Review Article

Probiotics: an update on mechanisms of action and clinical applications

Mehdi Goudarzi, Hossein Goudarzi, Marjan Rashidan*

Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Science, Tehran, Iran

Abstract

Probiotics are live microbial feed supplement and can provide health benefit to the host if administered in sufficient amounts. The most predominant species that have been used as probiotic include *Lactobacilli* and *bifidobacteria*. Proper administration of probiotics could be efficient in the treatment of various disorders. However; their mechanism of action is poorly understood. The effects of probiotics may be classified in following modes: reinforcement of the intestinal mucosal barrier against pathogens, competition with pathogens for adherence to the mucosa and epithelium, competitive exclusion of pathogenic microorganisms, production of antimicrobial substances, modulation of the immune system and interference with quorum sensing signaling. Exploration of the clinical features of probiotic strains, their modes of action and investigation based on probiotic therapy may be beneficial in treatment of various diseases..

Key words: Probiotics, *Lactobacillus*, *Bifidobacterium*, UTI

***Corresponding Author:** Marjan Rashidan, Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Science, Tehran, Iran. Tel: +98 21 23-87-25-56. E-mail: marjan.rashidan@yahoo.com

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Introduction

According to the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), probiotics are live microorganisms that can confer a health benefit to the host if administered in adequate amounts [1]. The name probiotic comes from the Greek 'pro bios' which means 'for life'. At the beginning of the 20th century, Probiotic was first conceptualized by the Russian Nobel Prize winner and father of modern immunology, Elie Metchnikoff [2]. In 1965, the first introduction of probiotic term was reported by Lilly and Still wellin to describe ‘substances secreted by one microorganism which stimulate the growth of another [3, 4]. In 1974 Parker proposed that probiotics are organisms and substances which contribute to intestinal microbial balance [5]. In 1989, Fuller introduced the modified definition of probiotic. According to the Fuller definition, probiotics are a live microbial feed supplement which beneficially affects the host by improving its microbial balance [6]. According to definition of Salminen et al, in 1998, probiotics are foods which contain live bacteria which can be beneficial to health, whereas Marteau et al in 2002 defined probiotics as microbial cell or components of microbial cells that have a beneficial effect on the health and well-being [7, 8].

There are several characteristics for assessment of a good probiotic product. many investigators believe that the definition of an ideal probiotic is based on the following options: resistant to gastric acid digestion and to bile salts, high ability to multiply in the gut,

strong adhesive capability with the digestive tract of the host, lacking side effect; do not have pathogenic and toxic effect on host cells, durable enough, ability to reduce the pathogenic microorganisms [9].

The most predominant species that have been used as probiotic bacteria are summarized in Table 1. However, probiotic bacteria can be also derived from the intestinal microbiota of healthy humans, nonhuman strains used in the fermentation of dairy products or species from genera such as Streptococcus, Bacillus and Enterococcus. Due to some of genera such as *Streptococcus*, *Bacillus*, and *Enterococcus* containing many pathogenic species there is some concerns about safety of such probiotics. Yeasts especially Saccharomyces have also been used as probiotics for many years [10-13].

Table 1. Commercial probiotic microorganisms

***Lactobacillus* species**	***Bifidobacterium* species**	**Other bacteria**
L. acidophilus	*B. bifidum*	*Bacillus cereus*
L. casei (rhamnosus)	*B. longum*	*Escherichia coli*
L. johnsonii	*B. breve*	*Saccharomyces cerevisiae*
L. bulgaricus	*B. infantis*	*Enterococcus faecalis*
L. plantarum	*B. lactis*	
L. reuteri	*B. adolescentis*	
L. lactis		

Discussion

A prebiotic is non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one species or a limited number of species in the colon. Oligosaccharides such as lactulose, galactooligosaccharides, inulin and fructooligosaccharides, are some of the prebiotics that are able to promote the growth and activities of probiotics. Synbiotics refer to nutritional supplements combining probiotics and prebiotics in a form of synergism [14].

Mechanisms of action of probiotics

Probiotic bacteria have various effects on the host cells and are advocated for the prevention and treatment of a wide range of disorders. The main mode of probiotic actions include 1) enhancement of the epithelial barrier 2) increased adhesion to intestinal mucosa and simultaneous inhibition of pathogen adhesion 3) competitive exclusion of pathogenic microorganisms 4) production of anti-microbial substances 5) modulation of the immune system. Recent data exhibit the effect of probiotics in interference with quorum sensing.[14-16]

Enhancement of the epithelial barrier

Intestinal barrier in healthy individual consists of the mucous layer, antimicrobial peptides, secretory IgA and epithelial cells that form tight junctions [15]. Disruption of the intestinal barrier function may result in enteric infections, celiac disease, some autoimmune diseases such as type1 diabetes and inflammatory bowel disease (IBD). Consumption of probiotic bacteria can contribute to increasing intestinal barrier function [16-19].

Intestinal barrier integrity may be increased by enhancing the expression of genes involved in tight junction signaling [20]. Some of the probiotics are able to induce signaling pathway involved in tight junction. For instance, *Lactobacilli* modulate the regulation of E-cadherin and β -catenin, in a T84 cell. Moreover, incubation of intestinal cells with *lactobacilli* modulates tight junction protein phosphorylation [21].

Probiotics may also repair the barrier function after damage. *Escherichia coli* Nissle 1917 (EcN1917) not only counteract the disruptive effects of enteropathogenic *E. coli* (EPEC) but also restores mucosal integrity in Caco-2 cells. This effect is

achieved by increasing expression and repartition of tight junction proteins of the zonula-occludens (ZO-2) and altering protein kinase C signaling [22]. In a TLR-independent pathway, Probiotics can protect the integrity of the mucosal gut barrier against the destructive action of enteropathogenic Escherichia coli. Alteration in levels of pro-inflammatory cytokines can lead to intestinal permeability in intestinal disease such as IBD. Consumption of probiotic bacteria prevents cytokine-induced epithelial damage [23, 17]. Soluble peptides secreted by *Lactobacillus rhamnosus* GG (LGG), p40 and p75 can prevent TNF-a-mediated cell apoptosis by activating the anti-apoptotic factor Akt and protein kinase B (PKB/Akt) and inhibiting the pro-apoptotic p38/mitogen-activated protein kinase (MAPK) [24,25].

Induction of mucous secretion by probiotic bacteria reinforces barrier function and the exclusion of pathogens [26]. Some of *Lactobacillus* species increases the expression of *MUC2*, *MUC3* and *MUC5AC* in HT29 cells [27].

Probiotic strains can also release antimicrobial proteins (AMPs) such as α defensins, β defensins, cathelicidins, C-type lectins and ribonucleases from epithelial cells. These small proteins can stabilize intestinal barrier function [28-32]. Defensins display antimicrobial activity against a wide range of bacteria fungi and viruses. Cathelicidins are involved in host defense against pathogens [33].

Increased adhesion to intestinal mucosa

Probiotic bacteria are able to adhere to epithelial cells, thereby, can block adherence of pathogens. The anti-adhesive effect might be the result of competition between probiotic strains and pathogens for the same receptor or the induction of mucin production by probiotics [34-36].

Several studies exhibited that different lactobacilli proteins promote mucous adhesions and bacterial surface adhesions mediate attachment to the mucous layer [37, 38]. MUB (mucus-binding protein) produced by Lactobacillus reuteri and MapA (mucous adhesion-promoting protein) by L. reuteri and L. fermentum has been reported to mediate the binding to mucous [38, 39].

Probiotic bacteria competitively inhibit adhesion of pathogenic bacteria. Lactobacillus GG and Lactobacillus plantarum 299V competitively inhibit the attachment of enterohemorrhagic E. coli 0157H7 to HT-29 cells [40, 41].

Competitive exclusion of pathogenic microorganisms

Based on several reported researches, probiotic bacteria are able to exclude or reduce the growth of pathogens by one of following ways: creation of a hostile microenvironment, physical blocking of available bacterial receptor sites, production and secretion of antimicrobial substances and selective metabolites and competition for essential nutrients [42].

Production of antimicrobial substances

Antimicrobial substances produced by probiotics can lead to inhibition of pathogen replication. These components are almost always low-molecular-weight (LMW) compounds (< 1,000 Da). The most important of these LMW compounds are short chain fatty acids. Organic acids especially acetic acid and lactic acid inhibit the growth of Gram-negative bacteria [43]. Also low-molecular-weight bacteriocins (LMWB) and high-molecular-weight bacteriocins produced by lactobacilli can inhibit pathogen replication. Some of *lactic acid bacteria* (LAB) produce bacteriocins. Lactacin B is produced by *L. acidophilus and* plantaricin by *L. plantarum*andnisin [45]. These bacteriocins have a narrow activity spectrum against related species such as other lactobacilli and taxonomically related Gram-positive bacteria or broad activity spectrum against across genera such as Gram-positive and Gram-negative bacteria as well as yeasts and molds [46]. Some of the most probable mechanisms of bacteriocins are formation of pore in cell membrane and inhibition of cell wall synthesis [47].

Studies done on probiotic bacteria exhibited that some of them can produce antibacterial compounds. Lactobacillus reuteri can produce reuterin antibiotic that is a broad spectrum antibiotic which not only is active against Gram-positive and Gram-negative bacteria but also against yeast, fungi, protozoa and viruses [44].

Intestinal *bifidobacteria* and *lactobacilli* also produce fatty acids such as conjugated linoleic acid (CLA) which is a potent anti-carcinogenic agent. CLA-

producing strains also show an anti-obesity effect and the ability to modulate the fatty acid composition of the liver and adipose tissue of the host in mice model [48, 49]. Finally, the production metabolite and derivatives of bile salts by some strains of probiotics can suppress the growth of fungi and other species of bacteria [51, 52]. For instance, *Lactobacillus* can produce antifungal substances, such as benzoic acid, methylhydantoin, mevalonolactone and short-chain fatty acids [50, 53, 54].

Probiotics and the immune system

Probiotic bacteria can effect on numerous cell types involved in the innate and adaptive immune responses such as epithelial cells, dendritic cells, monocytes/macrophages, B cells, T cells, regulatory T cells and NK cells and thereby exert their immunomodulatory effect [55].

Effects of Probiotic Bacteria on Dendritic Cells

Dendritic cells (DCs) are antigen-presenting cells that act as messengers between the innate and the adaptive immune systems. Specialized functions of DC in intestine lead to induction of oral tolerance to dietary antigens by development regulatory T cells and IgA-producing B cells through production of cytokines such as IL-10 and TGFβ [56-59]. Probiotic bacteria can down-regulate Th1 response and inhibit the production of pro-inflammatory cytokines, IL-12, TNF-α, and IFN-γ by DC [60].

Effects of Probiotic Bacteria on B Lymphocytes

Administration of *L. rhamnosus* GG to children with acute gastroenteritis increased a nonspecific humoral immune response by an enhancement in IgG, IgA, and IgM secretion from circulating lymphocytes [62]. In vaccination trial, the effects of probiotic bacteria on B lymphocytes have been proven. Studies indicate that combination of *L. rhamnosus GG* and Salmonella vaccination can increase *Salmonella*- specific IgA levels [63].

Effects of Probiotic Bacteria on Natural Killer (NK) Cells

More recently, in an animal study it have been proven that L. casei ssp. casei with prebiotic such as dextran enhanced the NK cell activities in spleen mononuclear cells of BALB/c mice and its oral administration increased production of IL-12 in human blood mononuclear cells [64]. Secretion of IL-22 by NK cells has anti-inflammatory effects on epithelial cells in vitro [65]. In addition, activity of NK cells will increase with consumption of a low fat milk drink with *B. lactis* HN019 [66].

Effects of Probiotic Bacteria on T cells

T cell response is mainly controlled by interactions between DC and T cells. Assessment of effect monocyte-derived dendritic cell cultured with *L. rhamnosus* exhibited that T-cell proliferation and T-cell cytokine production, particularly IL-2, IL-4, and IL-10 had decreased dramatically [67].

Interference with signaling factor of quorum sensing

Bacteria communicate with each other and their surrounding environment through chemical signaling molecules that are called auto-inducers. This phenomenon is known as quorum sensing (QS) that can measure the population density, nutrient concentration and other ecological characteristics. In addition to, QS can control the gene expression of the entire community in response to changes in cell number [68, 69].

Probiotic bacteria such as lactobacillus, bifidobacterium and B.cereus strains can produce auto-inducers that can control virulence gene expression in numerous microorganisms. Lactobacillus acidophilus La-5 secretes a compound that reduces the production of auto-inducer by E. coli O157 and through it, leads to significant reduction in the transcription of genes involved in colonization [70]. Recently, many studies have reported similar results for B. cereus and B. toyoi [71, 72].

Applications of probiotics

In the past century the beneficial roles of nonpathogenic bacteria in the establishment of health were described. Recently it is proved that there are clinical benefits to use specific nonpathogenic organisms and also there is strong evidence for their efficacy in some clinical scenarios. Considering beneficial roles of probiotics, they are now widely used in many countries by consumers and in clinical practice

Diarrheal diseases

Diarrhea is defined as the passage of 3 or more unformed stools for at least 2 consecutive days. Probiotics are able to protect the host against bacterial toxins and in this way impede diarrhea. *L. casei rhamnosus* (Lcr35) is found to inhibit colonization of large variety of pathogens in Caco-2 cell line and therefore prevent traveler's diarrhea [73].

Bifidobacteriumlactis HN019 reduced the severity of diarrhea associated with both rotavirus and *E. coli* in pigs. This effect is attributed to the increase of immune-mediated protection [74]. A meta-analysis done on probiotics exhibited that Saccharomyces boulardii and the bacterium Lactobacillus acidophilus in combination with L. bulgaricus, L. rhamnosus strain GG are effective in prevention of antibiotic-associated diarrhea. Another meta-analysis study done on probiotic bacteria showed that Lactobacillus species, Enterococcus species, and S boulardii are effective in the treatment of infective diarrhea in both adults and children (00). The preventive effect of *Lactobacillus GG* on antibiotic-associated diarrhea (AAD) has been assessed in children receiving different antimicrobial treatments [75].

Saccharomyces boulardii is effective in prevention and decrease of recurrent *C. difficile* infection (CDI). The effect of *S. boulardii* on *C. difficile* toxin in rats and human colonic mucosa is probably due to proteolytic digestion of toxins by secretion of protease. Although, they are used as a preventive and therapeutic measure but their role in treatment and prevention of CDI remains controversial. The best studied probiotic agents in CDI are Saccharomyces boulardii and Lactobacillus. Several studies shown that mixtures of probiotics can be useful in treatment and prevention of ADD and CDI [76, 77].

Urinary tract infections

There is a close correlation between the loss of the normal microbiota of genital tract, especially *Lactobacillus* species, and an increased incidence of UTIs. The role of probiotics in prevention of UTI is obvious [78]. *Lactobacillus plantarum* and *Lactobacillus rhamnosus* inhibit the adherence of E. coli to the gastrointestinal (GI) tract wall by inducing the production of mucin and leading to inhibition of the adhesion of pathogens [79]. Recent research has shown that two probiotics strains, *Lactobacillus acidophilus* PXN35 and *Lactobacillus plantarum* PXN47 have good anti-bacterial effects in inhibiting E. coli growth [80]. In study performed by Kontiokari et al. in 2003, they showed that consumption of fermented milk products containing probiotic bacteria more than three times a week was associated with a decreased risk of recurrent UTIs (RUTIs) [81].When *Lactobacillus fermentum* orally administer with low doses of ampicillin, was effective in the treatment of *E. coli* urinary tract infection in a mouse model [82].

Helicobacter pylori infections

During recent years, several studies in order to assess probiotics effect in the treatment of H. pylori infections have been done [83]. In vitro studies demonstrated that strains of lactobacilli, bifidobacteria and B. subtilis inhibit the growth or the adhesion of H. pylori to mucus cells [84-86]. In mice model, Lactobacillus salivarius eliminated the colonization of H. pylori [87].

Vaginal dismicrobism

The vaginal microbiota of healthy women consists of a wide variety of anaerobic and aerobic bacterial genera and species dominated by the genus Lactobacillus. Lactobacillus especially during pregnancy helps to maintain the natural healthy balance of the vaginal microbiota. Nad thereby decreases the rate of bacterial vaginosis (BV) and aerobic vaginitis (AV). In a study conducted by Hilton, oral administration of *L. johnsonii* effectively decreased candida vaginitis [88]. The production of hydrogen peroxide and lactic acid by *lactobacilli* contribute to reduction of pH and may inhibit growth of *Gardnerella* and other bacteria [89, 90].

Food allergy

Small peptides and amino acids produced by some *bacteria* are able to induce allergic reactions. Several investigators have shown that imbalance between *bifidobacteria* and *clostridia* can lead to allergies [84]. Enzymes derived from *L. casei* GG degrade Small peptides and amino acids, in result it will produce molecules with inhibitory effects on lymphocyte proliferation [91]. Mechanisms of anti-allergic probiotic bacteria include increasing intestinal barrier integrity,

modulation of the immune system through the production of inflammatory cytokines or enhancement of specific IgA responses and degradation of food antigens by means of productive enzymes of probiotics.

Lactose intolerance

Lactose intolerance (LI), also known as lactose malabsorption is the most common type of carbohydrate malabsorption. Due to low levels of lactase enzyme activity in patients, they are not able to digest lactose into glucose and galactose. Intensive intestinal distress with characteristic bloating, flatulence and abdominal pain could appear 30-minutes to 2 hours after consumption of food products containing lactose disaccharide. Lactases produced by L. acidophillis and Lactobacillis delbrlleckii ssp. blilgaricus by β-galactoside galactohydrolase (β-gal) and LactococcliSlactics ssp. lactis and Lactococcus lactis ssp crenzoris by β-D-phosphogalactoside galactohydrolase (β--P-gal) are able to hydrolyze and ease absorption of lactose. The production of hydrogen in breath is an indicator of bacterial metabolism of lactose in the colon [92].

Hypercholesterolemia

Several studies showed that the reduction of serum cholesterol can be created by oral administration of *L. johnsonii* and *L. reuterii* in pigs. Probiotic bacteria can decrease blood cholesterol level by cholesterol absorption, de-conjugation of bile acids through bacterial hydrolysis, attachment of cholesterol to bacterial cell walls and inhibition of hepatic cholesterol synthesis [93, 94].

Anticancer effects

There are claims for anti-cancer activity of probiotics. *Lactobacillus*, *Bifidobacteria* and *E. coli* Nissle 1917 strains have anti-mutagenic activities due to ability to metabolize and inactivate compounds of mutagen [95]. The anticancer effects of probiotics probably are due to several mechanisms such as inhibition of procarcinogen transformation to active carcinogens, inactivation of mutagenic compounds, production of anti-mutagenic compounds, suppression of the growth of pro-carcinogenic bacteria, reduction of the absorption of mutagens from the intestine and reinforcement of immune system function [96].

Conclusion

According to the clinically proven benefits of probiotics it is clear that probiotics can be used for the prevention of various diseases and even their management. Therefore, it is possible to conceptualize the use of probiotics due to their low cost, decrease in antibiotic resistance, modulation of immune system and inhibition of pathogens providing good alternative over the antibiotics.

References

1. FAO\WHO. Probiotics in food. Health and nutritional properties. FAO Food and Nutrition Paper 85. Rome, Italy. 2006.
2. Metchnikoff E. *The Prolongation of Life*. New York: Putmans Sons, 1908.
3. *Lactobacillus casei* strain Shirota, Yakult Honsha Co. Ltd,Yakult Central Institute for Microbiological Research,Tokyo, Japan (1998).
4. Lilly DM & Stillwell RH. Growth promoting factors produced by micro-organisms. *Science* 1965; 147: 747-48.
5. Parker RB. Probiotics, the other half of the antibiotic story. *Animal Nutrit Health* 1974; 29: 4–8.
6. Fuller R. Probiotics in man and animals. *Journal of Applied Bacteriology* 1989; 66: 365–378.
7. Salminen S, Bouley C, Boutron-Ruault MC et al. Functional food science and gastrointestinal physiology and function. *Br J Nutri* 1998; 80(supplement 1): S147–S171.
8. Marteau P, Cuillerier E, Meance S et al. *Bifidobacterium*animalis strain DN-173 010 shortens the colonic transit time in healthy women: a double-blind, randomized, controlled study. *Aliment Pharmacol Ther* 2002; 16: 587–593.
9. Brown M. **Modes of Action of Probiotics: Recent Developments**. *J.Anim.Vet.Adv.* 2011; 10: 1895-1900.
10. Guarner F, Malagelada J.R. Gut flora in health and disease. *Lancet* 2003; 361: 512–519.
11. CuttingS.M. *Bacillus* probiotics, *Food Microbiol.* (in press).
12. McFarland LV. Meta-analysis of probioticsfor the prevention of antibiotic associated diarrhea and the treatment of *Clostridium difficile* disease. *Am J Gastroenterol* 2006; 101: 812–822.
13. Madden AJ, Hunter OA review of the role of the gut microflora in irritable bowel syndrome andthe effects of probiotics. *Br J Nutrit* 2002; 88: Suppl. 1, S67–S72.
14. Gibson G. R, Roberfroid, M. B. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutri* 1995;125: 1401-1412.

15. Ohland C.L, Macnaughton W.K. Probioticbacteria and intestinal epithelial barrier function. *Am J Physiol Gastrointest Liver Physiol* 2010; 298:G807–G819.
16. Hooper L.V, Wong M.H, Thelin A, Hansson L, Falk PG, Gordon JI. Molecular analysis of commensal host-microbial relationships in the intestine. *Science* 2001; 291: 881–884.
17. Sartor R.B. Mechanisms of disease: pathogenesisofCrohn's disease and ulcerative colitis. *Nat ClinPractGastroenterol Hepatol*2006; 3: 390–407.
18. Sakaguchi T, Kohler H, Gu X and et al. Shigellaflexneri regulates tight junction-associated proteins in human intestinal epithelial cells. *Cell Microbiol.* 2002;4:367–381.
19. Watts T, Berti I, Sapone A and et al. Role of the intestinal tight junction modulator zonulin in the pathogenesis of type I diabetes in BB diabeticprone rats. *ProcNatlAcadSci U S A.* 2005;102:2916 –2921.
20. Anderson RC, Cookson AL, McNabb WC, Park Z, McCann MJ, Kelly WJ, Roy NC: *Lactobacillus plantarum MB452* enhances the function of the intestinal barrier by increasing the expression levels of genes involved in tight junction formation. *BMC Microbiol*2010; 10: 316.
21. Hummel S, Veltman K, Cichon C, Sonnenborn U, Schmidt M.A. Differential targeting of the E-cadherin/β-catenin complex by Gram-positive probiotic *lactobacilli* improves epithelial barrier function. *Appl Environ Microbiol* 2012; 78: 1140–1147.
22. Zyrek AA, Cichon C, Helms S, et al. Molecular mechanisms underlyingthe probiotic effects of *Escherichia coli*Nissle 1917 involve ZO-2 and PKCzeta redistribution resulting in tight junction and epithelial barrier repair. *Cell Microbiol.* 2007;9:804–816.
23. Bruewer M, Samarin S, Nusrat A: Inflammatory bowel disease and the apical junctional complex. Ann NY AcadSci 2006; 1072: 242–252.
24. Yan F, Polk DB: Probiotic bacterium prevents cytokine-induced apoptosis in intestinal epithelial cells. J BiolChem 2002; 277: 50959–50965.
25. Yan F, Cao H, Cover T.L., Whitehead R, Washington M.K., Polk D.B. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. Gastroenterology 2007; 132: 562–575.
26. Mack D.R., Ahrne S, Hyde L, Wei S, Hollingsworth M.A. Extracellular *MUC3*mucin secretion follows adherence of *Lactobacillus* strains to intestinal epithelial cells in vitro. *Gut* 2003; 52: 827–833.
27. Otte J.M., Podolsky D.K. Functional modulation of enterocytes by Grampositive and Gram-negative microorganisms. *Am J PhysiolGastrointest Liver Physiol.* 2004; 286:G613–G626.
28. Furrie E, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O'neil DA, Macfarlane GT.Synbiotic therapy (*Bifidobacteriumlongum*/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* 2005; 54: 242–249.
29. Ayabe T, Satchell DP, Wilson CL, Parks WC, Selsted ME, Ouellette AJ. Secretion of microbicidalalphadefensins by intestinal Paneth cells in response to bacteria. *Nat Immunol*2000; 1: 113–118.
30. O'neil DA, Porter EM, Elewaut D, Anderson GM, Eckmann L, Ganz T, Kagnoff MF. Expression and regulation of the human betadefensins hBD-1 and hBD-2 in intestinal epithelium. *J Immunol* 1999; 163: 6718–6724.
31. Takahashi A, Wada A, Ogushi K, Maeda K, Kawahara T, Mawatari K, Kurazono H, Moss J, Hirayama T, Nakaya Y. Production of betadefensin-2 by human colonic epithelial cells induced by *Salmonella enteritidis*flagella filament structural protein. *FEBS Lett* 2001; 508: 484–488.
32. Müller CA, Autenrieth IB, Peschel A. Innate defenses of the intestinal epithelial barrier. *Cell Mol Life Sci* 2005; 62: 1297–1307.
33. Kelsall B.L. Innate and adaptive mechanisms to control pathological intestinal inflammation. *J Pathol*214: 242–259, 2008.
34. Juntunen M, Kirjavainen PV, Ouwehand AC, Salminen SJ, Isolauri E. Adherence of probiotic bacteria to human intestinal mucus in healthy infants and during rotavirus infection. *ClinDiag Lab Immunol* 2001; 8: 293–296.
35. Schiffrin EJ, Brassart D, Servin AL, Rochat F, Donnet-Hughes A. Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. *Am J ClinNutr* 1997; 66: 515S–520S.
36. Hirano J, Yoshida T, Sugiyama T, Koide N, Mori I, Yokochi T. The effect of *Lactobacillus rhamnosus* on enterohemorrhagic *Escherichia coli* infection of human intestinal cells in vitro. *Microbiol Immunol* 2003; 47: 405–409.
37. Van Tassell ML, Miller MJ.*Lactobacillus*adhesion to mucus. *Nutrients* 2011; 3: 613–636.
38. Buck BL, Altermann E, Svingerud T, Klaenhammer TR. Functional analysis of putative adhesion factors in *Lactobacillus acidophilus* NCNCFM. *Appl Environ Microbiol* 2005; 71: 8344–8351.
39. Ouwehand AC, Salminen S, Tolkko S, Roberts P, Ovaska J, Salminen E. Resected human colonic tissue: new model for characterizing adhesion of lactic acid bacteria. *Clin Diag Lab Immunol* 2002; 9: 184–186.
40. Conway PL, Goldin BR, Gorbach SL. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *J Dairy Sci*, 1987; 70:1-12.
41. Mack DR, Michail S, Wei S, Macdougal L, Hollingsworth MA. Probiotics inhibit enteropathogenic E. coli adherence in vitro by inducing intestinal mucin gene expression. *Am J Physiol*, 1999; 39:G941-G950.
42. Rolfe RD: Population dynamics of the intestinal tract; in Blankenship LC (ed): Colonization Control of Human Bacterial Enteropathogens in Poultry. San Diego, Academic Press, 1991, pp 59–75.
43. Alakomi HL, Skytta E, Saarela M, Mattila- Sandholm T, Latva-Kala K, Helander IM. Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Appl Environ Microbiol* 2000; 66: 2001–2005.
44. Ouwehand AC: Antimicrobial components from lactic acid bacteria; in Salminen S, von Wright A (eds). Lactic Acid Bacteria: Microbiology and Functional Aspects. New York, Dekker, 1998, pp 139–159.
45. Nielsen DS, Cho GS, Hanak A, Huch M, Franz CM, Arneborg N: The effect of bacteriocin- producing *Lactobacillus plantarum strains* on the intracellular pH of sessile and planktonic Listeria monocytogenes single cells. *Int J Food Microbiol* 2010; 141:S53–S59.
46. Nemcova R. [Criteria for selection of lactobacilli for probiotic use.] *Vet Med (Praha).* 1997; 42:19 –27.
47. Hassan M, Kjos M, Nes IF, Diep DB, Lotfipour F: Natural antimicrobial peptides from bacteria:

characteristics and potential applications to fight against antibiotic resistance. *J ApplMicrobiol*. 2012, DOI: 10.1111/j.1365-2672.2012.05338.
48. O'Shea EF, Cotter PD, Stanton C, Ross RP, Hill C: Production of bioactive substances by intestinal bacteria as a basis for explaining probiotic mechanisms: bacteriocins and conjugated linoleic acid. *Int J Food Microbiol*2012; 152: 189–205.
49. Lee K, Paek K, Lee HY, Park JH, Lee Y: Antiobesity effect of trans-10,cis-12-conjugated linoleic acid-producing *Lactobacillus plantarum*PL62 on diet-induced obese mice. *J ApplMicrobiol* 2007; 103: 1140–1146.
50. Oelschlaeger TA: Mechanisms of probiotic actions – a review. *Int J Med Microbiol*2010; 300: 57–62.
51. Coloretti F, Carri S, Armaforte E, Chiavari C, Grazia L, Zambonelli C: Antifungal activity of *lactobacilli* isolated from salami. *FEMS MicrobiolLett* 2007; 271: 245–250.
52. Lindgren SE, Dobrogosz WJ: Antagonistic activities of lactic acid bacteria in food and feed fermentations. *FEMS Microbiol Rev*1990; 7: 149–163.
53. Prema P, Smila D, Palavesam A, ImmanuelG: Production and characterization of an antifungal compound (3-phenyllactic acid) produced by *Lactobacillus plantarum* strain. *Food Bioprocess Technol* 2008; 3: 379–386.
54. Sjogren J, Magnusson J, Broberg A, Schnurer J, Kenne L: Antifungal 3-hydroxy fatty acids from *Lactobacillus plantarum*MiLAB14. *Appl Environ Microbiol* 2003; 69: 7554–7557.
55. Gómez-Llorente C, Muñoz S, Gil A: Role of Toll-like receptors in the development of immunotolerance mediated by probiotics. *ProcNutrSoc* 2010; 69: 381–389.
56. Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure toantigen. *Nat Immunol.* 2001; 2:725–731.
57. Williamson E, Bilsborough JM, Viney JL. Regulation of mucosaldendritic cell function by receptor activator of NF-kappa B (RANK)/RANK ligand interactions: impact on tolerance induction. *J Immunol*.2002; 169:3606 – 3612.
58. Rescigno M, Urbano M, Valzasina B, et al. Dendritic cells express tightjunction proteins and penetrate gut epithelial monolayers to samplebacteria. *Nat Immunol.* 2001; 2:361–367.
59. Stagg AJ, Hart AL, Knight SC, et al. The dendritic cell: its role inintestinal inflammation and relationship with gut bacteria. *Gut*. 2003;52:1522–1529.
60. Hart AL, Lammers K, Brigidi P, et al. Modulation of human dendriticcell phenotype and function by probiotic bacteria. *Gut.* 2004; 53:1602–1609.
61. Pathmakanthan S, Li CK, Cowie J, et al. Lactobacillus plantarum 299:beneficial in vitro immunomodulation in cells extracted from inflamedhuman colon. *J GastroenterolHepatol.* 2004; 19:166 –173.
62. Kaila M, Isolauri E, Soppi E, et al. Enhancement of the circulatingantibody secreting cell response in human diarrhea by a human *Lactobacillus*strain. *Pediatr Res.* 1992; 32:141–144.
63. Fang H, Elina T, Heikki A, et al. Modulation of humoralimmuneresponse through probiotic intake. *FEMS Immunol Med Microbiol*.2000; 29:47–52.
64. Ogawa T, Asai Y, Tamai R, et al. Natural killer cell activities ofsynbiotic*Lactobacilluscasei ssp. casei* in conjunction with dextran.*ClinExpImmunol*. 2006; 143:103–109.
65. Cella M, Fuchs A, Vermi W, Facchetti F, Otero K, Lennerz JK, Doherty JM, MillsJC, and Colonna M. A human natural killer cell subset provides an innate source ofIL-22 for mucosal immunity. *Nature* 457: 722-725, 2009.
66. Chiang BL, Sheih YH, Wang LH, Liao CK, and Gill HS. Enhancing immunity bydietary consumption of a probiotic lactic acid bacterium (*Bifidobacteriumlactis*HN019): optimization and definition of cellular immune responses. *Eur J ClinNutr*54: 849-855, 2000.
67. Braat H, van den BJ, van Tol E, et al. Lactobacillus rhamnosusinducesperipheralhyporesponsiveness in stimulated CD4+ T cells via modulationof dendritic cell function. *Am J ClinNutr*. 2004; 80:1618 –1625.
68. Schauder, S. andBassler, B.L. (2001). The languages of bacteria. *Genes and Development* 15, 1468-1480.
69. Vilà B., Esteve-Garcia E and Brufau J. Probiotic microorganisms: 100 years of innovation and efficacy.Modes of action.*World's Poultry Science Journal*. 2010; 66: 369-380.
70. Medellin-Pena, M.J., Wang, H., Johnson, R., Anand, S. and Griffiths,M.W. (2007). Probiotics Affect Virulence-Related Gene Expression in *Escherichia coli O157:H7*. *Applied and Environmental Microbiology* 73, 4259-4267.
71. Medina-Martinez, M.S., Uyttendaele, M., Rajkovic, A., Nadal, P. andDebevere, J. (2007). Degradation of N-Acyl-L-Homoserine Lactones by *Bacillus cereus*in Culture Media and Pork Extract. *Applied and Environmental Microbiology* 73, 2329-2332.
72. Cerda-Cuellar, M., Badiola, I. and Castillo, M. (2009). In vitro degradationof N-acyl-L-homoserine lactones by *Bacillus cereus*vartoyoi. In *XI International Symposium on Digestive Physiology of Pigs*, Montbrió del Camp, Spain, S2.36.
73. Forestier C, De Champs C, Vatoux C, Joly B. Probiotic activities of *Lactobacillus caseirhamnosus*: in vitro adherence to intestinal cells and antimicrobial properties. *Res Microbiol* 2001; 152:167-73.
74. Shu Q, Qu F, Gill HS. Probiotic treatment using *Bifidobacteriumlactis* HN019 reduces weanling diarrhea associated with rotavirus and *Escherichia coli* infection in a piglet model. *J PediatrGastroenterolNutr* 2001; 33:171-7.
75. Arvola T, Laiho K, Torkkeli S, et al. Prophylactic *Lactobacillus GG* reduces antibiotic-associated diarrhoea in children with respiratory infections: a randomised study. *Pediatrics* 1999; 104:e64.
76. Castagliuolo I, Qiu BS, Lamont JT, Pothoulakis C. Saccharomyces boulardii protease inhibits *Clostridium difficile* toxin A effects in the rat ileum. *Infect Immun* 1996; 64:5225_ 32.
77. Castagliuolo I, Riegler MF, Valenick L, Lamont JT, Pothoulakis C. *Saccharomyces boulardii* protease inhibits the effects of Clostridium difficile toxins A and B in human colonic mucosa.*Infect Immun* 1999; 67:302_ 7.
78. Amdekar S, Singh V, Singh DD. Probiotic therapy: immunomodulating approach toward urinary tract infection. *CurrMicrobiol*.2011; 63(5):484-90.
79. Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA. Probiotics inhibit enteropathogenic*E. coli* adherence in vitro by inducing

intestinal mucin gene expression. *Am J Physiol*.1999; 276(4 Pt 1):G941-50.

80. Probiotics International Limited. Data on file.
81. Kontiokari T, Laitinen J, Järvi L, Pokka T, Sundqvist K, Uhari M. Dietary factors protecting women from urinary tract infection. *Am J Clin Nutr*.2003; 77(3):600-4.
82. Silva de Ruiz C, Lopez de Bocanera ME, Nader de Macias ME,Pesce de Ruiz Holgado AA. Effect *of lactobacilli* and antibioticson *E. coli* urinary infections in mice. *Biol Pharm Bull*1996; 19(1):88–93.
83. Cremonini F, Canducci F, Di Caro S, et al. *Helicobacter pylori*treatment: a role for probiotics. *Digest Dis* 2001; 19:144-7.
84. Midolo PD, Lambert JR, Hull R, Luo F, Grayson ML. In vitro inhibition of *Helicobacter pylori*NCTC 11637 by organic acids and lactic acid bacteria. *J ApplBacteriol* 1995; 79:475_ 9.
85. Mukai T, Asasaka T, Sato E, Mori K, Matsumoto M, Ohori H. Inhibition of binding of *Helicobacter pylori*to the glycolipid receptors by probiotic *Lactobacillus reuteri*. *FEMS Immunol Med Microbiol* 2002; 32:105_ 10.
86. Pinchuk IV, Bressollier P, Verneuil B, et al. In vitro anti-*Helicobacter pylori*activity of the probiotic strain *Bacillus subtilis3* is due to secretion of antibiotics. *Antimicrob Agents Chemother*2001; 45:3156_ 61.
87. Kabir AMA, Aiba Y, Takagi A, Kamiya S, Miwa T, Koga Y. Prevention of *Helicobacter pylori*infection by *lactobacilli* in a gnotobiotic murine model. *Gut* 1997; 41:49 _ 55.
88. Hilton E, Isenberg HD, Aplerstein P, France K, Borenstein MT. Ingestion of yoghurt containing *Lactobacillus acidophilus* as prophylaxis for candida vaginitis. *Ann Int Med* 1992; 116:353–7.
89. Hughes VL, Hilier SL. Microbiologic characteristics of *Lactobacillus* products used for colonisation of the vagina. *ObstetGynecol* 1990; 75(2):244–8.
90. Ocana VS, Pesce de Ruiz Holgado AA, Nader-Macias ME. Selection of vaginal H2O2-generating *Lactobacillus* for probiotic use. *CurrMicrobiol* 1999; 38(5):279–84.von der Weid T, Ibnou-Zekri N and Pfeifer A. Novel probiotics for the management of allergic inflammation.*Dig Liver Dis*. 2002 Sep; 34 Suppl 2:S25-8.
91. Sütas Y., Soppi E and et al. Suppression of lymphocyte proliferation in vitro by bovine caseins hydrolyzed with*Lactobacillus casei*GG–derived enzymes. *Journal of Allergy and Clinical Immunology*1996; 98: 216–224.
92. Kim HS, Gilliland SE. *Lactobacillus acidophilus*as dietary adjunctfor milk to aid lactose digestion in humans. *J Dairy Sci*1983; 66:959–66.
93. Ouwehand A.C. Antiallergic Effects of Probiotics. *The Journal of Nutrition* 2007; 794S- 797S
94. Lovegrove J, Jackson K. Coronary heart disease. In: Mattila Sandholm T, Saarela M, eds. Functional dairy products. Cambridge, UK: Woodhill Publishing, 2003:54–87.
95. LindneR D.J.D., Pandey A, Thomaz-Soccol V. The Potential of Probiotics: A Review. *Food Technol. Biotechnol*.2010; 48:413–434.
96. Gill H.S, Guarner F. Probiotics and human health: a clinical perspective. *Postgrad Med J* 2004;80:516–526.