PROBIOTICS, PREBIOTICS AND SYNBIOTICS— AN EMERGING AVENUE IN PHARMACEUTICAL BIOTECHNOLOGY

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There are bacteria present in the human intestine, which help in the digestion of food and also serve as a barrier against invading pathogens. These are the '**probiotics**'. They are friendly bacteria, which contribute to the health and balance of the intestinal tract.

In recent times, these bacteria have also been utilized for various therapeutic uses like to lower cholesterol levels in serum and also to serve as anti-cancer agents.

This article seeks to introduce the concept of probiotics and to explain their various modes of action against pathogens. It also explains three of the therapeutic uses of probiotics.

The latter part of the article talks about '**prebiotics**'. These are foods that cannot be digested by humans but which encourage the growth of the friendly bacteria in the gut.

Both pre- and pro-biotics, when administered together contribute '**synbiotics**'.

Intestinal Microflora

The human foetus in the uterus is sterile. However on passage through the vagina during birth it acquires microorganisms. These are rapidly added to after birth and the newborn baby acquires a gut microflora, characteristic for humans. The type of delivery, dietary constituents (such as if the baby was fed breast milk or formula milk) and the gestational age influence the colonization pattern.

The final indigenous gut microflora, which stabilizes in the gut, is a very complex collection of about 10^{14} microorganisms consisting of 400 different types of bacteria[1]. This establishment of bacterial activity complements the human digestive functions by extending the range of digestive enzymes and, under normal conditions, providing a barrier against invading pathogens.However, under stress or with dietary habits, the 'balance' of intestinal microflora may become disturbed and disorders in digestive functions may occur. Also antibiotics and chemotherapeutic agents which are widely used to inhibit pathogenic bacteria, may also depress non-pathogenic bacteria[2].

To prevent or cure such conditions, probiotics can be administered.

BOX 1: Factors Affecting the Human Colonic Bacteria.

Host factors:
Acids (hydrochloric, lactic and fatty acids).
Enzymes (gastric, pancreatic and epithelial).
Bile salts.
Peristalsis.
Local immune mechanisms.
Villous contraction/epithelial turnover.
Redox potential.

Microbial factors:
Bacterial interactions (antagonistic, symbiotic or synergiotic).
Traits of microorganism (egs— growth requirements and adhesion properties).

Environmental factors:
Diet.
Drugs.

'Probiotics'

Probiotics can be defined as *"a mono-or mixed-culture of live microorganisms which, when applied to man or animal (examples as dried cells or as a fermented product) affects the host beneficially by improving the properties of the indigenous microflora"*[3]. Thus probiotics contribute to the health and balance of the intestinal tract, protecting against disease and improving nutrition.

Although roughly 20 species of bacteria are described in depth as probiotic, many of these exert their beneficial effects in a transitory nature, and are either not resident in the gastrointestinal tract of humans, or reside only in the gastrointestinal tract of infants conferring immunity until their adult

complement of intestinal flora is acquired. However lactobacilli and biofidobacteria are two species of bacteria that take up permanent residence in the human intestine. This article will restrict itself to the therapeutic uses exerted by these two bacteria.

BOX 2 : Features of a Good Probiotic

1) Should be normal inhabitant of the intestinal tract.
2) Should be a strain, which is capable of exerting a beneficial effect on the host, eg. – increased growth or resistance to diseases.
3) Should be non-pathogenic or non-toxic.
4) Should be present as viable cells, preferably in large numbers.
5) Should be capable of surviving and metabolizing in the gut environment, eg— resistant to low pH and organic acids.
6) Should be stable and capable of remaining viable for long periods under storage and field conditions.

BOX 3: Possible Modes of Action of Probiotics

1) Suppression of viable count by :-
   a) competition for adhesion sites
   b) competition for nutrients
   c) production of antibacterial compounds
2) Enterotoxin neutralization
3) Prevention of toxic amine synthesis
4) Stimulation of immunity
   a) increased antibody levels
   b) increased macrophage activity

Modes of action : Probiotics act against various invading pathogens by the following mechanisms.

1) **Competitive attachment:** Surface action through attachment to the intestinal wall is necessary for enterotoxin producing ***E.coli*** to induce diarrhoea[4]. Thus the idea that lactobacilli compete with coliform for sites of adherence on the intestinal surface is an attractive hypothesis, as attachment is believed to support proliferation and to reduce peristaltic removal of organisms. However it is important to remember that adhesion is a host specific phenomenon. Also it can vary between strains of the same species and can be influenced by growth conditions of the bacteria.

2) **Bacteriocidal activity:** The decrease in pH of the stomach to less than 4.5, prevents the growth of many bacteria, including coliforms, but still allows the growth of some strains of lactobacilli. This decrease in pH is brought about by lactobacilli by production of organic acids like lactic acid.[5] Lactobacilli are also known to produce hydrogen peroxide which has bacteriocidal actions in vitro[6].

3) **Competition for nutrients:** Although theoretically, competition for nutrients between gut flora and and pathogens seems very likely, there is not much evidence for it occurring. In vitro studies have demonstrated a competition between gut flora and ***Shigella flexneii*** for carbon sources[7] but this antagonistic effect is changed when the medium is altered[8].

4) **Enterotoxin neutralization:** Investigations of ***L bulgaricus*** show that the organism produces a metabolite that is thought to neutralize the effect of enterotoxin relesed from coliforms. Although the neutralizing substance has yet to be identified, support for the anti-enterotoxic activity has been obtained from experiments with rats, pigs and calves[9,10,11].

5) **Prevention of toxic amine synthesis:** Coliforms and certain other bacteria in the pig gut, have the ability to decarboxylate amino acids and can yield amines which have toxic properties or perhaps cause subclinical pharmacological effects[12]. Certain probiotics can prevent such toxic amine synthesis.

5) **Enhanced immunity:** Conventional animals with a complete gut flora have increased phagocytic activity and immunoglobulin levels compared with germ free levels animals [13]. These findings of a systematic effect on immunity indicate that probiotics have the potential, not only to affect the balance of the gut flora, but also to influence the pathogenesis of diseases which occur in tissues remote from the intestinal tract.

BOX 4: Claimed Beneficial Effects and Therapeutic Application of Probiotic Bacteria in humans

Beneficial effects:
Maintenance of normal intestinal and urogenital microflora
Alleviation of lactose intolerance
Reduction of serum cholesterol levels.
Anticarcinogenic activity.
Stimulation of the immune system.
Improved nutritional value for food.

Therapeutic applications:
Prevention of urogenital infection.
Alleviation of constipation,
Protection against traveller's diarrhoea.
Prevention of antibiotic induced diarrhoea.
Prevention of hypercholesterolaemia.
Protection from colon/bladder cancer.
Reduction of side effects of hepatic encephalopathy.
Aid in cares of hypo- and hyperchlorohydria.
Prevention of osteoporosis.

Application of Probiotics: Of the various therapeutic uses and beneficial effects of probiotics mentioned in the box, this article will only concentrate on the following:-

1) Anticarcinogenic activity
2) Reduction in serum cholesterol levels
3) Control of intestinal pathogens.

1) **Anticarcinogenic activity**: The anticarcinogenic properties of lactobacilli fall under three categories:-

a) The inhibition of tumour cells[14].

b) The suppression of bacteria which produce enzymes such as beta-glucinosidase, beta-glucoronidase and azoreductase which are responsible for the release carcinogens from innocuous complexes[15] and

c) Destruction of carcinogens such as nitrosoamines[16] and the suppression of nitroreductase which is involved in the synthesis of nitrosoamines[17].

Thus lactobacilli suppress the metabolic activity of the colonic microflora and in this manner may reduce the formation of carcinogens in the large intestine. They can also delay the colonic tumor formation by prolonging the induction of the latent period[18].

The evidence for anti-tumor activity or inhibition of carcinogenesis is not extensive. However under certain defined conditions, specific strains of bacterial and fermented dairy products may have some protective role.

2) **Reduction in serum cholesterol levels**: Elevated plasma cholesterol has been positively associated with a higher rate of coronary heart disease. The various ways in which ***Lactobacillus acidophilus*** can be used to decrease cholesterol levels is given below:-

a) ***L.acidophilus*** deconjugate bile acids such as taurocholic acid and glycocholic acid. This is important because deconjugated bile acids do not support the absorption of lipids from the intestinal tract as much as conjugated ones[19]. This could result in reduced absorption of cholesterol from the intestines and thus influence serum cholesterol levels.

b) ***Lactobacilus acidophilus*** assimilates cholesterol. This could be because the organism binds cholesterol in the intestinal lumen, thereby reducing its absorption into the blood stream.[20]

c) The presence of organic acids, in fermented milk, such as uric, orotic and hydroxymethyl glutaric acids, inhibit cholesterol synthesis[21].

3) **Control of intestinal pathogens**: ***L acidophilus*** and bifidobacterium species possess anti-microbial properties. These have been demonstreted in vivo against a variety of undesirable bacteria including ***E.coli, Klebsiella, Salmonella, Straphaureus*** and ***Vibrio*** species. The inhibition may be due to a complex interaction of factors including lactic and acetic acids and antibiotic-like substances. Although the results of in vitro experiments do not guarantee antimicrobial activity in the intestine, it is likely that carefully selected strains i.e. of human origin and with demonstrated bile tolerance, have the potential to colonize the bowel and assist in the control of intestinal disorders[22].

Problems Associated with Probiotic Use

1) **Survivability:** Microorganisms introduced orally have to, at least, transiently survive in the stomach and the small intestine. Although this appears to be a rather minimal requirement, many probiotics often do not survive to reach the lower small intestine[23]. The reason for this appears to be the low pH of the stomach. In fasting individuals, the pH of the stomach is between 1 and 2 and most microorganisms, including lactobacilli, can only survive from thirty seconds to several minutes under these conditions[24]. Therefore, in order for a probiotic to be effective, even the selection of strains that can survive in acid at pH 3.0 for sometime would have to be introduced in a buffered system such as milk, yogurt or food.

2) **Bile resistance:** The small intestine and colon contain relatively high concentrations of bile acids which can inhibit growth or kill many bacteria.

In due course, the recombinant DNA strains could be developed for the exacting requirement.

3) **Adhesion:** It is not clear if adhesion to the intestinal epithelium is essential for the persistence of a probiotic in the human intestinal tract. However, adhesion seems to be a property that enhances long-term survival[18].

4) **Competition with bacteria already present:** The bacteria need to compete for nutrients and ecological sites of colonization with a previously established microbial flora comprising of several hundred other bacterial species[25].

One way of overcoming these problems associated with probiotic use, is to use prebiotics. These are not viable entities but are growth substrates which specifically benefit the potentially beneficial bacteria already growing in the colon[25].

Prebiotics

A prebiotic is a nondigestible food that beneficially affects the host by selectively stimulating the growth and/or activity of one or limited number of bacteria in the colon, and thus improves host health.

As shown in the figure, a food ingredient classified as a prebiotic must:

1) Be neither hydrolysed nor absorbed in the upper part of the gastrointestinal tract

2) Be a selective substrate for one or a limited number of beneficial bacteria commensal to the colon which are stimulated to grow and/or metabolically activated.

3) Be able to alter the colonic flora in favour of a healthier composition; and

4) Induce luminal or systematic effects that are beneficial to the host health.

Among the food ingredients, nondigestible carbohydrates (oligo- and polysaccharides), some peptides and proteins, and certain lipids (both ethers and esters) are candidate prebiotics. Because of their chemical structures, these compounds are not absorbed in the upper part of the gastrointestinal tract or hydrolysed by human digestive enzymes. Such ingredients could be called 'colonic foods' – i.e. foods entering the colon and serving as substitutes for the endogenous colonic bacteria, thus indirectly providing the host with energy, metabolic substrates and essential micronutrients. Among the colonic foods, nondigestible oligosaccharides are naturally occurring and are able to fulfill the criteria of prebiotics as defined above[25].

Among the natural nondigestible oligosaccharides, this article will discuss the fructo-oligosaccharides and their potential as prebiotic foods.

Fructo oligosaccharides as prebiotics : Among the natural nondigestible oligosaccharides that fulfill the criteria of a colonic food, fructo oligosaccharides are the only products presently recognized and used as food ingredients that meet all the criteria allowing them to be classified as prebiotics[25].

Fructo oligosaccharides (FOS) are presents in

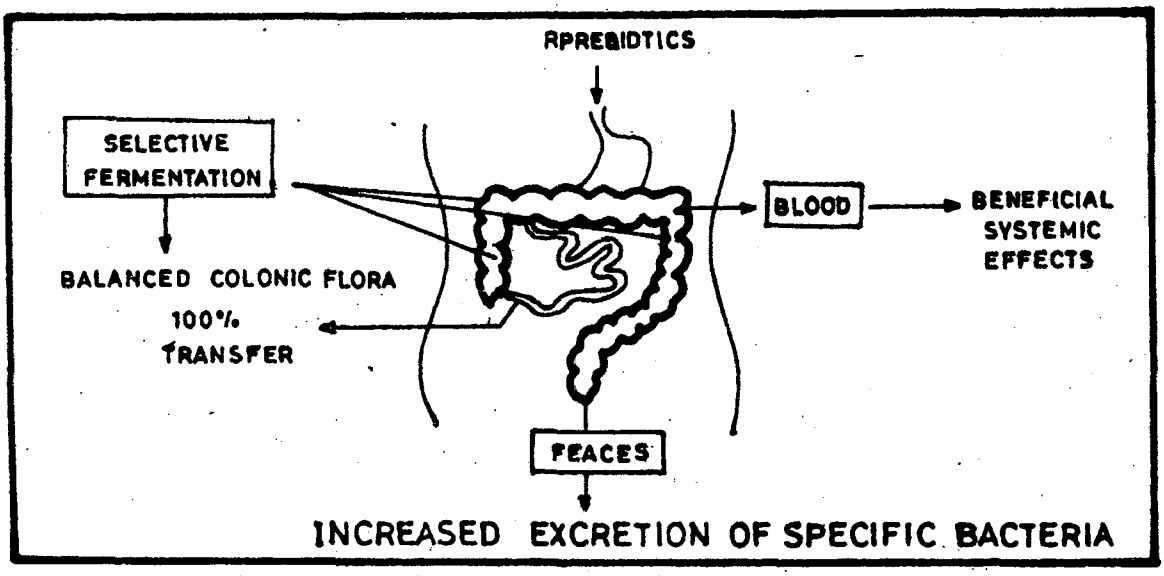

Fig.1:- Schematic representation of the properties that allow classification of food ingredients as prebiotics.

a number of common food-stuffs like garlic, onion, artichoke and asparagus[25].

Beneficial effects of FOS on human health:- FOS are selectively utilized by L-acidophilus and bifidobacterium for their better survival, stability, growth and proliferation. FOS promotes, stabilizes and enhances the proliferation of these beneficial bacteria into the hostile gastrointestinal tract. Incorporation of FOS as a supplement will intensify the viability and adhesion of the beneficial bacteria in the gastrointestinal tract. Conversely, pathogenic bacteria including ***Escherchia Coli, Clostridium Perfringers*** and others have been shown to be unable to utilize the FOS[26].

Benefits of FOS to human gastrointestinal ecology:-

1) Stimulates growth of acidophilus, bifidus and faecium group of microorganisms.
2) Causes reduction in fecal pH.
3) Causes reduction in toxic metabolites.
4) Causes reduction in serum cholesterol and triglyceride levels.
5) Reduces the blood pressure of elderly hyperlipemic people.
6) Alters metabolism of bile acid.
7) Modifies composition and rate of production of secondary bile acids.
8) Reduces carbohydrate and lipid absorption, thereby normalizing blood glucose and serum lipids.
9) Ameliorates derangements of carbohydrates and lipid metabolism in diabetics[26].

Compared to probiotics, prebiotics are likely to have distinct advantages such as the in-situ stimulation of the growth or certain resident bacteria already present in the host.. They also have their own physiological effects such as improved lipid profile and certain effects associated with dietary fiber[25].

Synbiotics

A combination of both probiotics and prebiotics together, constitute synbiotics. A synbiotic may be defined as *" a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare."*[25]

Nutritional Benefits of Synbiotics

1. Improved survival of live bacteria in food products with , as a consequence, prolonged shelf life.
2. An increased number of ingested bacteria reaching the colon in a viable form.
3. Activation of the metabolism of these bacteria

This approach will be particularly important if the bacteria that are targeted utilize specific substrates, as in the case of bifidobacteria, which utilizes fructo oligosaccharides.

Conclusion

Although the concept of probiotics has existed in practice since biblical times, we are still at a very early stage in the development of consistently effective probiotics for human use. In an increasingly health-conscious society, the market for 'health foods' is worth several billion dollars. However, if nutritional and health benefits are to be derived from products containing probiotic bacteria, it is imperative that we understand the mechanism by which these benefits are derived and we ensure that the cultures used maintain their desirable characteristics during production[21]. Also side effects of conventional drug therapy for corrective effect would undoubtedly be avoided by this natural way of curing the disorder. Hence a lot more research is required to understand which specific bacteria strain to use and also to produce an efficacious, viable and economical product to benefit the public.

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