한국유가공기술과학회지
Korean J. Dairy Sci. Technol.
Vol. 32. No. 2, pp. 141~145(2014)

프로바이오틱스가 생산하는 생리활성 물질의 장내 유해균 억제 효과

임광세[1] · Mansel W. Griffiths[2] · 박동준[3] · 오세종[4*]

[1]㈜매일유업 중앙연구소, [2]Dept. of Food Science, University of Guelph, Canadian Research Institute for Food Safety
[3]한국식품연구원, [4]전남대학교 동물자원학부

Bioactive Molecules Produced by Probiotics to Control Enteric Pathogens

Kwang-Sei Lim[1], Mansel W. Griffiths[2], Dong June Park[3] and Sejong Oh[4*]

[1]R & D Ctr., Maeil Dairy Co. Ltd, Pyeongtaek 451-861, Korea
[2]Dept. of Food Science, University of Guelph, Canadian Research Institute for Food Safety, Guelph, Canada
[3]Korea Food Research Institute, Seongnam 463-746, Korea
[4]Division of Animal Science, Chonnam National University, Gwangju 500-757, Korea

Abstract

There is a burgeoning number of products on the market that contain probiotics, but do they do you any good? What exactly are probiotics? They have been defined as living organisms that, when ingested in sufficient quantities, provide health benefits beyond basic nutrition. They are often referred to as "friendly bacteria" or “good bacteria.” Probiotics have been claimed, amongst other things, to (i) reduce the incidence of colon cancer and other diseases of the colon, such as IBS, (ii) stimulate the immune system, (iii) have anti-hypertensive and anti-cholesterolemic properties, (iv) mitigate against the effect of antibiotics on the intestinal microbiota, and (v) protect against gastrointestinal infections. However, the scientific basis for many of these claims is not well-established. Indeed, the European Food Safety Authority has denied the use of several health claims associated with probiotics, particularly those related to mitigation of diarrhea following consumption of antibiotics. Thus, there is a need for research on the mechanisms of action of probiotics. We have been mainly interested in the use of probiotics to control enteric infections. There are several possible modes of action to explain how probiotics may protect the host from enteric pathogens, including competitive exclusion and immunomodulation. We have shown that probiotics produce bioactive molecules that interfere with bacterial cell-cell communication (also called quorum sensing), and this results in a down-regulation of virulence genes that are responsible for attachment of the pathogen to the gastrointestinal epithelium. These bioactive molecules act on a variety of bacteria, including enterohemorrhagic and enterotoxigenic *Escherichia coli*, *Salmonella*, *Clostridium difficile* and *Clostridium perfringens*, and there is evidence that they can inhibit the formation of biofilms by *Listeria monocytogenes*. These bioactive molecules, which are peptidic in nature, can exert their effects not only *in vitro* but also *in vivo*, and we have shown that they mitigate against *E. coli* O157:H7 and *Salmonella* in mice and *Salmonella* and *E. coli* K88 infections in pigs. They can be delivered in foods such as yoghurt and maintain their activity.

Keywords: bioactive molecules, probiotics, quorum-sensing, pathogen

* Corresponding author: Sejong Oh, Division of Animal Science, Chonnam National University, Gwangju 500-757, Korea. Tel: +82-62-530-2116, Fax: +82-62-530-2129, E-mail: soh@chonnam.ac.kr

Probiotics and LGG

Probiotics are defined as live microorganisms that confer

beneficial effects to the health of the host. The health benefits that are attributed to probiotic bacteria in the literature can be categorized as nutritional, therapeutic, and/or prophylactic. Nutritional benefits include enhancing the bioavailability of minerals, increasing the digestibility of protein, and synthesizing vitamins, for example in yogurt (Breslaw and Kleyn, 1973; McDonough *et al*., 1983; Lee *et al*., 1999). The reported therapeutic benefits of probiotics include treatment of gastrointestinal tract diseases, relief from lactose intolerance, suppression of procarcinogenic enzymes, and alleviation of food-related allergies. Further beneficial aspects include the inhibitory effects that they have on *Ehrlich ascites* tumor cells and the enhancement of immunomodulation. Probiotics have also been shown to have a prophylactic effect on diarrheal illnesses of multiple etiologies such as *Clostridium difficile*-induced enteritis, traveler's diarrhea, and antibiotic-associated diarrhea (Biller *et al*., 1995; Hilton *et al*, 1997; Vanderhoof *et al*., 1999).

Lactobacillus is the largest genus within the group of lactic acid bacteria (LAB). It contains a very large number of species, which have been isolated mainly from humans, animals, plants and foods. The genus shows a large phenotypic, biochemical and physiological variability (Sanders, 1993; Schiffrin and Blum, 2001). *Lactobacillus* species are used as starter cultures in the fermentation of food. Several health benefits are also reported for lactobacilli strains which colonize the gastrointestinal tract such as *Lactobacillus* GG. These include the stimulation of immunoglobulin production, induction of interferon expression in macrophages, acidification of the local environment, hypocholesteraemic effects, binding of mutagenic compounds, production of bacteriocins, and prevention of the adhesion of pathogenic bacteria to epithelial cells.

Lactobacillus rhamnosus GG (ATCC 53103; LGG) is one of the most commonly used probiotic strains for human beings. It is also commonly known by the name *Lactobacillus* GG (Gorbach, 1996). The strain is undisputedly classified as a strain of the *L. rhamnosus* species, although the phenotypic characterization is not completely typical for the species (Saxelin, 1997). The first commercial probiotic products with LGG, under the Gefilus brand, were launched in Finland in 1990. *Lactobacillus* GG used in various product applications, including several dairy-based products, such as yogurt, fermented milk, daily-dose mini-bottles, pasteurized (uncultured) milk, and food supplements (Saxelin *et al*., 2005).

Lactobacillus GG has a balancing effect on the intestinal ecosystem, that is, it increases the level of lactobacilli and bifidobacteria, formation of SCFAs, lowers the activity of procarcinogenic enzymes, and improves as well as snormalizes the mucosal barrier.

Clinical trials have confirmed the following efficacies of LGG: prevention and treatment of antibiotic associated and traveler's diarrhea, as well as acute nonspecific and rotavirus-induced diarrhea in children. The suggested mechanisms involve the enhancement of immune response, balancing of intestinal microbiota, and restoration of the mucosal barrier (Tynkkynen *et al*., 1999). Furthermore, LGG-enriched milk reduced the risk of respiratory infections and the development of tooth decay in children (Hawrelak *et al*., 2005).

Controlling Enteric Pathogens

A luminescent phenotype of *C. jejuni* ATCC 33291 and ATCC 35921 was constructed from a transcriptional fusion between the *C. jejuni* flaA s28 promoter and *luxCDABE* genes from *Xenorhabdus luminecens* that had been incorporated into the plasmid pRYluxCDABE. A classical s28 flagellar promoter controls the transcription of the *flaA* gene. As the concentration of cell-free extracts of milk fermented by the probiotic strains LGG, ATCC 4356, LA-5, ATCC 15707, and ATCC 15697 increased, the growth of *C. jejuni* decreased (Ding *et al*., 2005).

For an enteric pathogen to be successful, it must be able to survive, grow, and ultimately exert pathogenicity in a highly competitive environment. This requires that the pathogen identify environmental factors so that appropriate genes are expressed or repressed. Current research on the health benefits of probiotics mostly focuses on the mechanisms of anti-infection, competitive attachment and colonization, and immunomodulation.

The improvement of host-acquired immunity, especially immunocompetence, has been proven to inhibit translocation, survival, and proliferation of bacterial pathogens in extra-intestinal tissues (such as the liver and spleen) and prevent bacteremia (Fuller and Gibson, 1997). These effects have been established mainly by measuring immunological parameters and correlating these parameters with pathogen counts in visceral tissues.

It has been established that probiotics exert their immunoenhancing effects by augmenting both nonspecific (e.g., phagocyte

function, natural-killer-cell activity) and specific (e.g., antibody production, cytokine production, lymphocyte proliferation, delayed-type hypersensitivity) host-immune responses. Although recent reports have determined some immuno-enhancing mechanisms (Schiffrin *et al*., 1997; Chiang *et al*., 2000; Gill *et al*., 2001), the precise way in which probiotics act on the immune system is not fully understood. The role of probiotics in disease prevention may also involve antagonistic effects on the adhesion, colonization, growth, and translocation of pathogens, such as *Staphylococcus aureus*, *Salmonella* Typhimurium, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Clostridium perfringens*, *Escherichia coli* O157:H7, and rotavirus (Perdigon *et al*., 1990; Gilliland and Speck, 1997).

To date, there has been no direct evidence to demonstrate competitive attachment and colonization *in vivo*. However, recent studies have shown that probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression to modulate the barrier effect of the gut. A recent study has compared the inhibitory effects of several *Bifidobacterium* strains against Shiga toxin-producing *E. coli* (Jordan *et al*., 2005). It was concluded that strains that were effective in preventing infection produced a high concentration of acetic acid and lowered the pH of the intestine and that this combination of high acetic acid concentration and low pH inhibited Shiga toxin production during Shiga toxin-producing *E. coli* growth *in vitro*. Acetate levels in supernatants used in the present study were not determined; however, *L. acidophilus* is obligately homofermentative, and acetate production by this organism will be low. There is some evidence that the expression of the locus of the enterocyte effacement pathogenicity island (*LEE*) in *E. coli* O157:H7 is down-regulated by probiotics, and this may be mediated by the interference of the AI-2 autoinducer signaling system (Medellin-Pena *et al*., 2007). *L. acidophilus* and bifidobacteria also exert antagonistic effects on the growth of pathogens such as *S. aureus*, *Salmonella* Typhimurium, *Y. enterocolitica*, and *C. perfringens*.

In recent studies, our research group found that the cell-free extracts of fermented milk by probiotics, including *L. acidophilus* and bifidobacteria, also has an inhibitory effect on the growth of *C. jejuni*. This effect is genus-, species-, and strain-dependent. These results are similar to those that show that the probiotic bacteria enhances resistance against intestinal pathogens via antimicrobial mechanisms. Using a luminescent phenotype of *Salmonella* Enteritidis to monitor infection in live mice, it has been demonstrated that oral ingestion of fermented milks and cell-free preparations of fermented milk can prevent infection (Brovko *et al*., 2002). Anti-infection mechanisms of probiotics may include the production of substances directly microbiocidal for pathogens; competition with pathogens for intestinal attachment sites; and effective enhancement of host immunity against pathogen infection.

Probiotics and Signaling

Probiotics produce small biologically active molecules that are able to interfere with cell-to-cell signaling between bacteria occupying their niche. The capacity of probiotics to inhibit the attachment of certain pathogenic bacteria to intestinal epithelium and their quorum quenching strategies against EHEC could offer potential novel therapeutic approaches to combat this pathogen (Medellin-Pena and Griffiths, 2009; Kim *et al*, 2012).

EHEC O157 inhabits the large intestine, which contains large amounts of resident microbiota, making the interspecies communication system essential for the bacterium's survival and infectivity. Probiotic lactic acid bacteria (LAB) are an important part of the microbial ecosystem of the human GI tract due to their protective roles against diseases. These protective roles include the prevention and amelioration of intestinal infections. Mechanisms of interference by probiotics include direct action against pathogens through adherence competition at colonization sites, antibacterial effects, and stimulation of the epithelial cell host-acquired immune response. The mode of action and molecular basis of probiotic effects are not yet fully understood but are likely to be multifactorial and strain specific. The increase of antimicrobial resistance has motivated the interest in therapeutic approaches other than antibiotics, focusing on the capacity of probiotics to inhibit attachment of certain pathogens. Determining the molecular basis and mechanisms of action of probiotics on bacterial pathogens is a promising new approach to protecting and controlling human infectious diseases.

If QS through the LuxS signaling system allows bacteria in the GI tract to communicate, it is possible that intestinal bacteria of other genera that also use QS as a main regulatory system may influence EHEC O157 gene expression. This

regulatory system, apart from being able to control genes involved in pathogenesis (flagellation and motility), regulates genes involved in bacterial metabolism, DNA repair, cell growth, and nucleotide and protein synthesis.

국문요약

시장에는 프로바이오틱스를 포함한 무수히 많은 제품이 판매되고 있는데, 우리에게 어떤 이로운 점을 줄 수 있는지 궁금해 한 적이 있었을 것이다. 프로바이오틱스는 기본적인 영양 측면을 배제하고, 우리가 유효한 양을 섭취하였을 때 건강상의 이점을 주는 살아있는 미생물로 정의된다. 프로바이오틱스는 유용하고 건강에 좋은 미생물로도 불려지고 있으며, 다음과 같은 다섯 가지 측면에서 건강 기능성이 있는 것으로 강조되어 왔는데, 1) 대장암 및 IBS와 같은 다른 대장(결장)관련 질병의 발병율 감소, 2) 면역 시스템의 촉진, 3) 항고혈압 및 항콜레스테롤 작용, 4) 장내세균에 작용하는 항생제의 효과를 경감시키고, 5) 위장관 감염을 예방시키는 점이다. 그러나 이러한 건강기능 작용들에 대한 과학적인 근거가 충분히 구축되어 있지는 않아, 유럽식품위생위원회(Euroupe Food Safety Authority)는 항생제 투여에 따른 설사증상의 완화와 같은 건강기능 표시를 금지하고 있어, 프로바이오틱스의 작용기전에 대한 연구가 필요한 시점이라 하겠다.

Acknowledgement

This study was supported by High Value-added Food Technology Development Program funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (A2012-0447).

References

1. Allen, K. J., and Griffiths, M. W. 2001. Effect of environmental and chemotactic stimuli on the activity of the *Campylobacter jejuni flaA* s28 promoter. FEMS Microbiol. Lett. 205:43-48.
2. Alm, R. A., Guerry, P. and Trust, T. J. 1993. The *Campylobacter* d54 flagellin promoter is subject to environmental regulation. J. Bacteriol. 175:4448-4455.
3. Biller, J. A., Katz, A. J., Flores, A. F., Buie, T. M. and Gorbach, S. L. 1995. Treatment of recurrent *Clostridium difficile* colitis with *Lactobacillus* GG. J. Pediatr. Gastroenterol. Nutr. 21:224-226.
4. Brassart, D., and Schiffirin, E. 1997. The use of probiotics to reinforce mucosal defense mechanisms. Trends Syst. Bacteriol. 46:102-111.
5. Breslaw, E. S., and Kleyn, D. H. 1973. *In vitro* digestibility of protein in yoghurt at various stages of processing. J. Food Sci. 38:1016-1021.
6. Brovko, L., Vandenende, C., Chu, B., Ng, K.-Y., Brooks, A. and Griffiths, M. W. 2002. *In vivo* assessment of fermented milk diet on course of infection in mice using bioluminescent *Salmonella*. J. Food Prot. 66:2160-2163.
7. Chiang, B. L., Sheih, Y. H., Wang, L. H., Liao, C. K. and Gill, H. S. 2000. Enhancing immunity by dietary consumption of a probiotic lactic acid bacterium (*Bifidobacterium lactis* HN019): optimization and definition of cellular immune response. Eur. J. Clin. Nutr. 54:849-855.
8. Ding, W., Wang, H. and Griffiths, M. W. 2005. Probiotics Down-Regulate flaA s28 Promoter in *Campylobacter jejuni.* Journal of Food Protection 68:2295-2300.
9. Fuller, R. and Gibson, G. R. 1997. Modification of the intestinal microflora using probiotics and prebiotics. Scand. J. Gastroenterol. 222(Suppl.):28-31.
10. Gill, H. S., Rutherfurd, K. J., Cross, M. L. and Gopal, P. K. 2001. Enhancement of immunity in the elderly by dietary supplementation with the probiotic *Bifidobacterium lactis* HN019. Am. J. Clin. Nutr. 74:833-839.
11. Gilliland, S. E. and Speck, M. L. 1997. Antagonistic action of *Lactobacillus acidophilus* toward intestinal and foodborne pathogens in associative culture. J. Food Prot. 40:820-823.
12. Gorbach, S. L. 1996. The discovery of *Lactobacillus* GG. Nutr. Today 31:2S-4S.
13. Guerry, P., Alm, R. A., Power, M. E., Logan, S. M. and Trust, T. J. 1991. Role of two flagellin genes in *Campylobacter* motility. J. Bacteriol. 175:4757-4764.
14. Hatakka, K., Savilahti, E., Ponka, A., Meurman, J. H., Poussa, T., Nase, L., Saxelin, M. and Korpela, R. 2001. Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. BMJ 322:1327-1329.
15. Hawrelak, J. A., Whitten, D. L. and Myers, S. P. 2005. Is *Lactobacillus rhamnosus* GG effective in preventing the onset of antibiotic-associated diarrhea: a systematic

review. Digestion 72:51-56.

16. Hilton, E., Kolakowski, P., Singer, C. and Smith, M. 1997. Efficacy of *Lactobacillus* GG as a diarrheal preventive in travelers. J. Travel Med. 4:41-43.
17. Jordan, D. M., Sperandio, V., Kaper, J. B., Dean-Nystrom, E. A. and Moon, H. W. 2005. Colonization of gnotobiotic piglets by a *luxS* mutant strain of *Escherichia coli* O157:H7. Infect. Immun. 73:1214-1216.
18. Kim, Y., Lee, J. W., Kang, S.-G., Oh, S. and Griffiths, M. W. 2012. *Bifidobacterium* spp. influences the production of autoinducer-2 and biofilm formation by *Escherichia coli* O157:H7. Anaerobe 18:539-545.
19. Lee, Y. K., Nomoto, S., Salminen, S. L. Gorbach, Probiotics. Y., Lee, K., Nomoto, S., Salminen, S. L. Gorbach. 1999. (Eds.), Handbook of probiotics, John Wiley & Sons, Inc, New York, NY, pp. 1-4.
20. McDonough, F., Wells, P., Wong, N., Hitchins, A. and Bodewell, C. 1983. Role of vitamins and minerals in growth stimulation of rats fed with yoghurt. Fed. Proc. 42:556-558.
21. Medellin-Pena, M. J. and Griffiths, M. W. 2009. Effect of molecules secreted by *Lactobacillus acidophilus* strain La-5 on *Escherichia coli* O157:H7 colonization. Applied and Environmental Microbiology 75:1165-1172.
22. 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.
23. Nase, L., Hatakka, K., Savilahti, E., Saxelin, M., Ponka, A., Poussa, T., Korpela, R. and Meurman, J. H. 2001. Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. Caries Res. 35:412-420.
24. Perdigon, G., de Macias, M. E. N., Alvarez, S., Oliver, G. and de Ruiz Holgado, A. A. P. 1990. Prevention of gastrointestinal infection using immunobiological methods with milk fermented with *Lactobacillus casei* and *Lactobacillus acidophilus*. J. Dairy Res. 57:255-264.
25. Sanders, M. E. 1993. Summary of conclusions from a consensus panel of experts on health attributes of lactic cultures: significance to fluid milk products containing cultures. J. Dairy Sci. 76:1819-1828.
26. Saxelin, M. 1997. *Lactobacillus* GG-a human probiotic strain with thorough clinical documentation. Food Rev. Int. 13:293-313.
27. Saxelin, M., Tynkkynen, S., Mattila-Sandholm, T, de Vos W. M. 2005. Probiotic and other functional microbes: from markets to mechanisms. Curr. Opin. Biotechnol. 16:204-211.
28. Schiffrin, E. J. and Blum, S. 2001. Food processing: probiotic microorganisms for beneficial foods. Curr. Opin. Biotechnol. 12:499-502.
29. Schiffrin, E. J., Rochat, F., Link-Amster, H., Aeschlimann, J. M. and Donnet-Hughes, A. 1995. Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. J. Dairy Sci. 78:491-497.
30. Szajewska, H., Skorka, A., Ruszczynski, M. and Gieruszczak-Bialek, D. 2007. Meta-analysis: *Lactobacillus* GG for treating acute diarrhea in children. Aliment. Pharmacol. Ther. 25:871-881.
31. Tynkkynen, S., Satokari, R., Saarela, M., Mattila-Sandholm, T. and Saxelin, M. 1999. Comparison of ribotyping, randomly amplified polymorphic DNA analysis, and pulsed-field gel electrophoresis in typing of *Lactobacillus rhamnosus* and *L. casei* strains. Appl. Environ. Microbiol. 65:3908-3914.
32. Vanderhoof, J. A., Whitney, D. B., Antonson, D. L., Hanner, T. L., Lupo, J. V. and Young, R. J. 1999. *Lactobacillus* GG in the prevention of antibiotic-associated diarrhea in children. J. Pediatr. 135:564-568.

(Received 26 November, 2014 / Accepted 6 December, 2014)