

Kertas Asli/Original Articles

The Health Benefits of Probiotics (Kebaikan Probiotik kepada Kesihatan)

YAP WEI BOON & RINA ANAK SUJANG

ABSTRACT

There has been a significant increase in research on probiotics-associated health benefits in the last 20 years. Many studies carried out in vitro and clinically show that consumption of probiotics inhibits the growth of pathogenic microorganisms. Furthermore, the consumption of probiotics also enhances the host immune response and decreases the levels of carcinogenesis-inducing enzymes. These positive outcomes have led to the use of probiotics in prevention and treatment of infectious diseases like bacterial or antibiotic associated diarrhea, chronic inflammatory bowel diseases and colon cancer. This review summarises literature pertaining to mechanistic actions of probiotics in improving the well-being of hosts.

Keywords: Probiotics; microorganisms; carcinogenesis; health benefits

ABSTRAK

Terdapat peningkatan yang signifikan dalam penyelidikan mengenai manfaat probiotik kepada kesihatan. Banyak kajian yang dijalankan secara in vitro dan klinikal menunjukkan bahawa pengambilan probiotik dapat merencat pertumbuhan mikroorganisma patogenik. Selain itu, pengambilan probiotik juga dapat meningkatkan tindak balas imun perumah dan mengurangkan tahap enzim yang terlibat dalam proses karsinogenesis. Hasil kajian yang positif ini menyebabkan probiotik digunakan dalam pencegahan dan rawatan penyakit seperti diareja yang disebabkan bakteria dan antibiotik, keradangan usus kronik dan kanser kolon. Ringkasan ini merumuskan kajian kesusasteraan yang berkaitan dengan mekanisme probiotik dalam meningkatkan kesihatan perumah.

Kata kunci: Probiotik; mikroorganisma; karsinogenesis; manfaat kesihatan

INTRODUCTION

The concept of probiotic was initially proposed by Elie Metchnikoff in 1910 who discovered that the relatively long life span of Bulgarians was due to daily intake of fermented dairy products (Bibel 1998). He believed that consumption of fermented milk products containing *Lactobacillus* contributed to the balance of microbial population in the colon. Since then, studies about probiotics continue to increase rapidly and a few definitions of probiotic have been proposed. Lilly & Stillwell (1965) defined probiotics as substances produced by microorganisms that promote growth of other microorganisms. Whereas Fuller (1989) described probiotics as food supplements consisting of live microorganisms that benefit hosts by balancing microorganisms in the intestines. Later in 2001, FAO/WHO recommended the standard definition of probiotics which is "live microorganisms which are beneficial to hosts when taken in sufficient amounts".

The majority of probiotic bacteria are gram positive and they produce lactic acid. The genera of *Lactobacillus* and *Bifidobacterium* compose most of members of probiotic bacteria. *Saccharomyces boulardii* (*S. boulardii*), *Saccharomyces cerevisiae* (*S. cerevisiae*), *Escherichia*

coli (*E. coli*) and *Bacillus* spp. are also included in some probiotic recipes (De Vrese & Schrezenmeir 2008). There are various mechanisms employed by probiotics in providing protection to the hosts. These mechanisms include inhibiting the growth of pathogenic microorganisms in the gastrointestinal tract, enhancing the host's immune system, producing beneficial enzymes and decreasing the level of enzymes involved in carcinogenesis.

INHIBITION OF PATHOGENIC MICROORGANISMS

Probiotics prevent colonization of pathogenic microorganisms by producing antimicrobial peptides and compounds such as bacteriosin, organic acids and hydrogen peroxide. Production of organic acids by probiotic bacteria lowers the intestinal pH which then inhibits the growth of pathogenic microorganisms (Ng et al. 2009). For instance, *Salmonella typhimurium* (*S. typhimurium*) which causes Salmonellosis is unable to survive acidic conditions (Salminen & Wright 1998). Meanwhile, bacteriosin such as nisin is very effective in preventing the growth of gram-positive and gram-negative

pathogenic bacteria and yeast (Cleusix 2008). Nisin is made of cationic and hydrophobic domains. Both of the domains interact with bacterial membrane and subsequently disrupt the membrane integrity, thereby forming pores in the membrane. Pore formation causes outflow of ions (K^+ and Mg^{2+}), amino acids (glutamic acid, lysine) and ATP passively from the cytoplasm and eventually results in microbial cell death. Corr et al. (2007) showed that *Lactobacillus salivarius* protected mice from *Listeria monocytogenes* (*L. monocytogenes*) infection through the direct action of bacteriosin Abp118. In addition, sakacin produced by *Lactobacillus sakei* (*L. sakei*) 1 was also shown to inhibit the adherence of *L. monocytogenes* on stainless steel surface (Winkelströter et al. 2011).

Probiotics also inhibit the growth of pathogens through competition for micronutrients and attachment site on the intestinal epithelia. Attachment of probiotics on the epithelial cell surface blocks the binding of pathogenic microorganisms (Kopp-Hoolihan 2001). In addition, probiotics are able to compete for micronutrients with pathogens, for example, competition for iron which is essential for the growth of bacteria. Hooper (2004) showed that *L. acidophilus*, *L. delbrueckii* and *E. coli* Nissle bound and chelated iron on the surface of the epithelial cells. This reduces the provision of iron to pathogens and eventually causes pathogen death.

The effectiveness of probiotics in inhibiting the growth of pathogenic bacteria has led to the use of probiotics in treating diarrhea and preventing pathogen infection in the intestine. For example, prevention of antibiotic associated diarrhea. Excessive intake of antibiotics disrupts the balance of microflora in the gastrointestinal tract, thus allowing the emergence of resistant pathogenic bacteria such as *Clostridium difficile* (*C. difficile*) which causes diarrhea in humans. However, by consuming probiotics, the growth of *C. difficile* can be suppressed and the balance of intestinal microflora is then restored. A number of clinical studies demonstrated that ingestion of *S. boulardii*, *Lactobacillus rhamnosus* GG (*L. rhamnosus* GG), *L. acidophilus*, *L. bulgaris* reduced the occurrence of antibiotic associated diarrhea by as much as 52% (Sazawal et al. 2006).

Helicobacter pylori (*H. pylori*) is commonly associated with chronic gastritis, peptic ulcers, and gastric cancer (Plummer et al. 2004). Apart from taking antibiotics and proton pump inhibitor as a treatment for *H. pylori* infection, probiotics have been employed in treating the bacterial infection. A number of animal studies showed that intake of *L. casei* Shirota reduced the colonization of *H. pylori* in the antrum and mucosa of the intestine (Sgouros et al. 2004). In addition, Wang et al. (2004) also showed that intake of yogurt containing *Bifidobacterium animalis* (*B. animalis*) Bb12 and *L. acidophilus* successfully suppressed *H. pylori* colonization and infection.

REDUCTION OF LACTOSE INTOLERANCE AND CARCINOGENESIS

Lack of β -galactosidase activity in the lower intestine causes lactose intolerance (Vasiljević & Shah 2012). Consumption of dairy products with high lactose content causes abdominal pain, diarrhea, nausea, and bloating in lactose intolerant individuals. The problems can be alleviated by consuming fermented dairy products supplemented with β -galactosidase producing *Lactobacillus* and *Bifidobacterium* spp. (Montalto et al. 2005). A few *in vivo* studies in lactase-deficient subjects showed that consumption of probiotics reduced bloating symptoms and this was likely due to the presence of β -galactosidase produced by lactic acid bacteria (Hirayama & Rafter 2000 for review, Gill & Guarner 2004).

Probiotics have been shown to decrease the activities of carcinogenic enzymes such as β -glucuronidase, azoreductase, and nitroreductase. According to Pedrosa et al. (1995), carcinogenic enzymes produced by intestinal microorganisms encourage the growth of cancers. Intake of probiotics not only inhibits the growth of harmful microflora but also reduces the levels of carcinogenic enzymes. Goldin (1996) showed that carcinogen-treated animals exhibited drastic tumor size reduction after being fed with *L. rhamnosus* GG. Furthermore, Salminen & Wright (1998) demonstrated that the levels of carcinogenic enzymes in human stool specimens were reduced significantly following the intake of *L. acidophilus* and *L. casei*.

ANTIOXIDANT ENZYMES

Probiotic lactic acid bacteria have been shown to produce antioxidant enzymes such as glutathione-S-transferase (GST), glutathione (GSH) reductase and GSH peroxidase (Hayes et al. 2007). Antioxidant enzymes are essential in biotransformation of xenobiotic compounds, carcinogens, free radicals and peroxides that cause oxidative stress in cells. Increase in oxidative stress results in cirrhosis, atherosclerosis and cancers (Rice-Evans & Burdon 1993). Challa et al. (1997) reported a significant increase in the GST level in rat colon following *Bifidobacterium longum* feeding to the rats. In addition, Ejtahed et al. (2012) also showed that consumption of yogurt containing *L. acidophilus* La5 and *Bifidobacterium lactis* Bb12 was capable of increasing the levels of erythrocyte superoxide dismutase and glutathione peroxidase activities in type II diabetic patients. In this light, probiotics are indeed able to improve anti-oxidative status in diabetic patients.

ENHANCEMENT OF HOST IMMUNE FUNCTION

Probiotics regulate host immune system by activating nuclear factor kappa beta (NF- κ B), balancing T-helper cell response, stimulating the production of IgA, controlling inflammatory reactions and increasing the activity of macrophages (Kalliomaki & Walker 2005). However, a balanced inflammatory response is relatively important in order to avoid excessive intestinal inflammation which leads to severe intestinal disorders such as inflammatory bowel disease (IBD) and necrotizing enterocolitis (NEC).

Intestinal lymphoid tissues are stimulated by probiotic bacteria attachment to Toll-like receptors (TLRs) expressed on macrophages, dendritic cells, B cells and epithelial cells (Pasare & Medzhitov 2005). Such attachment induces the production of cytokines by immune cells and secretion of polymeric IgA by plasma cells (Kohler et al. 2003). Bacterial ligands on the surface of probiotic bacteria such as lipopolysaccharide (LPS) and lipoteichoic acid interact with TLR-2 and TLR-4 of dendritic cells and subsequently activate NF- κ B which then migrates to the cell nucleus and binds to cytokine promoters. This binding then initiates inflammatory cytokine mRNA transcription. Pro-inflammatory cytokines such as TNF- α (tumor necrosis factors), IL (interleukin)-1 β , IL-8 and IL-6 are produced and subsequently involved in the activation of naïve T helper cells (Th0) into T helper (Th)1, Th2 and T-regulatory (Treg) cells (Momoko 2005). Th1 immune response is important in eliminating intracellular pathogens while Th2 response protects hosts against parasitic and extracellular pathogen infections (Delcenserie et al. 2008).

Probiotics also promote anti-inflammatory responses during bacterial infection which helps to avoid excessive inflammation in the intestine (Allan 2008). Interaction of CpG motifs in bacterial DNA with intracellular TLR-9 in dendritic cells causes the generation of IL-4 and IL-6. These cytokines stimulate the activation of Th3 cells (Rachmilewitz et al. 2004). The activated Th3 cells secrete anti-inflammatory cytokines such as IL-10 and TGF- β to alleviate Th1-mediated pro-inflammatory responses (Moore et al. 2001). The occurrence of Crohn's disease and NEC is attributable to excessive Th1-mediated inflammation. Several studies have shown that probiotics are able to suppress inflammation in Crohn's disease and NEC. Braat et al. (2004) reported a reduction in Th1 cytokines such as TNF- α , IL-6 and IL-12 levels in Crohn's patients given *L. rhamnosus*. Besides, a mixture of *S. boulardii* and mesalamine was administered in patients suffering from Crohn's disease (Guslandi et al. 2000). This recipe successfully helped to mitigate the severity of Crohn's disease. The leading factor of NEC is mainly due to continuous and excessive inflammation caused by pathogens or commensal bacteria in the distal small intestine in premature infants (Claud et al. 2004). Lin et al. (2005) showed that feeding of *L. lactis* and *Bifidobacteria infantum* to premature infants for a few days after the birth palliated the occurrence of NEC. These results manifest

the importance of probiotics in counterbalancing host's pro- and anti-inflammatory responses especially in patients suffering from intestinal disorders.

CONCLUSION

Consumption of probiotic has been shown to provide significant health benefits in preventing and treating intestinal infections and disorders and cancers. In view of this, it is worth noting that more studies and investigations are needed to uncover further health benefits of probiotics to humans.

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REFERENCES

- Allan, W. 2008. Mechanisms of action probiotics. *Clinical Infectious Disease* 46: 87-91.
- Bibel, D.J. 1998. Elie Metchnikoff's bacillus of long life. *American Society for Microbiology News* 54: 661-665.
- Braat, H., van den Brande, J., van Tol, E., Hommes, D., Peppelenbosch, M. & van Deventer, S. 2004. *Lactobacillus rhamnosus* induces peripheral hypo-responsiveness stimulated CD4⁺ T cells via modulation of dendritic cell function. *The American Journal of Clinical Nutrition* 80: 1618-1625.
- Challa, A., Rao, D., Chawan, C. B. & Shackelford, L. 1997. *Bifidobacterium longum* and lactose suppress azoxymethane-induced colonic aberrant crypt foci in rats. *Journal of Carcinogenesis and Mutagenesis* 18: 517-521.
- Claud, E.C., Lu, L., Anton, P.M., Savidge, T., Walker, W.A. & Cherayil, B.J. 2004. Developmentally-regulated I κ B expression in intestinal epithelium and susceptibility to flagellin-induced inflammation. *Proceedings of the National Academy of Sciences of the United States of America* 101: 7404-7408.
- Cleusix, V., Lacroix, C., Vollenweider, S. & Le Blay, G. 2008. Glycerol induces reuterin production and decreases *Escherichia coli* population in an in vitro model of colonic fermentation with immobilized human feces. *FEMS Microbiology Ecology* 63: 56-64.
- Corr, S.C., Li, Y., Riedel, C.U., O'Toole, P.W., Hill, C. & Gahan, C.G. 2007. Bacteriocin production as a mechanism for the anti infective activity of *Lactobacillus salivarius* UCC118. *Proceedings of the National Academy of Sciences of the United States of America* 104: 7617-7621.
- Delcenserie, V., Martel, D., Lamoureux, M., Amiot, J., Boutin, Y. & Roy, D. 2008. Immunomodulatory effects of probiotic in the intestinal tract. *Current Issues in Molecular Biology* 10: 37-54.
- De Vrese, M. & Schrezenmeir, J. 2008. Probiotics, prebiotics, and synbiotics. *Advances in Biochemical Engineering/ Biotechnology* 111: 1-66.

- Ejtahed, S.H., Mohtadi-Nia, J., Homayouni-Rad, A., Niafar, M., Asghari-Jafarabadi, M. & Mofid, V. 2012. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutrition* 28: 539-543.
- Fuller, R. 1989. Probiotics in man and animals. *Journal of Applied Bacteriology* 66: 365-378.
- Gill, H. S. & Guarner, F. 2004. Probiotics and human health: a clinical perspective. *Postgraduate Medicine* 80: 516-526.
- Goldin, B.R., Gualtieri, L.J. & Moore, R.P. 1996. The effect of *Lactobacillus* GG on the initiation and promotion of DMH-induced intestinal tumours in the rat. *Nutrition and Cancer* 25: 197-204.
- Guslandi, M., Mezzi, G., Sorghi, M. & Testoni, P.A. 2000. *Saccharomyces boulardii* in maintenance treatment of Crohn's disease. *Digestive Diseases and Sciences* 45: 1462-1464.
- Hayes, M., Stanton, C., Fitzgerald, G.F. & Ross, R.P. 2007. Putting microbes to work: Dairy fermentation, cell factories and bioactive peptides. Part II: Bioactive peptide functions. *Biotechnology Journal* 2: 435-449.
- Hirayama, K. & Rafter, J. 2000. The role of probiotic bacteria in cancer prevention. *Microbes and Infection* 2: 681-686.
- Hooper, L.V. 2004. Bacterial contributions to mammalian gut development. *Trends in Microbiology* 12: 129-134.
- Kalliomaki, M.A. & Walker, W.A. 2005. Physiologic and pathologic interactions of bacteria with gastrointestinal epithelium. *Gastroenterology Clinical of North America* 34: 383-399.
- Kohler, H., McCormick, B.A. & Walker, W.A. 2003. Bacterial-enterocyte crosstalk: cellular mechanisms in health and disease. *Journal of Pediatric Gastroenterology and Nutrition* 36: 175-185.
- Kopp-Hoolihan, L. 2001. Prophylactic and therapeutic uses of probiotics: a review. *Journal of the American Dietetic Association* 101: 229-238.
- Lilly, D.M. & Stillwell, R.H. 1965. Probiotics: Growth promoting factors produced by micro-organisms. *American Journal of Science* 147: 747-748.
- Lin, H.C., Su, B.H., Chen, A.C., Lin, T.W., Tsai, C.H., Yeh, T.F. & Oh, W. 2000. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Journal of Pediatrics* 115: 1-4.
- Momoko, N. 2005. Classical and alternative NF-KB activation pathways and their roles in lymphoid malignancies. *Journal of Clinical and Experimental Hematopathology* 45: 15-24.
- Montalto, M., Nucera, G., Santoro, L., Curigliano, V., Vastola, M. & Covino, M. 2005. Effect of exogenous beta-galactosidase in patients with lactose malabsorption and intolerance: A crossover double-blind placebo-controlled study. *European Journal of Clinical Nutrition* 59: 489-493.
- Moore, K.W., de Waal Malefyt, R., Coffman, R.L. & O'Garra, A. 2001. Interleukin-10 and the interleukin-10 receptor. *Annual Review of Immunology* 19: 683-765.
- Ng, S.C., Hart, A. L., Kamm, M.A., Stagg, A.J. & Knight, S.C. 2009. Mechanisms of action of probiotics: Recent advances. *Inflammatory Bowel Diseases* 15: 300-310.
- Pasare, C. & Medzhitov, R. 2005. Toll like receptor: Linking innate and adaptive immunity. *Advances in Experimental Medicine and Biology* 560: 11-18.
- Pedrosa, M.C., Golner, B.B., Goldin, B.R., Barakat, S., Dallal, G.E. & Russell, R.M. 1995. Survival of yogurt-containing organisms and *Lactobacillus gasseri* (ADH) and their effect on bacterial enzyme activity in the gastrointestinal tract of healthy and hypochlorhydric elderly subjects. *The American Journal of Clinical Nutrition* 61: 353-359.
- Plummer, M., Franceschi, S. & Munoz, N. 2004. Epidemiology of gastric cancer. *International Agency for Research Center Scientific Publications* 157: 311-326.
- Rachmilewitz, D., Katakura, K., Karmeli, F., Hayashi, T., Reinus, C., Rudensky, B., Akira, S., Takeda, K., Lee, J., Takabayashi, K. & Raz, E. 2004. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. *Gastroenterology* 126: 520-528.
- Rice-Evans, C. & Burdon, R. 1993. Free radical-lipid interactions and their pathological consequences. *Progress in Lipid Research* 32: 71-110.
- Salminen, S. & Von Wright, A. 1998. Current probiotics – safety assured? *Microbial Ecology in Health and Disease* 10: 68-77.
- Sazawal, S., Hiremath, G., Dhingra, U., Malik, P., Deb, S. & Black, R. E. 2006. Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials. *The Lancet Infectious Diseases* 6: 374-382.
- Sgouros, S.N., Bergele, C. & Mantides, A. 2004. Eosinophilic esophagitis in adults: a systematic review: Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea. *Clinical Gastroenterology and Hepatology* 2: 656-664.
- Vasiljević, T. & Shah, N. P. 2012. Probiotics: From Metchnikoff to bioactives. *International Journal of Dairy Technology* 18: 714-728.
- Wang, K.Y., Li, S.N., Liu, C.S., Perng, D.S., Su, Y.C. & Wu, D.C. 2004. Effects of ingesting *Lactobacillus* and *Bifidobacterium*-containing yogurt in subjects with colonized *Helicobacter pylori*. *The American Journal of Clinical Nutrition* 80: 737-741.
- Winkelströter, L.K., Gomes, B.C., Thomas, M.R.S., Souza, V.M. & De Martinis, E.C.P. 2011. *Lactobacillus sakei* 1 and its bacteriocin influence adhesion of *Listeria monocytogenes* on stainless steel surface. *Food Control* 22: 1404-1407.

Yap Wei Boon
 Rina Anak Sujang
 Program of Biomedical Sciences
 School of Diagnostic and Applied Health Sciences
 Faculty of Health Science
 Universiti Kebangsaan Malaysia
 50300, Jalan Raja Muda Abdul Aziz
 Kuala Lumpur, MALAYSIA

Corresponding author: Yap Wei Boon
 Email address: yapweiboon@fsk.ukm.my, yapweiboon@gmail.com
 Tel: +603-92897920; Fax: +603-26929032

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