽¹⁰³⁾, *Lactobacillus* GG, in the form of a milk preparation, was recently reported as having some modest but consistent benefits in terms of preventing and reducing the severity of respiratory infections at day care centers⁽¹⁰⁴⁾.

❖ ANTIPROLIFERATIVE EFFECT

Probiotics are able to modulate several intestinal functions such as detoxification, colonic fermentation, transit, and immune status, which may contribute to the development of colon cancer. The use of probiotics resulted in direct antiproliferative effects on tumor cells and immune cells⁽¹⁰⁵⁾. In rats, specific reduction of carcinogenic bacterial enzymes and modulation of gut and systemic immunity has been shown to have the potential to exert significant antiproliferative effects against colon cancer⁽¹⁰⁶⁾. Although the evidence is still mounting and more research is required, the data show promising evidence

supporting the protective role of probiotics in colon cancer⁽¹⁰⁷⁾.

CONCLUSION

It is apparent from the review that probiotics are beneficial to human being, are can be used for treatment of various disease such as inflammatory bowel diseases, colon cancer, allergic disorders, periodontal diseases (PD) and dental caries (DC). This review also provide information about mechanism of action of beneficial effect of probiotics.

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