Probiotics- Towards the Next Generation

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Abstract

Over the past decade, there has been considerable progress in identifying potentially beneficial roles for probiotics in human health. Probiotics are defined as "live microorganisms administered in adequate amounts that confer a health effect on the host". Interest in the role of probiotics for human health dates back to the beginning of the 20th century (1908) when the Nobel Prize winning Russian scientist, Elie Metchnikoff, linked the long, healthy life of Bulgarian peasants to their high intake of fermented milk products containing lactic acid producing microorganisms. He theorized that the lactic acid bacteria in fermented milk displace undesirable bacteria normally present in the intestine, resulting in a healthier life. This article shows scientific studies examining the health attributes of probiotics, especially those related to gastrointestinal health and immune system modulation.

Keywords: Functional foods, Probiotics, Synbiotics, Probiotic foods, Colon cancer, *Bifidobacterium*

1. Introduction

The functional food market is growing at a rate of 15- 20 % per year, and the industry is claimed to be worth $33 billion.[1] also known as, designer foods, medicinal foods, therapeutic foods, superfoods and medifoods- are defined as "foods that contain some health- promoting component(s) beyond traditional nutrients" foods can be modified by addition of phytochemicals, bioactive peptides, ω-3 polyunsaturated fatty acids, and probiotics to become "functional". Probiotics are officially defined as: 'Oral probiotics are living micro-organisms which, upon digestion in certain numbers, exert health benefits beyond inherent basic nutrition'.

Table 1- Functionalities of Functional Foods (Ref. No. 2)

Sr. No.	Food Modification	Possible Functionalities
1.	Addition of Phytochemicals (as Plant ingredients or extracts)	Antioxidant, lower risk of CHD, lower risk of cancer, lower blood pressure.
2.	Addition of bioactive peptides	Enhance immune function, enhances bioavailability of minerals, hypotensive.
3.	Addition of dietary fibre	Prevention of constipation, lower risk of Colon cancer, lowering of blood cholesterol.
4.	Addition of ω-3 polyunsaturated fatty acids	Lower risk of heart attack, lower risk of some cancers (colon), enhanced immune system.
5.	Addition of probiotics	Improved GIT function, enhanced immune system, and lower risk of colon cancer, improved digestibility.
6.	Addition of Prebiotics	Improved GIT function, enhanced immune system, and lower risk of colon cancer.

A more common definition is : 'A probiotic is a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance'.

The word 'probiotic' is derived from the Greek words for 'Pro- Life' and its three main aspects of the definition are: the micro-organisms (bacteria) are alive, the bacteria are administered orally, the bacteria should be capable of reaching the intestine alive, in order to have an influence on the microbial balance. Bacteria make up most of the flora in the colon and 60% of the mass of feces.

Probiotics are claimed to have beneficial effects on the host (human or animal) health.

2. History of Usage of Probiotics

Probiotics have been used for centuries as natural components in health-promoting foods. Henry Tissier, also from the Pasteur Institute, was the first to isolate a *Bifidobacterium*. He isolated the bacterium from a breast-fed infant and named it Bacillus bifidus communis. This bacterium was later renamed *Bifidobacterium bifidum*. After Metchnikoff's death in 1916 the centre of activity moved to the USA. It was reasoned that bacteria originating from the gut were more likely to produce the desired effect in the gut; German professor Alfred Nissle, in 1917 isolated a strain of *Escherichia coli* from the feces of a First World War soldier who did not develop enterocolitis during a severe outbreak of shigellosis. In those days, antibiotics were not yet discovered, and Nissle used the strain with considerable success in acute cases of infectious intestinal diseases (salmonellalosis and shigellosis). Escherichia coli Nissle 1917 are still in use and are one of the few examples of a non-LAB probiotic.

In 1920 Rettger demonstrated that Metchnikoff's "*Bulgarian Bacillus*", later called *Lactobacillus bulgaricus*, could not live in the

human intestine, and the fermented food phenomena pitted out. Metchnikoff's theory was disputable (at this stage) and people doubted his theory of longevity. In 1935 certain strains of *Lactobacillus acidophilus* were found to be very active when implanted in the human digestive track. In the 1960s the dairy industry began to promote new yoghurts containing *Lactobacillus acidophilus*. In the subsequent decades other Lactobacillus species have been introduced including *Lactobacillus rhamnosus*, *Lactobacillus casei*, and *Lactobacillus johnsonii*, because they are intestinal species with beneficial properties.

The term "probiotics" was first introduced in 1965 by Lilly and Stillwell, when it was described as growth promoting factors produced by microorganisms (protozoa) contrasting antibiotics, probiotics were defined as microbially derived factors that stimulate the growth of other microorganisms. In 1989 Roy Fuller's definition emphasizes the requirement of viability for probiotics and introduces the aspect of a beneficial effect on the host.

3. Localization

The colon has the greatest numbers of bacteria and the most different species, and the activity of these bacteria make the colon the most metabolically active organ in the body.

Most of the bacteria in the small intestine are Gram-positive, while those in the colon are mostly Gram-negative. The first part of the colon is mostly responsible for fermenting carbohydrates, while the latter part mostly breaks down proteins and amino acids.

Bacterial growth is rapid in the cecum and ascending colon, which has a low pH, and slow in the descending colon, which has an almost neutral pH. The body maintains the proper balance and locations of species by altering pH, the activity of the immune system, and peristalsis. Over 99% of the bacteria in the gut are anaerobes, but in the cecum, aerobic bacteria reach high densities.

4. Mechanism of Action and Functionality

Balance of Bacteria

This bacterial "balancing act" can be thrown off in two major ways:

a. By antibiotics, when they kill friendly bacteria in the gut along with unfriendly bacteria.
b. "Unfriendly" microorganisms such as disease-causing bacteria, yeasts, fungi, and parasites can also upset the balance.

Viability of probiotic organisms

To realize the health benefits, probiotic bacteria must be viable (able to sustain life) and available, typically 106 cfu/g of product .Factors responsible for the loss of viability of probiotic organisms: - acidity of products, acid produced during refrigerated storage (also known as post acidification), level of oxygen in products, oxygen permeation through package, sensitivity to antimicrobial substances produced by yoghurt bacteria and lack of nutrients in milk.

4.1 Lactase Activity for Improvement in Lactose Metabolism

Individuals with low levels of the intestinal enzyme lactase have a limited ability to digest lactose which results in lactose intolerance in which lactose, is not completely digested into its components

Fig. 1 *Lactobacillus acidophilus* (Ref. 3)

monosaccharides, glucose and galactose Compared to milk, viscous foods such as yogurt delays gastric emptying and slows intestinal transit which prolongs the action of lactase in the small intestine and decreases the osmotic load of the lactose.

A variety of probiotic bacteria such as *L. acidophilus* (See figure 1- Ref.3) and bifidobacteria improve digestion of lactose in lactose maldigesters, although the effects are less consistent than those achieved for yogurt with live, active cultures. Some dairy products formulated exclusively with probiotic bacteria, such as sweet milk (fermented by *L. acidophilus*), are ineffective in improving tolerance to lactose may be explained by the low level of probiotics in the product. Yoghurt bacteria produce higher lactase activity than probiotic bacteria. Proteolytic activity and lactase activity explain why yoghurt bacteria are faster than probiotic bacteria and why yoghurt bacteria are used as the main starter bacteria and probiotic bacteria as an adjunct starter. The traditional cultures used in making yoghurt *L. delbrueckii ssp. bulgaricus* and *S. thermophilus* contain substantial quantities of β-D- galactosidase. Some lactose is hydrolyzed by yoghurt bacteria during fermentation. Another reason for better tolerance could be that the bacterial enzyme auto digests lactose intracellularly before reaching the intestine and the third reason could be a slower oral caecal transit time. Gastric emptying and intestinal transit time play an important role in improved lactose tolerance. As a result, fermented acidophilus milk may be better tolerated than sweet acidophilus milk, since coagulated milk because of its viscous nature, may pass more slowly through the gut than unfermented milk.

4.2 Carbohydrate Fermentation and Absorption

Bacteria ferment carbohydrates; into short chain fatty acids, or SCFAs, which in turn are used by host cells, as a major source of useful energy and nutrients for humans. They increase the gut's absorption of water, reduce counts of damaging bacteria, increase growth of human gut cells and are also used for the growth of indigenous bacteria. The SCFAs are produced by a form of fermentation called saccharolytic fermentation and include acetic acid, propionic acid and butyric acid. Gases and organic acids like lactic acid are also produced by saccahrolytic fermentation. Acetic acid is used by muscle, propionic acid helps the liver produce ATP, and butyric acid provides energy to gut cells and may prevent cancer. In addition, the SCFAs they produce help the body absorb nutrients such as calcium, magnesium and iron.

4.3 Anti-Diarrhoeal Effects

Research supports a beneficial role for probiotics in the prevention and treatment of a variety of diarrheal illnesses, such as acute diarrhea caused by rotavirus infections, antibiotic-associated diarrhea and travelers' diarrhoea. Specific strains of lactobacilli, such as *Lactobacillus rhamnosus* GG and a combination of *Lactobacillus rhamnosus* 190702 and *Lactobacillus reuteri* DSM 12246, as well as fermented dairy foods such as yogurt, have been shown to reduce

the severity and duration of acute diarrhea caused by rotavirus infections in infants and young children. A common side-effect of antibiotic therapy is diarrhea, usually caused by the growth of pathogenic bacteria, specifically *Clostridium difficile*. Several placebo-controlled clinical trials have demonstrated that *L. rhamnosus* GG, *L. acidophilus* LA1, and *Saccharomyces boulardi*, as well as yogurt, reduce the incidence of or lessen the severity of antibiotic-associated diarrhea

4.4 Protection Against Infections and Immune Enhancement

Probiotics may help prevent or treat infections such as postoperative infections, respiratory infections and the growth of *Helicobacter pylori*, a bacterial pathogen responsible for type B gastritis, peptic ulcers, and perhaps stomach cancer. A 7-month randomized, double-blind, controlled study of more than 570 healthy children aged 1 to 6 years in day care centers found that intake of a probiotic milk containing *L. rhamnosus* GG reduced the number and severity of respiratory infections and the need for antibiotics. Regular intake of probiotics (i.e., a fermented milk drink containing a mixture of *L. rhamnosus GG, Bifidobacterium, L. acidophilus,* and *S. thermophilus)* has been demonstrated to reduce potentially pathogenic bacteria in the upper respiratory tract of humans (Ref. 4).

Probiotics such as *Lactobacillus casei* strain *Shirota*, bifidobacteria, and *Lactobacillus salivarius* have been shown to inhibit the growth and/or colonization of *H. pylori* in *vitro* and experimental animal studies. According to an in *vitro* study, yogurt containing *Bifidobacterium lactis* Bb12 reduced the growth of *H. pylori*, whereas *L. acidophilus La5* did not. When 59 adults with *H. pylori* infection consumed a mixture of these two probiotics in yogurt twice daily after a meal for 6 weeks, *H. pylori* was suppressed, but only when the probiotic yogurt was consumed regularly, whereas *H. pylori* continued to increase in the subjects consuming a placebo. Other human studies show that intake of fermented milks containing *Lactobacillus johnsonii* with or without antibiotics suppresses *H. pylori* infection or diminishes the severity and activity of gastric inflammation caused by *H. pylori*. Although these preliminary findings in humans indicate that some specific probiotic strains may help control *H. pylori* infection, there is no evidence that they can kill this pathogen in *vivo*.

4.5 Anticarcinogenic Activity

Epidemiological studies in human's link intake of yogurt or other fermented milk products to decreased cancer risk, however, the findings are inconsistent. Although human intervention studies demonstrate the ability of specific probiotics to inhibit biomarkers of colon cancer risk, such as bacterial enzyme activities, there is no direct evidence that probiotics reduce colon cancer incidence in humans *B. longum* and *B. infantis* are effective antitumor agents. Their mode of action may be by suppression of bacterial enzymes, activation of host immune system and reduction of intestinal pH. LAB and fermented products from them have potential anticarcinogenic activity. Oral dietary supplements, containing viable cells of *L. acidophilus*, decreased β- Glucuronase, azoreductase, and nitroreductase, bacterial enzymes, which catalyze the conversion of procarcinogens to carcinogens. Anticarcinogenic effects of *L. acidophilus* or bifidobacteria may be due to direct removal of procarcinogens and activation of the body's immune system.

4.6 Proteolytic Activity (Ref. 2)

Proteolytic enzymes are required to degrade milk casein to oligopeptides, which are degraded by peptidases to peptides and amino acids. Milk does not contain sufficient free amino acids and peptides to allow the growth of LAB, particular fastidious organisms such as *L. acidophilus* and *Bifidobacteria*. Yoghurt has been traditionally been made using *Streptococcus thermophilus* and *L. delbrueckii ssp. bulgaricus* (See figure 2- Ref.3) as starter culture. The above latter organism produces essential amino acids owing to its proteolytic nature, and the symbiotic relationship of *L. delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* is well established, the former producing amino nitrogen for the latter. These two bacteria grow rapidly. On the other hand, *L. acidophilus* and *bifidobacteria* grow slowly in milk because of the lack of proteolytic activity; so the usual practice is to add yoghurt bacteria (main starter culture) to probiotic products (bacteria act as adjunct started culture) to reduce the fermentation time. With probiotic bacteria only, the fermentation time came be as long as 24 hr, compared to approximately 4 hr with yoghurt bacteria. This causes the loss of viability of the probiotic bacteria when in presence of the yoghurt bacteria due to competition.

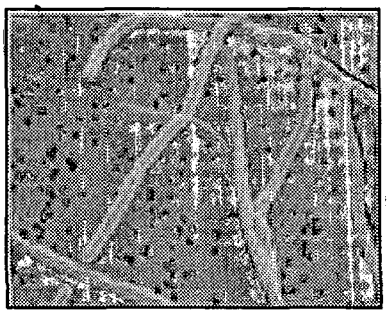

Fig. 2 Lactobacillus Delbrueckii SSP Bulgaricus (Ref. 3)

4.7 Antimicrobial Properties and Antagonism Against Bacteria

With the emergence of antibiotic resistant bacteria, the concept of probiotics has attained much attention.

Probiotic bacteria produce organic acids such as lactic and acetic acids, H_2O_2, and bacteriocins. Lactic and acetic acids account for more than 90% of the acids produced Lowering of the pH due to lactic or acetic acid produced by these bacteria in the gut has a bactericidal or bacteriostatic effect. Probiotic bacteria show strong anti-microbial properties against gram positive bacteria such as *Staphylococcus aureus* and *Clostridium perfringens* rather than against gram negative bacteria such as *Salmonella typhimurium* and *E. coli*. Yoghurt bacteria are reported to produce bacteriocin against probiotic bacteria and vice-a-versa. Also yoghurt may contain one

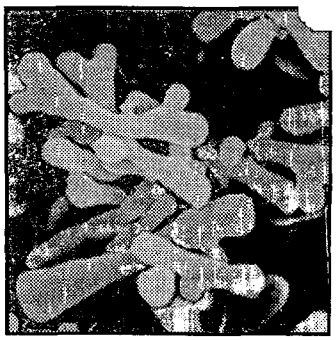

Fig. 3 Bifidobacteria- as Under Scanning Electron Microscope (Ref. 3)

or more groups of *L. acidophilus* or *L. casei* organisms, and thus affect the viability of *L. delbrueckii ssp. Bulgaricus.*

4.8 Probiotics and Allergy (Ref. 3)

Allergy is caused by an immune reaction that is out of all proportion to the antigenic stimuli. Classical allergy is a type I hypersensitivity reaction mediated by the interaction of mast cells (and eosinophils) coated with allergen-specific IgE and a cross-linking allergen. On bacterial colonization of the colon after birth the appropriate microbiological stimuli is essential to redress the balance of the skewed T-helper 2 immune response present in the newborn. This normal interaction between baby and microbes is thought to be compromised in the Western world, with a reduction in bifidobacteria and an increase in clostridial species, particularly in bottle-fed infants. The use of probiotic therapy to prevent allergic disease has been demonstrated in two studies using a probiotic *Lactobacillus rhamnosus* GG in neonates. Management of allergy through probiotics has also been demonstrated in infants, using lactobacilli to control atopic eczema and cow's milk allergy.

4.9 Anti-Mutagenic Activity

Antimutagenic activity of fermented milk has been demonstrated in vitro against a large spectrum of mutagens, including 4-nitroquinoline-N'-oxide, 2-nitrofluorene and Benzopyrene, and against range of mutagens and promutagens in various test systems based on microbial and mammalian cells. Microbial binding of mutagens could be a possible mechanism. While acetic acid bacteria showed higher anti mutagenic activity than lactic or pyruvic acids; butyric acid showed a broader spectrum of Antimutagenic activity against mutagens or promutagens. Butyric acid is claimed to prevent carcinogenic effects at molecular level. Live bacterial cells showed higher anitmutagenicity than killed cells against mutagens. This suggests that live bacteria may bind or metabolize the mutagens. Inhibition of mutagens and promutagens by probiotic bacteria appeared to be permanent for live cells and temporary for killed cells. Killed cells release mutagens and promutagens when extracted with dimethyl sulfoxide.

4.10 Reduction in Serum Cholesterol

Feeding of fermented milks containing very large numbers of probiotic bacteria (~109/ g) to hypercholesterolemic individuals has lowered cholesterol levels from 2000ppm to 1000ppm. A decrease in serum cholesterol levels is observed in men fed with large quantities of milk fermented with *Lactobacillus*, which may be due to the production of hydroxymethyl glutarate by lactic acid bacteria, which inhibit hydroxymethyl glutaryl CoA reductases required for the synthesis of cholesterol Removal of cholesterol from the culture medium by *L. acidophilus* and other species is not due to bacterial uptake of cholesterol but due to conjugation of bile acid by *L. acidophilus*. *L.acidophilus* and *Bifidobacteria* actively assimilate cholesterol and organic acids; the former itself may take up cholesterol during its growth in the small intestine and make it unavailable for absorption into the blood stream.

4.11 Miscellanous Potential Health Benefits

Some experimental animal and human investigations suggest that probiotics may reduce the risk of heart disease by their beneficial effects on blood lipid levels and blood pressure. Different strains of lactobacilli and fermented milk products containing probiotic bacteria may help prevent and treat urinary tract infections, bacterial vaginosis, and yeast vaginitis in women. Probiotics may also help relieve constipation, reduce colic in infants, alleviate kidney stones, decrease inflammation associated with arthritis and protect against dental caries.

5. Criteria of Selection of Probiotics with Debates on Effectiveness

The desired effects of probiotic microorganisms are produces only if they are able to adhere, colonize, and multiply in the intestine. The adherence ability if these organisms will improve their chances of winning competition against "**unfriendly bacteria**" to occupy the intestinal "niches". Adherence to the intestinal cell- wall is an important prerequisite for the colonization in the gastrointestinal tract. Not all but only a few *Bifidobacteria* strains adhere and colonize in the intestinal cells. *L. acidophilus* strains inhabit in the upper parts of the intestine, whereas the bifidobacteria in the lower part of the intestine, particularly the colon. In general, *Bifidobacterium spp.* adhere better (22.2% of the available) than *L. acidophilus* (16.6% of the available). Especially, *B. infantis* and *B. longum* adheres the best of all.

This means that probiotic bacteria should be resistant against acid (stomach), bile, capable of growing under anaerobic conditions and be non-toxic. These criteria limit the number of bacterial species and strains to the following groups of bacteria: *Lactobacillus, Streptococcus* and *Bifidobacterium species*, although some other species can be used in some cases (such as yeasts and Bacillus species in animal nutrition). Reduction of diarrhoea, improved digestion of lactose, reduced constipation and beneficial effects on *Candida* have been scientifically proven for a number of strains. For most other claims there is no or very little scientific evidence. Even scientifically proven probiotic strain may not be effective in every person, since the intestinal flora is individual specific.

6. Composition of Probiotics

Probiotic preparations mostly are composed of bacteria naturally found in human intestines such as *L.acidophilus, L.casei, Enterococcus faecium* and *Bifidobacterium bifidum*. Yoghurt starter bacteria (*L.bulgaricus* and *Streptococcus thermophilus*) are also included.

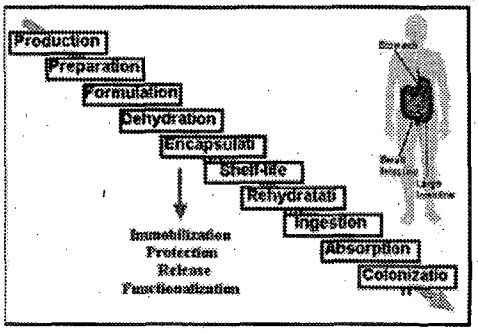

Fig. 4 Probiotic Processing (Ref.3)

Formulators- What Levels of Probiotics Need to be delivered in Products?

Probiotic levels used in product offerings must be based on levels found to be efficacious in human studies. General recommendation for a minimum amount of probiotic to be effective cannot be accurately made. This is because efficacious doses vary widely in documented human studies. For example, several studies of *L. reuteri SD2112* and of *B. infantis* 35264 have documented that 1x108 (100 million)/day is an adequate dose for several different health targets. However, it is recommended at 1.8x1012 (1.8 trillion)/day for management of recurrence of certain inflammatory bowel conditions. The required dose is dependent on a variety of factors, including physiological characteristics of strains being used, types of clinical endpoints being tracked, whether the endpoint is prophylactic or therapeutic, length of time of administration of probiotic, and if other bioactive ingredients are used in conjunction with the probiotic (See figure 4- Ref. 3).

Table- 2- Health Effects of Different Probiotic Strains (Ref- 11.1.7)

Sr. No.	Strain	Proven effect in humans
1.	Bifidobacterium animalis subsp. lactis BB-12	Immune stimulation, to prevent diarrhoea in children. Stabilizes intestinal passage.
2.	Bifidobacterium lactis HN019 (DR10)	Immune stimulation
3.	Bifidobacterium infantis 35624	Irritable Bowel Syndrome (IBS)
4.	Lactobacillus casei DN114-001	Immune stimulation
5.	Bifidobacterium longum BB536	positive effects against allergies
6.	Lactobacillus acidophilus NCFM	reduces symptoms of lactose intolerance, prevents bacterial overgrowth in small intestine
7.	Saccharomyces cerevisiae (boulardii) lyo	against antibiotic-associated diarrhoea and Clostridium difficile infections
8.	Streptococcus salivarius ssp thermophilus	reduces symptoms of lactose intolerance

7. Prebiotics and Synbiotics (Ref 10.6)

The beneficial effects of the presence of bifidobacteria in the GIT are dependent on their viability and metabolic activity. Their growth is dependent on the presence of complex carbohydrate known as oligosaccharides. Certain oligosaccharides are considered, which are defined as "non-digestible food that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon". To maximize the effects of bifidus products, prebiotics are used in probiotic foods. Some oligosaccharides, because of their chemical structure, are resistant to digestive enzymes and therefore pass into the large intestine, where they become available for fermentation by saccharolytic bacteria. Compounds which are either partially degraded or not degraded by the host and are preferentially utilized by bifidobacteria as carbon and energy source are referred to as "bifidogenic factors". Some of these factors of commercial significance include fructooligosaccharides, lactose derivatives such as lactulose, lactitol, galactooligosaccharides, and soybean oligosaccharides (SOS). Resistant starch and non-starch oligosaccharides are classified as colonic foods, but not as prebiotics because they are not metabolized by certain beneficial bacteria. Fructooligosaccharides (FOS) and galactooligosaccharides (GOS) increased the bifidobacteria number by 2 log cycles. Likewise fructans and inulin are prebiotic in nature and are widely used in USA and Europe. Some other prebiotics are lactosucrose, isomalto-oligosaccharides, xylo- oligosaccharides, gentio-oligosaccharides.

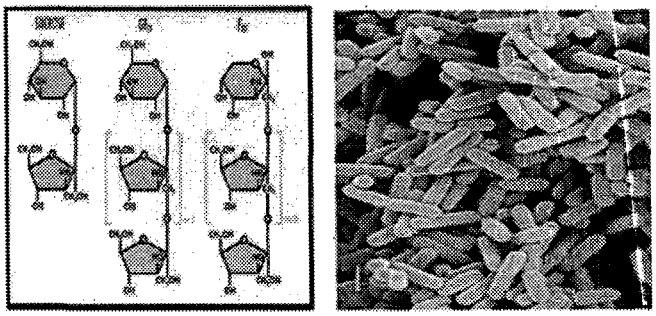

Fig. 5 Prebiotic Dependent Bacteria (*Lactobacillus*) (Ref. 11.3)

Synbiotics have also been defined as metabolites produced by ecoorgan or by synergistic action of prebiotics and probiotics e.g. short chain fatty acids, other fatty acids, amino acids, peptides, polyamines, carbohydrates, vitamins, numerous antioxidants and phytosterols, growth factors, coagulation factors, various signal molecules such as cytokine-like bacteriocins.

Examples of Working of Prebiotics and Synbiotics

a. Strains of *B. longum, B. catenulatum* and *B. animalis* grow best on FOS and much lower growth rate on inulin.
b. From the in vivo studies, rats that were fed FOS and synbiotic displayed higher levels of bifidobacteria and lower levels of coliforms. FOS has been reported to increase bile resistance in *Bifidobacteria.*
c. Soygerm powder contains SOS and also is a rich source of isoflavones, which are believed to be protective against some forms of oestrogen- related cancer, osteoporosis and cardiovascular conditions. Soygerm powder (4.0 g/l) increased resistance of *L. reuteri* to bile salts. In addition, the lactobacilli (See Figure 5- Ref.3) cleaved the isoflavones glycosides to liberate the aglycone isoflavones, increasing its bioavailability.

8. Conclusion

Research indicates that probiotics confer a variety of health benefits. However, when drawing conclusions regarding this research, it is important to recognize that different strains, species, and genera of bacteria may have unique effects. *In vitro*, experimental animal and human studies of probiotics have used different bacterial strains and combinations of strains at different doses. Because human health issues are very difficult to study directly, different end points (e.g., blood cholesterol as an indicator of heart disease risk) are employed. Also, the mechanisms by which probiotics exert their effects are largely unknown. For the above reasons, generalizations about the health effects of probiotics can be misleading. Nevertheless, some promising health benefits (e.g., alleviation of lactose intolerance symptoms, anti-diarrheal effects, and immune stimulation) are attributed to specific strains of probiotics consumed at adequate levels.

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