

Mechanism of Action of Probiotics

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ABSTRACT

The modern diet doesn't provide the required amount of beneficial bacteria. Maintenance of a proper microbial ecology in the host is the main criteria to be met for a healthy growth. Probiotics are one such alternative that are supplemented to the host where by and large species of Lactobacillus, Bifidobacterium and Saccharomyces are considered as main probiotics. The field of probiotics has made stupendous strides though there is no major break through in the identification of their mechanism of action. They exert their activity primarily by strengthening the intestinal barrier and immunomodulation. The main objective of the study was to provide a deep insight into the effect of probiotics against the diseases, their applications and proposed mechanism of action.

Key words: Anti-allergic, Gastrointestinal problems, Immunomodulation, Infectious diseases, Probiotics

INTRODUCTION

The gut microenvironment has an effect on the nutrition, feed conversion and disease of the host, thereby maintaining the microbial ecology of the gut (Guarner and Malagelada 2003). During the periods of stress, illness or antibiotic treatment, the gut flora is often changed in favor of harmful bacteria that may cause diarrhea and loss of appetite (Cremonini et al. 2002; Harish and Varghese 2006). Overgrowth of the harmful bacteria and its subsequent invasion of the system lead to inflammatory, immunological, neurological and endocrinological problems. Induction of the growth of beneficial bacteria is one of the possible solutions to normalize the health conditions. This could be achieved by the supplementation of viable bacterial cells into the host. Probiotics can help to build up the beneficial bacterial flora in the intestine and completely exclude the pathogenic bacteria. These bacteria also release some enzymes which help in the digestion of the feed (Jean et al.

2003). A daily intake of 10^9 - 10^{10} colony forming units (CFU) viable cells has been shown to have positive effect on the host health.

There are many microorganisms that could potentially function as probiotic, of which *Lactobacillus* and *Bifidobacterium* species are the most commonly used. Probiotics are live microorganisms thought to be beneficial to the host organism. According to the currently adopted definition by FAO/WHO, probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit on the host. In addition, nonpathogenic species belonging to the class of *Saccharomyces*, *Streptococcus* and *Lactococcus* are also used as probiotics. Probiotics affect the host beneficially, which may be direct or indirect, including enhanced barrier function, modulation of the mucosal immune system, production of antimicrobial agents, enhancement of digestion and absorption of food and alteration of the intestinal microflora (Fig. 1) (Jean et al. 2003).

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The efficacy of a probiotic effect often depends on the mechanism by which they exert their activity. By and large, to treat a disease, the probiotics follow a set of mechanisms, which is discussed in this review. The effective performance of the probiotic depends on their strong adherence and colonization of the human gut, which in turn improves the host immune system (Sarah et al. 2008). The mechanism of adherence is still under

investigation, but *Lactobacillus plantarum* 299v has been shown to exhibit a mannose specific adhesion by which it can adhere to human colonic cells. Once the probiotic adheres to the cell, various biological activities take place, which primarily include the release of cytokines and chemokines. These then exert their secondary activity such as stimulation of mucosal and systemic host immunity (Delcenserie et al. 2005).

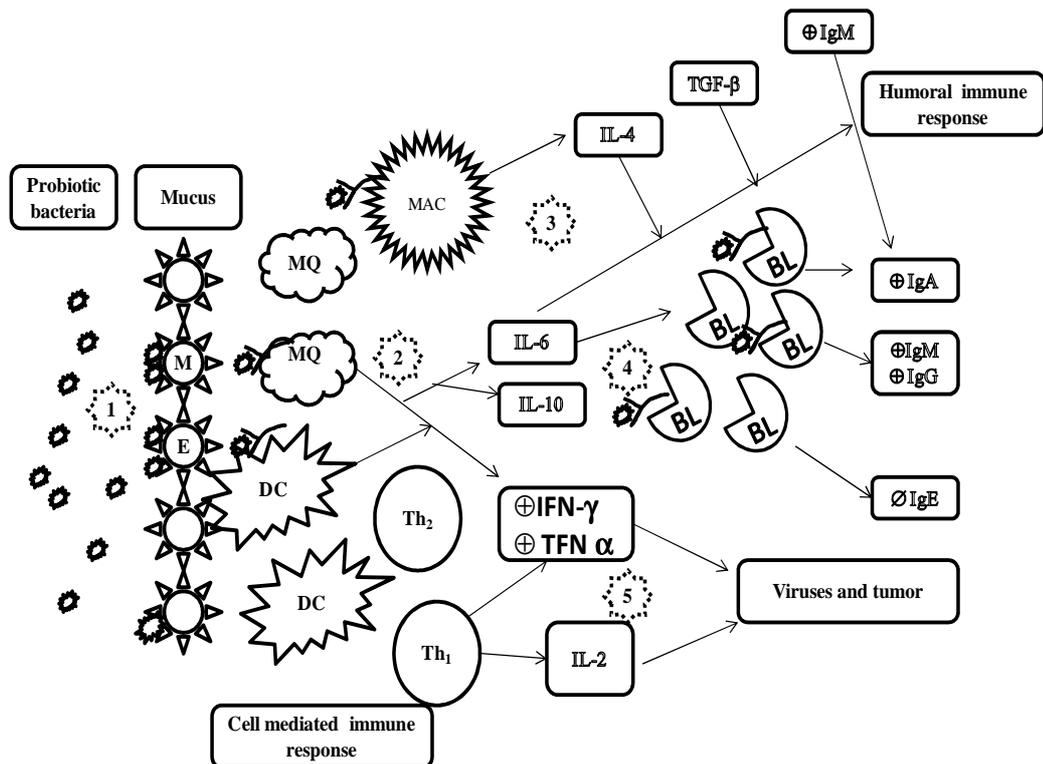


Figure 1 - Hypothesised mechanism of immunomodulation by probiotics. (1). Interaction of probiotic bacteria with epithelial cells (E) or M cells (M) or the Dendritic cells (DC) results in the internalization of the bacteria or its components. (2). This interaction stimulates the release of IL-6 by epithelial cells and stimulates macrophages (MQ) and dendritic cells to produce TNF- α and IFN- γ . (3). Mast cells (MAC) or also stimulated to produce the cytokine IL-4, which together with IL-6 and TGF- β induce the T-independent switch from IgM to IgA on the surface of B lymphocytes (BL), thereby enhancing the production of IgA. (4). IL-6 favours the clonal expansion of IgA B lymphocytes. There is also an associated increase in the production of antibodies such as IgM, IgG and reduced secretion of IgE. (5). Th1 cells produce pro-inflammatory cytokines such as IFN γ , TNF α and IL-2, which stimulate the phagocytosis and destruction of microbial pathogens and induce macrophages, natural killer cells and cytotoxic T-lymphocytes to kill viruses and tumors (Galdeano et al. 2007).

Prevention of infectious diseases

Probiotic strains inhibit the pathogenic organisms by competing for the limited substrates required for fermentation or the receptors. They prevent the adherence of the pathogenic bacteria to the host

cells by strengthening the barrier effect of the intestinal mucosa (Eizaguirre et al. 2002; Mangell et al. 2002) and release of gut-protective metabolites (arginine, glutamine, short-chain fatty acids and conjugated linoleic acids). Probiotic acts

as antimicrobial by secreting the products called bacteriocins and substances such as organic acids (lactic, acetic and butyric acid) and H₂O₂ (De Keersmaecker et al. 2006). For example, *Lactobacillus acidophilus* has been shown to produce two compounds, bacteriocin lactacin B and acidolin. Lactacin B was shown to inhibit *Lactobacilli in-vitro*, whereas acidolin inhibited enteropathogenic organisms. Silva et al. (1987) demonstrated an inhibitory substance produced by *Lactobacillus* GG with similar broad spectrum activity. They also lower the intestinal pH (De Keersmeacker et al. 2006), agglutinates pathogenic microorganisms, binds and metabolise toxic metabolites (Fonden et al. 2000; Oatley et al. 2000; Haskard et al. 2001) or regulate the intestinal motility (Marteau et al. 2002) and mucus production (Mattar et al. 2002; Vrese and Marteau 2007).

The clinical data show that the intake of

Lactobacillus reuteri significantly reduces the incidence or severity of diarrhoea of different origins and reduces the gastrointestinal illness or infections. The ability of *L. reuteri* to influence the basic immune responses in the human gastrointestinal tract may be the basis for an improved protection against the pathogenic infection (Connolly 2004). Many different strains are known to treat diarrhea caused by (primary) lactose intolerance; acute diarrhea from viral and bacterial infections, e.g. nosocomial rotavirus infections in children, gastrointestinal infections in children in day-care centers and travelers' diarrhea; antibiotic-associated diarrhea (AAD); *Clostridium difficile* gastroenteritis; diarrhea in tube-fed patients; chemo- or radiotherapy induced diarrhea; inflammatory bowel diseases (Crohn's disease, ulcerative colitis and pouchitis); small bowel bacterial overgrowth; and irritable bowel syndrome (IBS) with diarrhea (Fig 2) (Michael de Vrese and Marteau 2007).

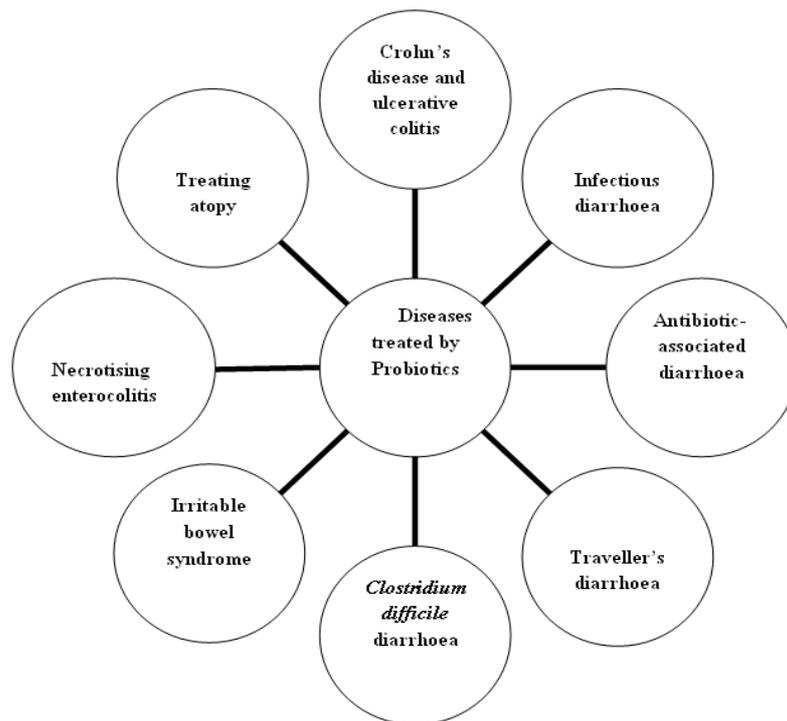


Figure 2 - List of various diseases that are possibly treated by probiotics.

The daily intake of *L. reuteri* in humans significantly reduced the number of reported sick days due to common infections, and this effect was even more pronounced in the shift workers (Tubelius et al. 2005). The daily consumption of *L.*

reuteri at the dosage of 10¹⁰ CFU for 21 days was shown to be safe and had no side effects on the adults infected with HIV (Wolf 1998). A short term daily ingestion of *L. reuteri* delivered by drinking straw or tablets reduced the levels of

salivary *Streptococci* in young adults (Caglar et al. 2006). The *L. reuteri* supplementation during and after *Helicobacter pylori* eradication therapy significantly reduced the frequency and intensity of antibiotic associated side effects (Lionetti et al. 2006). The administration of *L. reuteri* significantly decreased UBT (Urea Breath Test) values in *H. pylori* positive subjects, demonstrating that *L. reuteri* suppressed *H. pylori* density (Imase et al. 2005). The probiotic supplementation with *L. reuteri* has a beneficial effect on *H. pylori* infection in humans (Saggiaro et al. 2005).

Stimulation of mucosal and systemic host immunity

There is considerable evidence from the animal studies that probiotic organisms can modulate the mucosal and systemic immune systems. Link-Amster et al. (1994) fed 16 volunteers with fermented milk supplemented with *L. acidophilus*, *Bifidobacterium bifidum* Bb12 and *Streptococcus thermophilus* for three weeks during when they injected attenuated *Salmonella typhi* Ty21a vaccine. Results showed that the specific serum IgA titre rise was significantly higher in the controls, denoting an improvement in the humoral immune response.

Stimulation of the immune system in response to rotavirus infection was studied by Kaila et al. (1992a). The 39 children with acute rotavirus diarrhoea were randomly assigned to receive *Lactobacillus casei* GG or a placebo milk product. They found an increased IgA specific antibody secreting cell response to rotavirus in the probiotic group, associated with a reduction in diarrhoea. When they repeated this study with heat inactivated *Lactobacillus casei* GG, specific IgA levels were unaltered but the duration of diarrhoea was reduced. Finally, they concluded that probiotics must have interacted beneficially with the mucosal immune system. The microorganisms present in the gastrointestinal tract (including the endogenous flora), interact with the epithelial and immune cells. The cytokine response is initially manifested by the release of interleukin 8 (IL-8), which leads to migration of neutrophils and monocytes to the mucosa (Salminen et al. 1998). Activated monocytes and dendritic cells in the lamina propria produce tumor necrosis factor α (TNF- α) as well as IL-1 and IL-6 (Venkat et al. 2004). The IL-1 and IL-6 stimulate CD41 T cells (type 1), and these produce a variety of cytokines

including IL-4, -5, IL-6, and interferon- γ . This response is unable to clear the infection and sustains inflammation. Different species of lactobacilli exert different activation patterns on the dendritic cells (Raja 2003). Further, *L. reuteri* DSM12246 inhibited the activities of other species. Clearly, not all the probiotics share the same immunomodulating properties and can even have opposite effects on some parameters.

Probiotics on non-gastrointestinal problems

Apart from the gastrointestinal diseases, probiotics possess functions such as antiallergic activity. Probiotics are used in the treatment of allergic conditions, including rhinitis, atopic dermatitis and food allergy (Norin et al. 2004). Although initial results in the studies of children have been promising, a definitive role for any of these indications remains unproven. Intestinal microflora can contribute to the processing of food antigens in the gut, resulting in food hypersensitivity, of which atopic disease is a manifestation. Probiotics have the potential to modify the structure of potential antigens, reduce their immunogenicity, reduce intestinal permeability and generation of proinflammatory cytokines that are elevated in the patients with a variety of allergic disorders. They directly modulate the immune system through the induction of anti-inflammatory cytokines or through increased production of secretory, which contribute to an exclusion of antigens from the intestinal mucosa (Kaila et al. 1992b). Probiotics could have a potential effect on bone accretion by microbial production of metabolites or enzymes or synthesis of vitamins (Norin et al. 2004; Katharina et al. 2007) because several vitamins are also involved in calcium metabolism and are required for bone matrix formation and bone accretion, e.g. vitamin D, C, or K or folate (Weber 1999). Mechanisms that may contribute to the bone health-supporting effect include degradation of mineral complexing phytic acid, stimulation of calcium uptake by enterocytes and probiotic antiarthritic effect (Katharina et al. 2007).

Probiotics as a feed for animals and aquaculture

The usage of probiotics is not limited to the humans. They have many beneficial effects when they are used in animal feeds and aquaculture. These effects include exclusion of pathogens, improved digestion and absorption of nutrients.

The effect of feeding *Lactobacillus* on the gut microflora, growth and survival of post-larvae of *Macrobrachium rosenbergii* (de Man) has been studied by Venkat et al. (2004). The commercial products can be administered through water or incorporated in the feed. A wide range of microalgae such as *Dunaliella salina* (Raja et al. 2007a,b), *Spirulina platensis* (Raja et al. 2008), *Chlorella vulgaris* (Raja et al. 2008) and yeasts such as *Debaryomyces*, *Phaffia* and *Saccharomyces* Gram-positive bacteria such as *Bacillus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Micrococcus*, *Streptococcus* and *Weissella* (Reid 2002) and Gram-negative bacteria such as *Aeromonas*, *Alteromonas*, *Photobacterium*, *Pseudomonas* and *Vibrio* have been evaluated.

Probiotics actively inhibit the colonization of potential pathogens in the digestive tract by antibiosis or by competing for the nutrients and space, alteration of microbial metabolism or by the stimulation of host immunity. Probiotics may stimulate appetite and improve the nutrition by the production of vitamins, detoxification of compounds in the diet, and by the breakdown of indigestible compounds into simpler compounds. There is evidence that probiotics are effective at inhibiting a wide range of fish pathogens, but the reason for the inhibitions is often unstated (Irianto and Austin 2002). In addition, probiotics have been particularly useful in the early stages of chick growth since the gut of the newly hatched chick is sterile and administering probiotics through water at this stage helps to build up the beneficial bacteria in its gut much faster than the normal course (Singh et al. 2008).

The balance between the phytoplankton, zooplankton and beneficial bacteria during the culture process play a crucial role in the maintenance of pond health (Irianto and Austin 2002). Probiotics have also been used in a big way as pond cleaners in aquaculture (Balcazar et al. 2006). Probiotic bacteria directly uptake or decompose the organic matter or toxic material and improve the quality of water. The microbial cultures produce a variety of enzymes such as amylase, protease, lipase, xylanase and cellulase in high concentrations than the native bacteria, which help in degrading the waste. These bacteria have a wide range of tolerance for salinity, temperature and pH, which are the normal ambience of

aquaculture operations. The use of antibiotics in aquaculture is banned due to the possible side effects of antibiotics on the marine food (Balcazar et al. 2006). Hence, the usage of probiotics is recommended to counter the effect of viral and bacterial infections in commercial aquaculture. The pond probiotics also have a special blend of denitrifying bacteria that remove the algae's primary source of food namely nitrogen from the pond water. This drastic reduction in nitrogen concentration makes it difficult for the algae to bloom (Hallegraeff 1993).

CONCLUSION

The use of probiotics in the day-to-day medicine in the treatment of gastrointestinal disorders is increasing with the discovery of the beneficial effects of these agents. It is important to assess the degree of effects on the population and activity of gut microbes. There are several reports on the role of probiotics in reducing the colon cancer but the reoccurrence of cancer due to such treatments has not yet been established. Studies documenting the probiotic effects in humans are limited, although results in several biological systems are intriguing. The degree of evidence required to substantiate the bioactivity of food ingredients is not clearly established. This is a complex issue involving both regulatory and scientific considerations. The commercial use of probiotics, however, has proceeded because essentially no risk is associated with the consumption of well-defined probiotics in foods and many benefits are possible. Perhaps the most compelling evidence for probiotic efficacy is in the areas of anti-diarrheal effects and improved digestion of lactose in lactose-intolerant people, because these findings have been substantiated in human studies.

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