



REVIEW ARTICLE

Potential Role of Probiotics in Mechanism of Intestinal Immunity

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ABSTRACT

Probiotics are nonpathogenic bacteria exert a constructive influence on health or physiology of the host. Effect of probiotics in the intestinal defense against variety of diseases is well known. The probiotics are involved in the mechanism of intestinal defense, support as antagonist against pathogens, improve intestinal epithelial layer and boost the innate as well as adaptive immunity. However these responses are also exerted by intestinal components. The intestinal components as well as probiotics play a reciprocal role to enhance the immune response of the individual. The possibilities of mechanism of action include the stimulation of epithelial cells, activation of dendritic cells via toll-like receptors (TLRs), conversely produce cytokines. These observations reviewed together advocate that specific immunomodulatory properties of probiotic bacteria should be focusing on mechanism of action via antigen presenting cells (APC).

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INTRODUCTION

Probiotics are live microbial feed/or food supplements or components of bacteria which have beneficial effects on animal and human health. The healthy animal and/or human consist of about 100 trillion cells, carries about ten times as many microorganisms in the intestines including both groups' useful and potentially harmful microorganisms (Guarner and Malagelada, 2003). The most frequent passage for the entry of bacteria is digestive tract, and natural security of the digestive tract is controlled by useful bacteria called 'friendly' bacteria. When these friendly bacteria are not capable to defend the host against pathogens, the harmful bacteria invade the tissues and produce hazardous toxins and their metabolites (Brock and Madigan, 1991) conversely; the host can fight against pathogens through their defense mechanisms. But, in the war of survival, occasionally pathogen succeeds, and need a complementary preventive and control measures (Oyetayo and Osho, 2004). Chemotherapeutic drugs have been applied to control the pathogenic microorganisms, but with the passage of time these drugs developed resistance in the animals as well as in population of the bacteria, herewith, liberate toxic effects in the host. The live biotherapeutic alternative such as probiotics (Gillingham and David, 2009) has been regarded as alternative of

antibiotics. Probiotics have supportive effect on the health of host by producing an affirmative balance of digestive microflora, increase in antioxidant functioning and restraining the damage caused by pathogenic microorganisms (Shen *et al.*, 2011), improving epithelial cell integrity and increasing immune response (Vanbella *et al.*, 1990; Jin *et al.*, 2000; Wenk, 2000; Panda *et al.*, 2001; Linge, 2005). Preventive application of probiotics achieves better utilization of nutrients and has a positive effect on environment of gastrointestinal tract (Capcarova *et al.*, 2009). Useful probiotic strains include the members of genera *Enterococcus*, *Bifidobacterium*, *Lactobacillus*, *Bacillus*, *Lactococcus*, *Propionibacterium*, *Saccharomyces*, *Streptococcus*, and their probio-active (cellular) substances that have awesome influence on the intestinal and non intestinal physiology of host. Many species of probiotics are used in prevention of intestinal affliction, such as constipation, diarrhea, reverse peristaltic movement and accumulation of gases etc (Miles *et al.*, 2006; Huang *et al.*, 2012). The current review presents an overview on beneficial effects of probiotics mainly focusing on maintaining healthy intestine, their mechanism of action and enhancing the cytokine response by antigen presenting cells (APC) via TLR signaling, that is one of the strong tools of multicellular organisms against pathogens by supporting signaling response.

Beneficial Effects of Probiotics to Intestinal Afflictions

Lactose Intolerance: The lactose intolerance is an inability of gastro intestinal tract (GIT) to absorb lactose (predominant sugar of milk), resulting in an increased osmotic pressure in the small intestine with fluent secretion of fluids (Launiala, 1968; Lasser *et al.*, 1975). Fermented milk products have been observed to be tolerated well by lactose maldigesters as compared to milk, these properties might be valuable for lactose intolerant individuals (Vesa *et al.*, 2000). Somewhat probiotics species producing lactase, such as *Lactobacillus*, contain (beta) galactosidase or intracellular lactose; have a role in getting relief from lactose intolerance symptoms (Sanders, 2000). However, the mechanism of action of probiotics against lactose intolerance requires resolving mounting issue.

Diarrhea: Diarrhea is an increase in the frequency of defecation, bowel evacuation and water content of the stool. Now-a-days, various mechanisms have been described to avert the diarrhea. The first mechanism was active blockage of receptor sites (Bernet, 1994) and this theory might be logical if there is an evidence for competition of specific receptors (Lundgren and Svensson, 2001). Somehow lactobacilli response to compete with the peptides released from villous endocrine cells or toxin, and the force that leads to diarrhea could be prevented (Reid *et al.*, 2002). The other mechanism is supported by the local immunoglobulin A (IgA) antibody effective against the rotavirus (Kaila *et al.*, 1992). Animal studies indicate that secretory IgA can be triggered by lactobacillus ingestion, but the negative aspect of this theory is that high concentration of causative agents may drop the effect of secretory IgA (Reid *et al.*, 2002). However, the third mechanism involves the signals from probiotics (*Lactobacilli*) to the host that regulate the intestinal defense to eliminate perceived noxious secretions (Yolken, 1994). Mucins (Glycosylated in nature) secreted by intestine play an active role to inhibit rotaviruses. It has been confirmed that *MUC2* and *MUC3* mRNA expression increase in response to lactobacillus signaling, protect cells against harmful bacterial adhesion (Mack *et al.*, 1999). The last theory involves that probiotics could produce substances that inactivate the active particles (Cadieux *et al.*, 2002). The probable effect may be due to acid, but more specific antiviral properties have not been ruled out yet (Reid *et al.*, 2002). The mechanism of probiotic may be different depending on the cause of diarrhea. The cell receptors have a vital role in signaling to initiate and control the cellular response to develop the immunity.

Constipation: Constipation is one of the major GIT problems in animals as well as human beings, and provides an opportunity for pathogens to invade intestinal mucosa. Peristaltic movement of the intestine is responsible for defecation, and helps in excretion of the harmful agents from the body. Constipation may be due to insufficient liquid intake, low-fiber diets, physical inactivity and some drugs etc. A modified faecal microflora with reduced levels of *bifidobacteria*, *Bacteroides* and particularly reduced levels of clostridia were reported while investigations were made on affected

individuals (Shimoyama *et al.*, 1984; Arthur, 2002). Probiotics have proved to be effective to get relief from constipation (Goldin, 1998), hence a comprehensive study on the mechanism of action of probiotics in constipation is the prime need of time.

Colon Cancer: Procarcinogens convert into carcinogens in intestinal tract and become the cause of colon cancer, while specific strains "probiotic" (*L. acidophilus*) neutralize the procarcinogens. A number of super strains are capable to metabolize procarcinogens that could revert them into noncarcinogenic material. Such step may be preceded by an activation of metabolites a precursor of carcinogen production (Walker, 20008). Certain active enzymes (such as nitro-reductase and beta-glucuronidase) that convert procarcinogen substances into carcinogenic material are tainted by helpful microbes by removing their damaging properties (Wen *et al.*, 2009). Literature manifest that some species of probiotics might be applied for the treatment of cancer. However reliable findings are not accessible in citations.

Inflammatory Bowel Disease: Inflammatory bowel disease (IBD) is characterized clinically by two overlapping phenotypes, ulcerative colitis (UC) and Crohn's disease (CD), both chiefly affect the colon (UC and CD) and/or the distal small intestine (CD). The etiology of disease is not absolutely understood, but a genetic tendency and the friendly intestinal microflora are thought to perform an imperative role (Mattila-Sandholm *et al.*, 1999). Modification in the composition and general activity of the useful microflora may give good results against the disease. Some selected probiotics have been investigated to condense the number of relapses and extend the period of remission (Hamilton-Miller, 2001). Interestingly, not only *L. salivarius* UCC118, and *L. rhamnosus* GG, but also *S. cerevisiae* (*boulardii*) and a strain of *E. coli* (Nissle) have been benefited in alleviating the symptoms of IBD (Gupta *et al.*, 2000; Guslandi *et al.*, 2000). But, additional investigations on the mechanism of action in detail are obligatory to elucidate the mitigation of IBD, symptoms by probiotics.

Necrotizing Enterocolitis: Necrotizing enterocolitis (NEC) is one of the challenging diseases in infants. NEC pathophysiology has been observed multifactorial and only been elucidated to some extent (Vander *et al.*, 2005; Lin and Stoll, 2006). Theoretically, association of targets is a positive adaptation in the intestinal flora which could consist of an efficient process of preventing the commencement of NEC (Deshpande *et al.*, 2007). Contemporary data have shown that a lower incidence of NEC in the group supplemented with *Lactobacillus* GG than in the placebo-supplemented control group (1.4 vs 2.7%) (Dani, *et al.*, 2002), *B. breve* administration can promote colonization by *Bifidobacterium*, and may also stimulate mucosal immunity and immunological development of VLBW infants (Li *et al.*, 2004). *Bifidobacterium infantis* supplementation extensively reduces the incidence of NEC (Caplan *et al.*, 1999). It is confirmed that probiotics have their own functioning mechanism and capacity to reduce the possibility of NEC.

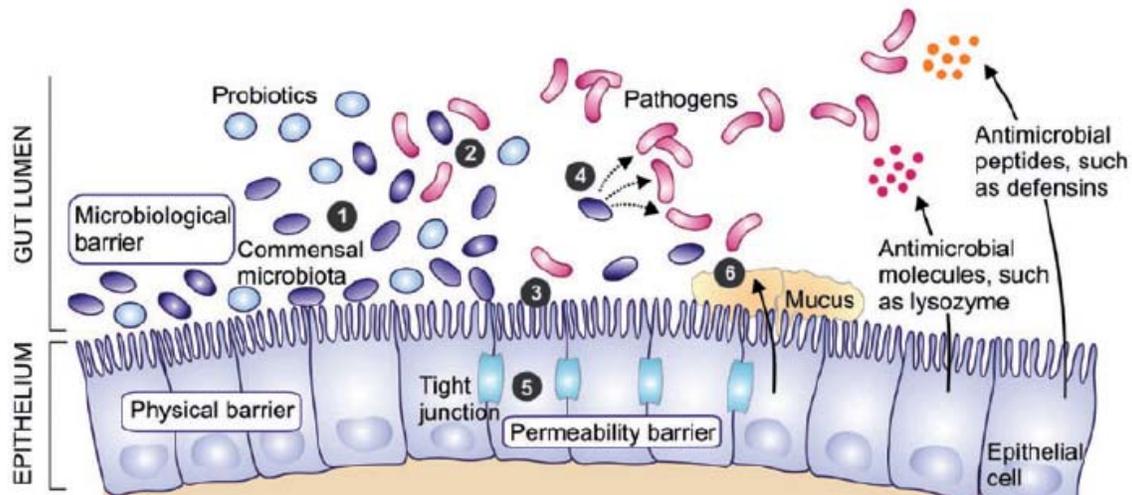


Fig. 1: A systemic presentation of intestinal cells and possible mechanism of action of probiotic and response of intestinal cells (Riina, 2008). 1) Modulation of commensal bacteria; 2) Competition with pathogen (receptor and nutrients); 3) Inhibition of pathogen attachment with intestinal wall; 4) Production of antimicrobial agents against pathogen; 5) Strengthening of the mucosal barrier; 6) Inducing mucin production.

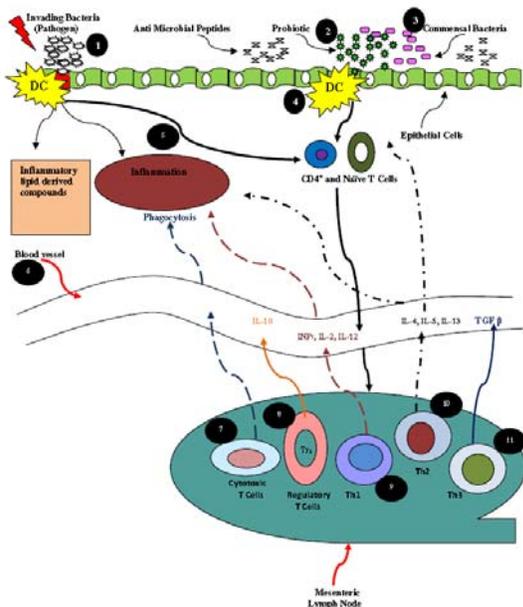


Fig. 2: The diagram shows systemic defense of intestinal lining, cytokine production in response of invading pathogen and beneficial probiotics, while key cells interacting with systemic response between pathogen and probiotics. 1) Invading bacteria adhering on epithelia cells wall; 2) Attachment of probiotic without invading epithelial cells; 3) Commensal bacteria incorporation with probiotics; 4) Dendritic cells (APC) presenting antigen of probiotics to immunomodulation; 5) Phagocytosis in inflammation region; 6) Blood vessel containing cellular production, in response of pathogen; 7) Cytotoxic T cell to Phagocytosis activity in inflammation part. Tr₁ production to maintain the balance of Th₁ and Th₂; 8) Response of Th₁ and synthesis of INF- γ , IL-2 and IL-12; 9) Production of IL-4, IL-5 and IL-13; 10) Th₃ active to produce TGF.

Beneficial effects for Homeostasis: In the contemporary emergent globe, use of the probiotics in healthy subjects has become the part usual diets. However, information about probiotics is complex, although it plays a significant role since probiotics have mainly been marketed for the betterment of healthy subjects and widely used in food, medicine, and husbandry industries (Qin *et al.*, 2007). The effects of probiotics on healthy subjects are anticipated to

be limited to hazard risk reduction (Tuomola *et al.*, 2000; Ouwehand *et al.*, 2001). Generally probiotics are often available in markets having adhering antagonizing, defensive and epithelial supporting characteristics. The enduring evidence suggests that, long term use of probiotics may decrease the risks for infections, absence of illness in early age (Hatakka *et al.*, 2001). The conclusion of this investigation showed that probiotics can also be utilized by healthy animal as well as human beings, by keeping in view their beneficial effects.

Mechanism of Probiotics in the Intestinal Defense: The defense system of intestine can be enhanced by the application of probiotics as they produce their beneficial effects by diverse mechanisms such as antagonist against pathogen, improves intestinal epithelial layer and boost the innate immunity as well as support in adaptive immunity.

Antagonistic effects against pathogens: Probiotics act as an antagonist against pathogens by producing antibacterial and antimicrobial compounds such as cytokines and butyric acid (Kailasapathy and Chin, 2000), also reduce gut pH by stimulating lactic acid producing microflora and favorably adjust the intestinal microflora balance (Langhendries *et al.*, 1995). They have the capacity to change the metabolism and composition of commensal microbiota to enhance the defense against pathogens (Siigur *et al.*, 1996; Kuisma *et al.*, 2003) and also inhibit lethal organisms adhesion by competing exclusion, *in vitro* models, as has been reported in *Latobacillus*, *Propionibacterium* and *Bifidobacterium* strains (Collado *et al.*, 2006; Collado *et al.*, 2007). Probiotics compete for binding on receptor sites that can be occupied by pathogens (Fujiwara, 1997; Kailasapathy and Chin, 2000); also boost immune system and stimulate immunomodulatory cells (Isolauri *et al.*, 1995). Furthermore, compete with pathogens for accessible nutrition and other growth factors (Rolfe, 2000). These all findings confirm that probiotics are strong tool as antagonist and further detail investigations are need of time.

Improving Epithelial Structure: The intestinal epithelium is rapidly and constantly transformed by epithelial stem cells inhabitants. The stem cell population varies into numerous intestinal epithelial cell types, specific in their functions such as Goblet cells, Entero endocrine cells, Enterocytes, Paneth cells and M cells. These epithelial cells serve as a physical barrier between the luminal contents (commensal microbiota, food antigens and also probiotics) and the complex mucosal immune system (McCracken and Lorenz, 2001). The secretions of probiotics improve the continuous interaction of healthy intestinal linings with interacting pathogens. With this, also play a critical role to initiate the innate as well as adaptive immunity in the host.

Boost Intestinal Immunity: Immunological reactions are divided into two parts by their specificity and speed of the reaction; in response to innate and adaptive immunity (Parkin and Cohen, 2001). Furthermore, the probiotics application *in vivo* illustrated that; modern approaches could activate innate and adaptive immunity (Li *et al.*, 2011). The innate immunity provides instant but, non-specific immunity to host defense, and includes physical, chemical, and microbiological barriers as well as support in several fundamentals of the immune system, such as neutrophils, monocytes, macrophages, complement, cytokines, and proteins active responses (acute-phase). However, their activation is most imperative which is initiated by intestinal epithelial cells, the most important APC and macrophages respectively. The adaptive immunity comprises antigen-specific reactions through T and B lymphocytes, take several days to develop, that works against specific invading pathogens. However, the cells are most reliable source for initiation of innate and adaptive immunity via their receptor signaling and transduction activity. Furthermore, several aspects of further studies related to mechanism of action of probiotics still requires focal point for researchers to hypothesize the mode of action, and response of dendritic cell (TLRs).

Importance of Intestinal Cells Receptor Signaling:

Activation of the host defense mechanisms is based on hasty detection of specific structural components of microorganisms also known as pathogen-associated molecular patterns (PAMPs), it includes peptidoglycan, lipopolysaccharide (LPS), lipoprotein, lipoteichoic acid, flagellin and CpG-containing (unmethylated) DNA (Sansonetti, 2004; Alexopoulou and Kontoyiannis, 2005; Winkler *et al.*, 2007). These components are identified by pattern-recognition receptors, and the best known are Toll-like receptors (TLRs) (Underhill, 2007). TLR family includes at least 13 proteins that recognize microbe derived ligands. For example, TLR1 and TLR2 recognize lipoproteins and lipid-modified sugars TLR4 recognizes LPS, TLR5 is activated by flagellin whereas TLR9 recognizes CpG motifs in bacterial DNA (Underhill, 2007). In general, the stimulation of TLRs by PAMPs consequences to the activation of adaptor proteins and this initiates a signaling cascade involving several kinases. However, some of Gram positive probiotics (highly flagellin in their structure), their membrane secretes lipoproteins and lipid-modified sugars, and activates

TLR1-2 and TLR5 initiated to transcription of gene leading to the synthesis of immunomodulatory molecules. But, estimation of TLR1-2 and TLR5 and the mechanism of action are ambiguous.

Cytokine Production in Response of TLR Signaling:

Cytokines are known as diminutive proteins secreted by immune and many other types of cells (Sarah *et al.*, 2010). Each cytokine may have multiple activities in unusual circumstances and cell types. Their action depends on binding with specific receptors present on the surface of cell and by stir up changes in the development, growth, and/ or activity of the target cells (Kidd, 2003). Different production patterns of cytokine are applied to segregate T helper cell (Th) responses to dissimilar classes. Th1-type cells generate IFN- γ , IFN- α , IL-2, IL-12 and also endorse cell-mediated immunity (Kidd, 2003). The second type of T-Helper cells (Th2-type) produce different interleukins mainly including IL-4, IL-5, IL-6, and IL-13 and trigger antibody-mediated immune response as well as mast and eosinophils cells (Wu *et al.*, 2007). Th3-type cells function against Th1 and Th2, and they down regulate the inflammatory retort through TGF- β production and Tr1 cells are responsible to maintain the balance in between immunity through IL-10 production (Izcue and Powrie, 2008; Taylor *et al.*, 2006). Cytokines can be categorized according to their production, including pro inflammatory, inflammatory and anti-inflammatory interleukins. Pro inflammatory cytokine, TNF- α is responsible to activate local inflammation concerned with immune system (Bertazza and Mocellin, 2008). TNF- α is production of professional immune cells like NK cells, T cells and macrophages, but non-immune cells also take part to produce like epithelial cells. These cells are capable to induce TNF- α , IL-1 β , but mainly produced by macrophages and epithelial cells and further participate in macrophage and T cell activation (Barksby *et al.*, 2007).

The second one is inflammatory cytokines that include IL-1 α/β (Barksby *et al.*, 2007), TNF- α (Bertazza and Mocellin, 2008), IFN- γ (Schoenborn and Wilson, 2007) and IL-6 (Gabay, 2006). Whereas IFN- γ , produced by T cells and NK cells, also possess antiviral activity and activates macrophages and suppresses Th2 responses (Schoenborn and Wilson, 2007). And involve in systemic effects of inflammation, such as weight loss, fever and hepatic protein synthesis (acute-phase). IL-1 and IL-6 also mediate the same response in systematic inflammation. IL-2, produced by T cells and its proliferation is promoted, but concentration may fluctuate in different immune supporting organs (Yu *et al.*, 2010). IL-4 and IL-5 are produced by mast and T cells respectively. IL-4 activates B cells, and is involved in the control of IgE and suppression of Th1 cells, while IL-5 induces growth and differentiation of eosinophils. IL-6 is produced by endothelial cells, macrophages, T cells and is concerned with T- and B-cell growth and differentiation, acute phase response (Gabay, 2006). IL-12 induces the production of IFN- γ and differentiates Th2 cells to Th1-type cells and plays a key role to form a link between innate and adaptive immunity (Trinchieri, 2003). Dendritic cells and phagocytes produce IL-12 during infection in response to pathogens and also activate NK cells (Trinchieri, 2003). IL-10 is considered to be an anti-inflammatory or

regulatory cytokine and it can be produced by many types of cells (Couper *et al.*, 2008). All through the infection, it inhibits the activity of Th1 cells, NK cells, & macrophages, required for optimal pathogen clearance, but in addition contributes in tissue damage (Couper *et al.*, 2008). Further study is mandatory for documentation of APC cells receptor signaling and in response, production of cytokine in favor of host innate as well as adaptive immunity.

Conclusions: Immunomodulatory functions of bacteria from the resident intestinal microflora, and the mechanism involved in the TLR signaling are essential for the better understanding of immunomodulatory effects. The differences in genus, species or strain of probiotic organisms are reflected in their virtual immunomodulatory efficiency and signaling mechanism. However, production of antibacterial substances is also decisive incident in the generation of a successful probiotic effects in the development of innate and adaptive immunity. Although the mechanisms whereby probiotic components enhance the immune response have not been resolved, however, possibilities include activation of toll-like receptors, of dendritic cells or stimulation of intestinal epithelial cells and release of cytokines and chemokines. Although, the documentation pertaining to the TLR signaling mechanisms is lacking, whereby, probiotics elicit beneficial effects to facilitate animal as well as human beings. It is prime requisite to be acquainted with the mechanism that enhances the functioning prospects of probiotics.

Future Prospects: It has been revealed from the literature that, studies concerning to antigen presenting cells (APC) receptors, (TLRs), signaling and transduction allied to the effects of probiotics is inadequate, whereby the effective understanding of signaling and transduction mechanism will be a milestone for prospect studies, and better indulgent of mechanism will support to acquire more imminent effects of probiotics, that may depend upon the physiological condition of the subject, consequently, the variation in same genus and species may be possible.

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