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FUNCTIONAL MECHANISMS OF PROBIOTICS

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ABSTRACT

Probiotics are the live microorganisms which when ingested in adequate amounts confer health benefits. The strains most frequently used as probiotics include Lactic acid bacteria, bifidobacteria and yeast *Saccharomyces boulardii*. However, several other bacterial strains are being investigated for potential probiotic value viz. *Enterococcus*, *Streptococcus*, *Bacillus*, among others. Significant therapeutic potential of probiotics has been demonstrated in several *in vitro* studies and that involving animal models and humans. Despite intense focus on probiotics research the mechanisms responsible for health benefits are not yet completely understood. Several important mechanisms have been proposed such as improvement of gut epithelial barrier function, Immunomodulatory effects, degradation of toxin receptors, competition for nutrients, production of inhibitory substances, antiproliferative effects, blocking of adhesion sites and modulation of gut microbiota. Bacterial cell components such as DNA or peptidoglycan may also be involved in functional mechanism of probiotics. Effectiveness of a probiotic for potential application as prophylactic or treatment agent for certain ailment is determined by its ability to possess all or most of these characteristic features. The current article describes the general functional mechanisms of probiotics.

Keywords: Probiotics, health benefits, functional mechanisms

INTRODUCTION

The concept of probiotics (as beneficial microorganisms replacing harmful microbes in the intestine by useful ones) dates back to the times of Elie Metchnikoff (1845-1916), a Russian professor of biology, who later worked as a director at the Institut Pasteur in Paris. Élie Metchnikoff is considered as the inventor of probiotics. Intrigued by the longevity of the Caucasian population and its frequent consumption of fermented milks, Metchnikoff proposed that the acid-producing organisms in fermented dairy products could prevent "fouling" in the large intestine and lead to a prolongation of the life span of the consumers **(Smug *et al.*, 2014)**. Although Metchnikoff's ideas were clearly related to lactic acid bacteria in dairy products, the interest of other scientists soon turned to lactic acid bacteria of intestinal origin. This observation initiated intensive research interest on probiotics. As a result probiotic concept expanded to include bacteria from intestinal origin in addition to those isolated from fermented dairy products. 'Probiotics' is Greek word which means 'for life', and was initially defined by Fuller in 1989 as 'a live microbial feed supplement which beneficially affects the host by improving its microbial balance' **(Fuller, 1989)**. Since then, several broad definitions have been proposed by experts **(Schrezenmeir and De Vrese, 2001)** and by the Food and Agriculture Organization of the United Nations and World Health Organization **(FAO/WHO, 2001)**. According to these definitions, probiotics are 'live microorganisms that, when ingested in adequate quantities, exert a health benefit to the host', by stimulating the growth of other microorganisms, modulating mucosal and systemic immunity, and improving the nutritional and microbial balance in the intestinal tract. Probiotics mostly include strains of lactic acid bacteria (*Lactobacillus* spp.) and *Bifidobacterium* spp. but also include certain yeast (*Saccharomyces boulardii*) and some other bacterial and yeast spp. **(Bermudez-Brito *et al.*, 2012; Bajaj *et al.*, 2014)**. Safety of probiotic consumption is no issue as probiotics have been consumed in naturally fermented products since ancient times.

The health benefits associated with probiotic consumption have been extensively investigated in animal models and human studies, and include (Fig. 1) prevention and treatment of diarrhoeal disease (acute infantile diarrhoea, antibiotic associated diarrhoea, nosocomial infections), prevention of systemic infections, management of inflammatory bowel disease, immunomodulation, prevention and treatment of allergies, anticancer effects, treatment of cholesterolaemia, and alleviation of lactose intolerance **(Gill and Guarner, 2004)**. Recently immense health benefits of probiotics have motivated the food industries to develop probiotic foods **(Liong *et al.*, 2011)**.

However, the health benefits of probiotics cannot be generalized as probiotics show huge differences at the level of genus, species and strains as far as their health benefitting attributes are concerned. The health benefits associated with one strain cannot be extrapolated to other strains without experimentation **(Bermudez-Brito *et al.*, 2012)**. Moreover one probiotics may not possess all the proposed health benefits. Monostrain probiotics are defined as probiotics containing one strain of a certain species, while multistrain probiotics contain more than one strain of the same species or closely related species, for instance *Lactobacillus acidophilus* and *Lactobacillus casei*. Multispecies probiotics are defined as strains of different probiotic species that belong to one or preferentially more genera, e.g. *Lb. acidophilus*, *Bifidobacterium longum*, *Enterococcus faecium* and *Lactococcus lactis* **(Timmerman *et al.*, 2004)**. The probiotic strain must tolerate and survive gastric and bile secretions during transit through the upper gastrointestinal tract and then flourish and colonize in the intestine. Metabolic products of the strain should not have any pathogenic, toxic, mutagenic, or carcinogenic reactions to the host. Probiotic strain must be genetically stable with no plasmid transfer mechanism. Furthermore, the probiotics must possess good technological properties to withstand and survive conditions during manufacturing, processing, storage and transport of the food products, and have a satisfactory level of viability at the time of consumption, **(Schiffrin and Blum, 2001).**

A major health challenge in developing countries is provision of diet which is augmented with physiological functional components that boost and maintain high immunity. This challenge is further complicated by high incidences of malnutrition, HIV-AIDS, and non complete diet and low sanitation. Furthermore, industrialization, urbanization, economic development and market globalization, had significant impact on the health and nutritional status of populations in the developed/developing world. To combat these challenges directly, WHO advocates the implementation of alternate disease control strategies such as exploiting prophylactic and therapeutic potential of probiotics **(FAO/WHO, 2001).**

Most probiotics delivery vehicles are based on dairy products which are very nutritious and satisfy the nutritional requirements of fastidious LAB, and have

high acceptance among consumers. Probiotics have been incorporated in a wide range of dairy food products like milk, ice cream, yogurt, cheese, among others (**Champagne *et al*., 2005; Kailasapathy *et al*., 2008; Daneshi *et al*., 2013**). Physicochemical properties of food carriers used for probiotic delivery, such as buffering capacity, water activity, redox potential, protein, sugar content, pH and temperature are significant factors that influence survival of the probiotics during gastric transit. Cheeses have a strong potential for delivering probiotics due to their specific chemical and physical characteristics (**Karimi *et al*., 2011**). Ice cream and frozen dairy desserts have great potential as probiotic vehicles due to the lower storage temperature and less risk of temperature abuse (**Cruz *et al*., 2009**). Dairy desserts such as chocolate mousse have also been considered as potential probiotic delivering agents (**Possemiers *et al*., 2010**). However, due to some issues in dairy products like high cholesterol content, casein and cold storage, among others, there is substantial thrust on development of non-dairy food products as probiotic vehicles such as coconut milk, vegetable/fruit juices/drinks, nutrition bars, soy products and cereal-based products (**Ranadheera *et al*., 2010**). The development of new non-dairy probiotic food products is demanding considering the consumer's expectancy for products that are simultaneously relish and healthy (**Rad *et al*., 2014**).

Probiotics exert numerous health benefits on host through diverse mechanisms such as by influencing the composition and/or function of the commensal microbiota, altering host epithelial and immune system **(Hyland *et al*., 2014)**, and by combating the toxins or products of microbial, food or host origin which may have ill effects on host health **(Rupa and Mine, 2012; Sanders *et al*., 2013)**. Despite, the field of probiotics has made stupendous strides though there is no major breakthrough in the identification of the mechanisms by which probiotic strains enhance the health of the host. Intense research focus on mechanistic details of health benefits of probiotics is currently desired. The efficiency of probiotics 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 and several studies have been done on how probiotics work. Figure 2 outlines some of the suggested general functional mechanisms for probiotics. So many mechanisms from these studies are trying to explain how probiotics could protect the host from the health disorders. The current article presents an over view of the various proposed mechanisms of probiotic action.

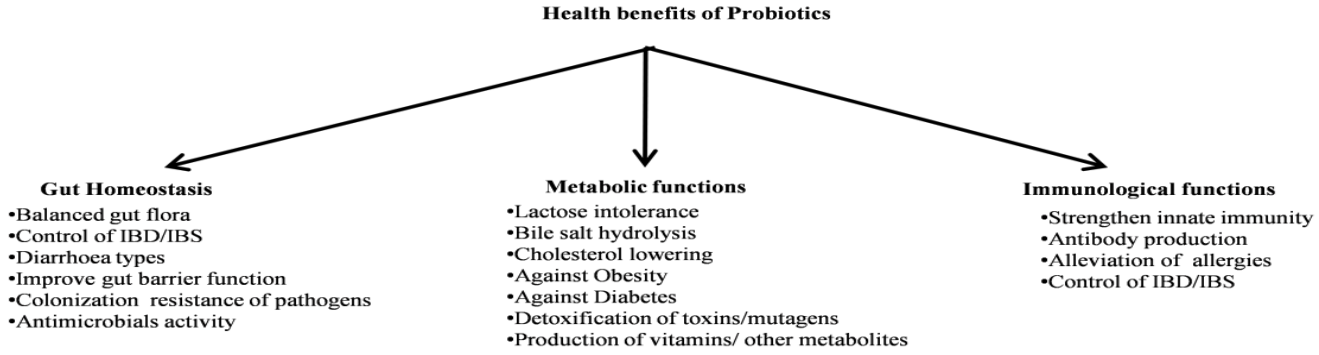

Figure 1 Health benefits of probiotics

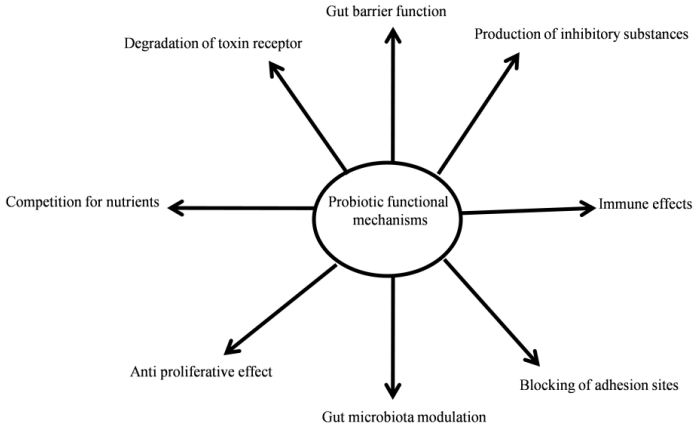

Figure 2 Proposed functional mechanisms of probiotics

PROBIOTICS ROLE ON THE GUT BARRIER FUNCTION

The mucus layer, the epithelial lining of the mucosal tissues as well as the immune cells, present at sub-epithelial level, are all part of the mucosal barrier. Thus, modulation at all these levels can positively affect barrier robustness and thereby, influence disease states **(Liu *et al*., 2011; Hyland *et al*., 2014)**. At cellular level, epithelial cells are at the centre stage of the barrier effect, receiving molecular signals from the gut lumen, exchanging signals with the underlying immune cells but also communicating with the entire organism by means of circulating signalling molecules. The gut barrier plays a crucial role in the pathogenesis of numerous gastrointestinal diseases such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), coeliac disease and infectious enterocolitis **(Blaut and Klaus, 2012)**. Therefore, selecting probiotic strains that can strengthen the gut barrier appears to be a relevant strategy with broad impact on different types of diseases **(van Hemert *et al.*, 2013).**

Several studies using Caco-2 intestinal cells and mice showed that *L. rhamnosus* GG (LGG) or the probiotic mix VSL#3 could interact directly with intestinal epithelial cells and maintain the integrity of the epithelial barrier. LGG persistence capacity in the GIT was linked to its *in vivo* expression of pili containing a mucus binding domain **(Lebeer *et al.*, 2012)**. In addition, LGG and its soluble factors (p75 and p40) were shown to prevent epithelial cell apoptosis *in vitro* through activating anti-apoptotic Akt and suppression of NF-kB, and an additional effect observed in the study was that LGG enhances mucin secretion by epithelial cells **(Yan and Polk, 2002)**. These effects can potentially contribute to pathogen exclusion and maintenance of homeostasis if reproducible *in vivo*. In addition, it shows that probiotic strains affect the same tissue –in this case the epithelium – by different pathways, all contributing to the preservation of the barrier effect. In the clinical context, administration of *Lactobacillus plantarum* in the small intestine of healthy subjects induce structural changes in epithelial tight junctions, resulting in increased tight junction specific proteins occludin and zonula occludens-1. Since loss of tight junction integrity and the resulting increased intestinal permeability to macromolecules are associated with several diseases such as IBD, IBS and coeliac disease, the data obtained with the *L. plantarum* strain provide relevant information towards an intervention in the corresponding subjects **(Sawada *et al.*, 2003)**.

Treatments with several spp. and strains of *Lactobacillus* such as *L. plantarum*, *L. acidophilus*, *L. casei*, and *L. rhamnosus* induce differential gene-regulatory networks and pathways in the human mucosa involving up-regulation of IL-1b, an activator of NF-kB signalling cascade, which may drive the transcription of genes involved in lymphogenesis and B-cell maturation, thus contributing to enhancement of barrier function. Differential expression of genes involved in wound repair and healing, angiogenesis, IFN response, calcium signalling and ion homeostasis, are relevant for the vascularization/nourishment of epithelial cells **(van Baarlen *et al.*, 2011)**. Furthermore, the observed changes in transcriptional networks display similarity with responses obtained with bioactive molecules and drugs, which reflect upon potential novel application of probiotics in areas of therapeutic and/or prophylactic nutritional regimes.

Probiotics like *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* show curative effects on post-infectious irritable bowel syndrome (PI-IBS) induced by *Trichinella spiralis* in a mouse model. *Bifidobacterium* or *Lactobacillus* treated PI-IBS mice exhibited decreased abdominal withdrawal reflex score and contractile response, reduced plasma diamine oxidase (DAO) and D-lactate. Furthermore, probiotic treatments suppressed the expression of proinflammatory cytokine IL-6 and IL-17 and promoted the expression of major tight junction proteins claudin-1 and occludin. The mixture of the three probiotic strains performed better than the individual in up-regulating these tight junction proteins and suppressing IL-17 expression **(Wang *et al*., 2014).**

PRODUCTION OF INHIBITORY SUBSTANCES BY PROBIOTICS

Probiotics exert antibacterial effects against pathogenic and/or food spoilage gram-positive and gram-negative bacteria through production of antibacterial substances such as bacteriocins, organic acids, hydrogen peroxide, among others **(Arauz *et al*., 2009; Razdan *et al.*, 2012; Bajaj *et al.*, 2014; Bajaj *et al.*, 2014[a];**

Dec *et al*., 2014). Probiotic-derived antibacterial substances show their effects individually or synergistically to inhibit the growth of pathogenic bacteria. Probiotics have been reported to produce a wide range of different bacteriocins such as nicin **(Arauz *et al*., 2009)** which constitute the major mechanism for their antimicrobial action. *Lactobacillus acidophilus* has been reported to produce acidophillin, lactocidin, and acidolin and *Lactobacillus planatarum* produces lactolin **(Vila *et al*., 2010)**. Bacteriocin produced by probiotic strain *Lactobacillus salivarius* UCC118, protect the mice against infection with the invasive foodborne pathogen *Listeria monocytogenes.* From the studies it was also confirmed that antimicrobial effect was mediated by the bacteriocin Abp118 produced by direct antagonism between *Lb. salivarius* and the pathogen **(Corr *et al*., 2007)**. Lactobacilli and bifidobacteria have been shown to inhibit a broad range of pathogens, including *E. coli*, *Salmonella*, *Helicobacter pylori*, *Listeria monocytogenes* and Rotavirus **(Bermudez-Brito *et al.*, 2012)**. Bacteriocins produced by Gram-positive bacteria have a narrow activity spectrum and act only against closely related bacteria, however, some bacteriocins inhibit food-borne pathogens like *Listeria monocytogenes* **(Nielsen *et al.*, 2010)**.

Several *Bifidobacterium* strains have been reported to produce a unique bacteriocin (bifidocin B, from *B. bifidum* NCFB 1454) which is active towards Gram-positive bacteria. Two *Bifidobacterium* strains exhibited a strong killing activity against several pathogenic bacteria, including *Salmonella enterica* ser. Typhimurium SL1344 and *E. coli* C1845 **(Bermudez-Brito *et al.*, 2012)**. Twenty *Lactobacillus* strains inhibited enteropathogenic *Yersinia enterocolitica* while two strains *Lactobacillus casei* C1 and *Lactobacillus plantarum* C4 inhibited *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* in addition to *Y. enterocolitica*. Mechanism of inhibition was decrease in pH resulting from dextrose fermentation by lactobacilli. However, protective effects of these probiotic strains could not be established in mouse experimental infection models against *S. Typhimurium*. Although *L. plantarum* C4 showed partial protective effect that was attributable to an immunostimulatory mechanism. Thus, *in vitro* study of antibiosis may provide useful information on the probiotic potential of *Lactobacillus* strains **(Bujalance et al., 2014).**

The common mechanisms of bacteriocin-mediated killing include the destruction of target cells by pore formation and/or inhibition of cell wall synthesis. For example, nisin forms a complex with the ultimate cell wall precursor, lipid II, thereby inhibiting cell wall biosynthesis, and subsequently, the complex aggregates and incorporates peptides to form a pore in the bacterial membrane. Bacteriocin production confers producing strains with a competitive advantage within complex microbial environments as a consequence of their associated antimicrobial activity, and at the same time inhibits pathogens in GI tract **(Nielsen *et al*., 2010; Hassan *et al*., 2012; O'Shea *et al*., 2012).**

BLOCKING OF ADHESION SITES BY PROBIOTICS

Adhesion to intestinal mucosa is one of the major selection attribute for probiotics as it is required for intestinal colonization, and is also important for modulation of the immune system and antagonism against pathogens. Lactic acid bacteria (LAB) display various surface determinants that are involved in their interaction with intestinal epithelial cells and mucus which help competitive exclusion of pathogens from the mucus. Several *Lactobacillus* proteins (along with saccharide moieties and lipoteichoic acids) have been shown to promote mucous adhesion **(Van Tassell and Miller, 2011; Bermudez-Brito *et al.*, 2012)** and bacteria display surface adhesions that mediate attachment to the mucous layer. Bacterial adhesins (MUB, mucus-binding protein) have been reported from *Lactobacillus reuteri* **(Buck *et al.*, 2005).** Probiotics, such as *L. plantarum*, have been reported to induce MUC2 and MUC3 mucins and to inhibit the adherence of enteropathogenic *E. coli*. Thus, enhanced mucous layers and glycocalyx overlying provides protection against pathogen invasion. Furthermore, probiotic organisms adhere to intestinal gut epithelial surfaces and block the adhesion sites, therefore, prevent colonization of pathogenic bacteria **(Ohland and MacNaughton, 2010)**. When lactobacilli are ingested, they compete for binding sites, leaving less binding sites open for pathogens. Pathogens pass through gut and leave the body sooner when no binding site is available. Adhesion of *L. plantarum* Lp6 to rat mucus is mediated by the mannose specific adhesion proteins that reversibly bind to the cell surface components and important for competing with pathogens binding sites in gut, and therefore, resist the colonization of the pathogens **(Sun *et al*., 2007)**. Acid-resistant strains of *Bifidobacterium longum* and *B. catenulatum* showed better adhesion to human intestinal mucus as compared the acid-sensitive strains **(Collado *et al.*, 2005)**. Acid resistance in bifidobacteria enhances potential functionality by improving stability and surface properties. The mixture of probiotics and VSL#3 enhance the synthesis of cell surface mucins and modulate mucin gene expression thus improving the adhesion of bacterial cells to the intestinal epithelium **(Caballero-Franco *et al*., 2007).** Candidate probiotics *Lactobacillus reuteri* ATCC 55730, *Lactobacillus rhamnosus* AC413, but not *L. salivarius*, reduced *Staphylococcus aureus* -induced keratinocyte cell death in both undifferentiated and differentiated keratinocytes. Keratinocyte survival was significantly higher if the probiotic was applied prior to or simultaneously with infection with *S. aureus*. *S. aureus* utilizes the α5β1 integrin to adhere to keratinocytes, and blocking of this integrin resulted in a protective effect similar to that observed with probiotics. This suggests that the protective mechanism for *L. reuteri*-mediated protection of keratinocytes was by competitive exclusion of the pathogen from its binding sites on the cells. Thus use of a topical probiotic prophylactically could inhibit the colonization of skin by *S. aureus* and aid in the prevention of infection **(Prince *et al*., 2012).**

ROLE OF PROBIOTICS IN COMPETITION FOR NUTRIENTS

Competition for nutrients may be one of the mechanisms for colonisation resistance of pathogens in human gut. When health promoting bacteria are present in the gut, they utilize more nutrients, leaving fewer nutrients for pathogenic bacteria, which may suffer starvation, and not survive. The competitive exclusion takes place in two ways; firstly inhibiting the pathogens by consuming the nutrients and energy source which pathogens need, thus preventing them from proliferation and growth in the gut environment. Second is producing several organic acids and volatile fatty acids because of their metabolism and fermentation, resulting in lowering of the gut pH below that essential for pathogenic bacteria e.g. *Salmonella* and *E. coli* **(Bermudez-Brito *et al*., 2012)**. Continuous flow culture model of the mouse caecal flora was used to investigate the colonisation resistance against *Clostridium difficile*. It was reported that the levels of carbohydrates within a continuous flow culture colonised with mouse intestinal flora were insufficient to support *C. difficile* growth. In particular, it appeared that an unidentified organism competed more efficiently than *C. difficile* for monomeric glucose, N-acetylglucosamine, and sialic (N-acetylneuraminic) acid in the continuous flow culture model **(Wilson and Perini, 1988).** Probiotics may similarly over compete with pathogens for nutrients, and cause exclusion of pathogens and, thus providing protection to the host. Thus, competition for nutritional substrates amongst probiotics, intestinal pathogens and microbiota may play a significant role. *Bifidobacterium adolescentis* S2-1 compete with *Porphyromonas gingivalis* for utilization of vitamin K other growth factors **(Hojo *et al.*, 2007)**, and inhibit growth of *P. gingivalis*. Germ-free mice colonised with human baby microbiota, showed diverse alteration of pathways including the metabolism of amino acid, methylamines and short chain fatty acids (SCFA) upon exposure to a probiotic strain of *Lactobacillus paracasei* or *Lactobacillus rhamnosus* **(Martin *et al.*, 2008).**

Iron constitute one of the essential nutrients for most of the bacteria, is often available in limited amounts. However, lactobacilli not require iron and hence have edge over other iron requiring bacteria (pathogens); furthermore, some probiotics such as *L. acidophilus* and *L. delbrueckii* bind ferric hydroxide at their cell surface, and make it unavailable to pathogenic microorganisms **(Elli *et al*., 2000).** Thus, probiotic bacteria alter the physical environment in such a way that the pathogenic bacteria cannot survive. Probiotic strains *L. paracasei* and *L. rhamnosus* exert inhibitory effects on pathogens *Salmonella typhimurium* and *Listeria monocytogenes* biofilm formation by mechanism involving competition, exclusion and displacement. *L. monocytogenes* biofilm cells were reduced by more than 3 log cycles **(Woo and Ahn, 2013).**

IMMUNE EFFECTS OF PROBIOTICS

Different pathways have been identified by which probiotics modulate immune system **(van Hemert *et al.*, 2013; Hyland *et al*., 2014)**. One possible mechanism of probiotics to protect the host from intestinal disease is by stimulating specific and nonspecific immunity. LAB products exert immunomodulatory activity via inhibition of inflammatory responses, regulation of the expression of TLRs (Toll like Receptors), activation of DCs (Dendritic cells) and NK (Natural Killer) cells in innate immunity; proliferation of lymphocytes, balancing T-helper (Th1/Th2) cells responses, secretion of specific IgA, among several other ways **(Tsai *et al*., 2012)**. Role of *Saccharomyces boulardii* and *Bacillus subtilis* B10 play a potential role in modulating immunological functions of chicken bone marrow dendrite cells by targeting specific toll like receptors (TLRs) and associated factors. Probiotics attached on the surface of dendrite cells. Gene expression levels of MHC-II, CD40, CD80 and CD86 was up-regulated. Furthermore, toll-like receptors TLR1, TLR2, TLR4, and chicken specific TLR15 expressions were improved and downstream associated factors MyD88, TRAF6, TAB1, and NFκ-B mRNA levels increased (Rajput *et al*., 2014).

Probiotic bacteria exert its beneficial effects and modulate the immune system of the host against potentially harmful antigens via activation of lymphocytes and production of antibodies. The colonization of healthy microbes leads to maturation of the humoral immune mechanisms, particularly circulation of the IgA and IgM secreting cells. After priming, memory B and T cells migrate to effectors sites followed by active proliferation, local induction of certain cytokines and production of secretory antibodies IgA. The entrance of probiotics in the gut stimulates the production of IgA. The production of IgA in the immune system has become clear from the studies performed in mice, which are kept germ-free after birth **(Ng e*t al*., 2009)**. A lot of reports showed that lactic acid bacteria (LAB) as *Lactobacillus* and *Bifidobacterium* and their fermented products are effective at enhancing innate and adaptive immunity, prevent gastric mucosal lesion development, alleviate allergies, and put up defense against intestinal pathogen infection **(Tsai *et al*., 2012)**.

Lactobacilli also stimulate immune cells to release pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and interleukin-12 (IL-12). Mechanisms of innate immunity include enhancement of antigen presentation, phagocytosis on antigen presenting cells (APCs), and cytotoxicity by natural killer (NK) cells, all of which can kill transformed cells in a seemingly nonspecific fashion. DCs play a crucial immunoregulatory role in immune responses under antigen uptake and processing, such as the induction of Ag-specific immune responses and Th1/Th2 balance. Probiotics regulate secreted cytokines by DC to improve the morbidity of intestinal diseases **(Tsai *et al.*, 2012)**. Oral administration of *Lactobacillus* enhances specific adaptive immune responses through innate immunity in mice **(Paturi *et al.*, 2007)**. The interaction between CD40 and CD154 on DCs and CD4+ T cells, respectively, induces the priming and expansion of CD4+ T cells, and subsequently leads to activation, proliferation, and differentiation **(Grewal and Flavell, 1998)**. The immunomodulation of gut mucosal immunity by *Lactobacillus* species induces B cell migration and protective IgA production through intestinal DC modulation **(Mora *et al.*, 2006).** Several *Lactobacillus* strains have the effect of skewing T cells from T helper 2 (Th2) toward Th1 responses, thus promoting humoral immunity **(Mohamadzadeh *et al.*, 2005)**. Many types of LAB products have anti-allergic effects in a murine model. Recent research demonstrates that the *L. rhamnosus* GG derived soluble protein p40 activates EGFR to inhibit cytokine-induced apoptosis and disrupted barrier in intestinal epithelial cells. Furthre, it also showed the effects of LGG p40 through EGFR activation play a significant role, in the development of probiotic-derived proteins as novel reagents for protecting the intestine from injury and inflammation **(Yan *et al.*, 2011)**. Supplementation of the diet with probiotics can persistently modulate both innate and adaptive immune responses locally and systemically. Up-regulation of helper T cell activation induces stronger DC/NK and DC/CD4+ T cell interaction, lymphocyte proliferation, and cytokine expression **(Tsai *et al.*, 2012)**.

Lactobacillus rhamnosus-feeding to 16 months old mice resulted in alleviated immunosenescence-associated Th1/Th2 imbalance, improved antioxidant capacity, and enhanced resistance of aged mice to *E. coli* infection. In probiotic fed mice splenocytes showed increased IFN-γ and decreased IL-4 and IL-10 production, neutrophil respiratory burst enzymes and phagocytosis increased while no aggravation in plasma levels of MCP-1 and TNF-α occurred, IgG1/IgG2a ratio and IgE levels decreased, antioxidant enzymes activities increased and *E. coli* translocation to organs significantly reduced **(Sharma *et al.*, 2014)**.

ROLE OF PROBIOTICS IN DEGRADATION OF TOXIN RECEPTORS

Probiotics modify toxin receptor through an enzymatic mechanism, because of the degradation of toxin receptor on the intestinal mucosa *Saccharomyces boulardii* protects the host against *Clostridium difficile* intestinal disease. Some other offered mechanisms are suppression of toxin production, reduction of gut pH, attenuation of virulence **(Bermudez-Brito *et al.*, 2012)**. Probiotics can also modify toxin receptors and block toxin-mediated pathology. *Saccharomyces boulardii* degrades *Clostridium difficile* toxin receptors in the rabbit ileum and blocks cholera-induced secretion in rat jejunum by the production of polyamines. Probiotics may also promote nonspecific stimulation of the host immune system, including immune cell proliferation, enhanced phagocytic activity of macrophages, and increased production of secretory immunoglobulin IgA and IgM **(Kaur *et al.*, 2009).** The protective effect of a multi-strain probiotic and synbiotic formulation (*Lactobacillus plantarum* F44, *L. paracasei* F8, *Bifidobacterium breve* 46, *B. lactis* 8:8, galacto-oligosaccharides, isomalto-oligosaccharides and resistant starch) was evaluated in C57BL/6 mice infected with *Clostridium difficile* NAP1/027. Feeding of the formulation resulted in increase of total bifidobacteria and lactobacilli counts, and absolutely no caecal toxins were detected. qPCR of caecal content showed significant reduction in *C. difficile* DNA copies **(Kondepudi *et al.*, 2014).**

ANTI PROLIFERATIVE EFFECT OF PROBIOTICS

Probiotics have been claimed to possess anti-cancer activity which may be attributed to the reduction of putrefactive bacteria like *Clostridium*, coliforms or Bacteroides species and an enhanced level of lactobacilli and bifidobacteria that help reducing incidence for colorectal cancer. The incidence of adenocarcinoma in the colon of IL-10 knockout mice was factually reduced in mice treated with probiotic *Lactobacillus salivarius* ssp. salivarius **(O'Mahony *et al.*, 2001).** Treatment with probiotics able to interfere with chronic recurrent inflammation of the gut might also be helpful in preventing colon carcinoma, because chronic inflammation promotes the appearance of this disease. An example of an anti-inflammatory active probiotic is *Streptococcus thermophilus* strain TH-4 which also produces high amounts of folate important for DNA repair in epithelial cells **(Van Guelpen *et al.*, 2006; Tooley *et al.*, 2006).**

Marked anti-mutagenic activity of many lactobacilli, some bifidobacteria strains may be due to their ability to metabolically inactivate the mutagenic substances. Furthermore, certain probiotics bind N-nitroso compounds and heterocyclic aromatic amines. This can lead to reducing the levels of carcinogenic compounds and reducing DNA damage **(Geier *et al.*, 2006).**

Another mechanism for anti-tumour activity of probiotics may be due to their ability to amplify the immune response to tumour tissue by modulation of cytokine production and T cell function **(Hirayama and Rafter, 2000).** Administration of the cytoplasmic fraction of *L. acidophilus* SNUL, *L. casei* YIT9092 and *B. longum* HY8001 lead to reduction of tumour cell proliferation *in vitro* and increased survival rate of mice injected with tumour cells **(Lee *et al.*, 2004)**. Peptidoglycan from *Lactobacillus* species reduced in a dose-dependent manner growth of CT26 cancer cells originating from the colon of BALB/C mice by increasing apoptosis (**Sun *et al.*, 2005).** Factors secreted by *Lactobacillus reuteri* ATCC PTA 6475 potentiated apoptosis in myeloid leukemia-derived cells induced by tumour necrosis factor **(Chandra *et al.*, 2008).** *Lactobacillus* and *Lactococcus* strains isolated from food products can be introduced as probiotics because of their health-promoting characteristics including anticancer activity. Cytotoxicity assessments of *Lactococcus lactis* subsp. Lactis 44Lac were used to analyze the effects of the secreted metabolite on different cancer cell lines, including HT29, AGS, MCF-7, and HeLa, as well as a normal human cell line (HUVEC). Results showed acceptable cytotoxic properties for secreted metabolites (40 µg/ml dry weight) of *Lactococcus lactis* subsp. Lactis 44Lac. Such performance was similar to that of Taxol against all of the treated cancer cell lines; however, the strain exhibited no toxicity on the normal cell line. Cytotoxic assessments through flow cytometry and fluorescent microscopy demonstrated that apoptosis is the main cytotoxic mechanism for secreted metabolites of *L. lactis* subsp. Lactis 44Lac. By contrast, the effects of protease-treated metabolites on the AGS cell line verified the protein nature of anti-cancer metabolites. However, precise characterizations and *in vitro/in vivo* investigations on purified proteins should be conducted before these metabolites are introduced as potential anti-cancer therapeutics **(Haghshenas *et al.*, 2014).**

PROBIOTICS FOR GUT MICROBIOTA MODULATION

Gut microbiota has been involved in regulating several physiological functions, ranging from energy regulation and cognitive processes to toxin neutralization and immunity against pathogens. Development and onset of various chronic diseases occurs when there is alteration in the composition of the gut microbiota. Studies have shown that gut microbiota play a critical role in the development of different disease conditions, including obesity, fatty liver disease, and lung infection. Interventions with the potential application of prebiotics and probiotics helps maintaining optimal gut health, and preventing/treating chronic inflammatory and immunity related diseases **(Lin *et al.*, 2014).** Potential health benefits of probiotics may be due to direct effects of probiotic cells, by means of secreted cell components, metabolic effects and cell to cell interactions. The impact of probiotics strains on the human GIT microbiota seems to play a very important role in the gut microbial network interactions. Probiotic bacteria, generally ingested at a level of 10^{8-9} cells, reach the colon in an amount based on survival rate in stomach and small intestine. The impact of ingested probiotics on the colonic environment is essentially attributed to the fecal persistence of the ingested strains. They colonize the gut temporarily and disappear once the consumption stops. Modulation of commensal microbiota by transiting probiotics can be expected due to antimicrobial compounds with broad spectrum such as reuterin or plantaricins or indirectly through modulation of the immune system or gut barrier function. Use of probiotics has been reported in the prevention of antibiotic-induced diarrhea, and acute infectious diarrhea, and helpful in other gut related disease such as IBS or colics that have been associated with microbiota dysbiosis; the gut microbial community composition remains more stable during the period of probiotics treatment and that it positively correlates with improvement of disease symptoms **(Ceapa *et al.*, 2013).** Most evidence available on the impact of probiotics on the microbiota composition and functions has been obtained by using methods targeting specific bacterial genera like *Lactobacillus* and *Bifidobacterium* while this type of nutrition may have very subtle influence on other relevant genera as well (**Rautava *et al.*, 2012).**

Microbiota dysbiosis in immune-related disease such as allergy or IBD can be managed by a successful probiotic intervention which may be associated with a targeted modulation of the microbiota to repress specific pathobionts or stimulate endogenous beneficial groups on top of direct molecular interaction with immune cells in the small intestine. Transiting probiotics are therefore not always expected to affect the global intestinal microbiota structure in a major way, but rather to directly modulate with the immune system and miscellaneous epithelial receptors all along the digestive tract. As a consequence low abundance but metabolically active bacteria can still be meaningful in microbiota modulation, by for example modulating existing microbiota-interactive metabolic networks. All evidence taken together, probiotic strains that are able to combine specific and direct interaction with the host with transient impacts on the residing microbiota can elicit complex multifaceted but more optimal health **(Bajaj *et al.*, 2014[a]; Ceapa *et al.*, 2013; Lin *et al.*, 2014).** Role of probiotics in correcting dysbiosis of the normal microbiota resulting from disease or disruptive events was reviewed **(McFarland, 2014)** based on the studies published on probiotic intervention for the prevention or treatment of various diseases. The outcome was the degree of microbiota correction by specific probiotic strains, and the association between the degree of dysbiosis correction and clinical efficacy. Assessment of the degree of dysbiosis improvement was dependent on the

enrolled population and the timing of microbiological assays. However, the functional claim for correcting dysbiosis was poorly supported for most probiotic strains and necessitates further research.

Although numerous studies have been conducted more insight is needed for the characterization of a 'normal' microbiota at a functional level, screening for probiotic strains with a high protective potential is necessary, the mechanisms of action of single probiotic strains and combinations are essential for their use in the clinical practice, and finely the clinical studies with better design and larger cohorts are necessary to support concepts fitting in the 'health by means of diet' concept.

CONCLUSION

Though probiotics have significant therapeutic and/or prophylactic potential in various gastrointestinal or other diseases/disorders. However, several of the health claims of probiotics are yet to be established experimentally through animal models/human studies, and underlying mechanisms of action still needs to be fully elucidated. Moreover, the functional role of gut microbiota and potential invention by probiotics has yet to be worked out for human health and disease. The current article attempted to review the available scientific information on mechanisms of action of probiotics. Major health-benefitting mechanisms of probiotics included enhanced gut epithelial barrier function, Immunomodulatory effects, degradation of toxin receptors, competition for nutrients, production of inhibitory substances, antiproliferative effects, blocking of adhesion sites, modulation of gut microbiota, among others.

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