FUNCTIONAL MECHANISMS OF PROBIOTICS

Bijender Kumar Bajaj1,2*, Ingmær J.J. Claes2 and Sarah Lebeer2

Address(es):
1 School of Biotechnology, University of Jammu, Jammu-180006 INDIA Phone: +91-94191-02201; Fax: +91-191-2456534.
2 Department of Bioscience Engineering, University of Antwerp, Groenenborgerlaan 171, B-2020, Antwerp, Belgium.

*Corresponding author: bijenderbajaj@yahoo.com; bajajbrijenderk@gmail.com
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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.

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. Elie 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 “foulness” 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 cholesterolemia, and alleviation of lactose intolerance (Gill and Guarner, 2004). Recently immense health benefits of probiotics have motivated the food industries to develop probiotic foods (Liang 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 multispecies 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 (Karrimi 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). Dairied desserts such as chocolate mousse have also been considered as potential probiotic delivering agents (Posemiers 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 overview of the various proposed mechanisms of probiotic action.

ProbioticsRole 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-κB, 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 induces 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).

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-κB, 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 induces 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 differentially gene-regulatory networks and pathways in the human mucosa involving up-regulation of IL-1b, an activator of NF-κB 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 cell 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 dopamine 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;
Dec et al., 2014). Probiotic-derived antibacterial substances show their effects either individually or synergistically to inhibit the growth of pathogenic bacteria. Probiotics have been reported to produce a wide range of different bacteriocins such as nisin (Araya et al., 2009) which constitute the major mechanism for their antimicrobial action. Lactobacillus acidophilus has been reported to produce acidophilin, lactocidin, and acidolin and Lactobacillus plantarum 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 revealed that the bacteriocin effect was bacteriostatic and bactericidal. Lactobacillus acidophilus produces by direct antagonism between Lb. salivarius and the pathogen (Corry 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 probiotics can inhibit growth of pathogens from within the gut wall. In addition, bacteria have a mechanism to inhibit growth of a wide range of pathogens 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 serovar 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 adjuvant-induced mice (Mechanics, et al., 2008). Probiotics in general have a protective effect in vivo resulting from dextrlose 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 (Souza et al., 2013), it was not possible to show any beneficial effect 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 attributes 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 modulation of immune responses in the GI tract (Mechanics, et al., 2008). Protein C4 inhibited L. plantarum C4 to adhere to keratinocytes, and simultaneously with infection with L. reuteri, S. aureus, S. aureus utilizes the α5β1 integrin to adhere to keratinocytes, and block the adhesion sites, therefore, prevent colonization of pathogenic bacteria (Ohland and MacNaughton, 2010).

**ROLE OF PROBIOTICS IN COMPETITION FOR NUTRIENTS**

Competition for nutrients may be one of the mechanisms for colonization and exclusion of pathogens (Abu-Mostafa et al., 2012). 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 reducing their growth. The second way 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 colonization resistance against the complex pathogenesis. 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 monosaccharic, N-acetylgalactosamine, and stialic (N-acetylneuraminic acid) in the continuous flow culture model (Wilson et al., 2000). The second way is to utilize the nutrients, and cause exclusion of pathogens and, thus providing protection to the host. Thus, competition for nutritional substrates amongst probiotics, intestinal pathogens and microbriata 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, methamylamines 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 (Elb 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 pathogens 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 NFκB 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 maturation of the host against potentially harmful antigens via activation of lymphocytes and production of antibodies. The presence 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 et al., 2009). A lot of reports showed that lact acid bacteria (LAB) as Lactobacillus and Bifidobacterium and their fermented products as prebiotics and probiotics improved better the mucusal lesion development, alleviate allergies, and put up defense against intestinal pathogen infection (Tsai et al., 2012).
Lactobacilli also stimulate immune cells to produce 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 (APC), and cytokine by natural killer (NK) cells, which can lead to cell kill transformation 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). Lactobacillus amylovorus induces B cell proliferation, enhanced phagocytic activity of macrophages, and increased secretion of cytokines responses through innate immunity in mice (Paturi et al., 2007). The interaction between CD40 and CD154 on DCs and CD+ T cells, respectively, induces the priming and expansion of CD+ 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 Th helper 2 (Th2) toward Th1 responses, thus promoting humoral immunity (Mohammadzadeh et al., 2005). Many types of LAB products have anti-inflammatory effects (Kondepudi et al., 2012). Recent research demonstrates that the L. rhamnosus GG derived soluble protein p40 activates EGRF to inhibit cytokine-induced apoptosis and disrupted barrier in intestinal epithelial cells. Further, it also showed the effects of LGG p40 through EGRF activation play a significant role, in the development of probiotic-derived proteins as novel reagents for protecting the intestine from injury and inflammation (Van et al., 2011). Similar, probiotic benefits on gut health may be due to the modulation of 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 inflammatory responses (Th1/Th2), improved gut antioxidant capacity, and enhanced resistance of aged mice to E. coli infection. In probiotic fed mice splenocytes showed increased INF-γ and decreased IL-4 and IL-10 production, neutrophil respiratory burst enzymes and phagocytosis increased without any 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 polyanines. Probiotics may also promote specific stimulation of the host immune system, including immune cell proliferation, enhanced phagocytic activity of macrophages, and increased production of interleukin-10 (IL-10) and IgA and IgM (Kaur et al., 2009). The protective effect of a multi-strain probiotic and synthetic 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 (Kondrupi et al., 2014).

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 strain GG derived p40 activation led 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 27135, particularly Lactobacillus sanfranciscensis, induced leukemia-derived cells 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 B. longum cultures showed the effects of probiotics 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. The strain also exhibited no toxicity on the normal cell line. Cytotoxic assessments through flow cytometry and fluorescence 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 therapies (Haghshenas et al., 2014).

PROBIOTICS FOR GUT MICROBIOTA MODULATION

Lactobacilli has been involved in regulating several physiological functions, ranging from energy regulation and cognitive processes to toxic neuronatalization 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 probiotics 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 probiotics, by means of modifying the gut microbiota composition, metabolic effects and cell-to-cell signaling. 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^{10} 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 metabolic syndrome components including protective effects against the oxysterol based metabolic syndrome, high blood pressure, diabetes, and increased survival rate of mice injected with tumour cells (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 commensal facultative biota 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 resident microbiota (Steidler et al., 2014; 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 successful and led to a better understanding of the effects of the secreted metabolites on the gut microbiota and immunosenescence. It was observed that dysbiosis and dysbiosis improvement was dependent on the
enrolled population and the timing of microbial 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 present article attempted to review the available scientific information on mechanisms of action of probiotics. Major health-benefiting mechanisms of probiotics included enhanced gut epithelial barrier function, Immunomodulatory effects, degradation of toxin receptors, competition for nutrients, production of inhibitory substances, antiinflammatory effects, blocking of adhesion sites, modulation of gut microbiota, among others.

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