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Probiotic viability – does it matter?

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Probiotics are viable by definition, and viability of probiotics is often considered to be a prerequisite for the health benefits. Indeed, the overwhelming majority of clinical studies in the field have been performed with viable probiotics. However, it has also been speculated that some of the mechanisms behind the probiotic health effects may not be dependent on the viability of the cells and, therefore, is also possible that also non-viable probiotics could have some health benefits. The efficacy of non-viable probiotics has been assessed in a limited number of studies, with varying success. While it is clear that viable probiotics are more effective than non-viable probiotics and that, in many cases, viability is indeed a prerequisite for the health benefit, there are also some cases where it appears that non-viable probiotics could also have beneficial effects on human health.

Keywords: *probiotics*; *non-viable probiotics*; *viability*; *efficacy*; *mechanisms*

Viability is an inherent property of probiotics since the current definition of probiotics, issued by the Joint FAO/WHO Working Group (1), defines that probiotics are 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host'. Therefore, by definition, viability is an essential requirement for probiotics. This does not necessarily implicate that viability is an essential requirement for the health benefits conferred by probiotics or their derivates. As indicated later, there may be situations in which the health benefits of probiotics do not necessarily depend on the viability status of the cells – despite that it is widely acknowledged that, in general, viable probiotics are more effective than non-viable probiotics and that the health effects of viable probiotics have been explored to far greater extent than the potential health effects of non-viable probiotics. Research (and reviews) on this topic are hampered by lack of satisfactory terminology. No proper term or definition exists for the non-viable forms of probiotics. Terms such as non-viable probiotics and inactivated probiotics have been used, but these terms are self-contradictory since the word 'probiotic' as such indicates viability. In this discussion, the term non-viable probiotics is used in the lack of better terminology.

Mechanisms of probiotic health effects – is viability essential?

While probiotics have been linked with different health benefits in a plethora of clinical trials with a variety of different outcomes, study populations, and probiotic ingredients, it is acknowledged that, in most cases, the exact mechanisms of the health benefits are not fully understood. Mechanistic studies have provided several plausible and possible modes of action, but in many cases, it has not been possible to identify direct and undisputed cause-effect relationships. In many cases, there are several potential mechanisms that could explain a certain clinical health benefit, and it has not been easy to exclude the other potential mechanisms in favour of a single mode of action. Perhaps, this is only natural, since a clinical health benefit may be a combined result of a number of different mechanistic effects occurring at cellular and molecular levels. The potential health efficacy of non-viable probiotics depends on whether the mechanism of the probiotic health effect itself is dependent on the viability of the cells. Given that there are multiple potential mechanisms, it is clear that this consideration should be taken case-by-case.

Adhesion to host tissues is thought to facilitate the host–microbial interactions such as the effects of microbes on the immune system of the host. Therefore, adhesion may be a key determinant for probiotic efficacy. In the gut, administered probiotics are clearly outnumbered by the resident gut microbiota. As such, this may reduce the chances of probiotics for having a major effect on the host health – however, adhesion to host mucosa may change the balance in the favour of probiotics locally and temporarily. Thus, at mucosal level, probiotics may become a major member of the local microbial population and become an important effector of host–microbial interactions. This may be particularly relevant in the small intestine, where the resident microbial numbers are

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smaller than in the colon. The effect of viability on adhesion is not fully understood and may be strain dependent (2). Some reports suggest that viable and non-viable lactobacilli are equally adherent to intestinal mucus (3). The adherence may be dependent on the way by which the cells have been killed; one study suggested that heat-killing and protease treatments were detrimental to the ability of probiotics to adhere to human mucus, but other means of cell killing had no effect (4). An *in vivo* mouse study suggested that heat-killing of lactobacilli affects the localization of the cells in the intestine; viable bacteria were reported to be located in the Peyer's patches and lamina propria shortly after administration to mice, whereas most heat-killed bacteria were located in the lumen and were rapidly cleared (5). While adhesion to host tissues may be equally efficient between viable and non-viable bacteria, prolonged colonization in the mucosa obviously requires formation of a viable colony. Production of antimicrobial compounds is one potential mechanism of probiotic action against pathogens and clearly a property of viable bacteria only. However, in addition to *in situ* production in the intestine, antibacterial compounds may also be produced during manufacturing process and then used as bacterial lysates or extracted ingredients. It has also been suggested that heat-killed lactobacilli may inhibit pathogen adhesion to host tissues by competitive exclusion (6).

Reduction of gut permeability is another potential mechanism of probiotic action, which has been reported for several viable probiotics, although mainly in cell cultures or in animal models. The molecular mechanisms by which the integrity of the epithelial layer is improved are not fully understood. It is known that production of short chain fatty acids such as acetic acid improves the epithelial integrity locally. Clearly, *in situ* production of short chain fatty acids is a property of a viable cell only. While research assessing the efficacy of non-viable probiotics is minimal, some studies have suggested that inactivated lactobacilli (7) and cell-free supernatants of probiotics (8) may improve epithelial integrity.

Interactions between probiotics and host immune system have been investigated in numerous studies with viable probiotics, but in many cell culture studies, non-viable probiotics have also been used. Probiotic cell components associated with *in vitro* immunomodulatory properties include cell wall extracts (9), lipoteichoic acids (10), bacterial DNA (11, 12), and S-layer proteins (13). Some clinical studies have also suggested that non-viable probiotics can modulate human immune system, e.g. by enhancing salivary IgA production (14) and by modulating host T-cell responses (15) and gene expression (16). Limited number of *in vitro* and animal studies have directly compared the effects of viable and inactivated probiotics on innate immunity, and in many cases, these have been found to be equally effective (17–19). A study by Gill and Rutherfurd (20) suggested that viable and killed cells of *Bifidobacterium lactis* HN019 were able to enhance cell phagocytic responses in mice peripheral blood cells, but only viable cells increased the phagocytic activity of peritoneal cells. In some studies, viable probiotics have proved to be more effective than non-viable probiotics (21–23). In the case of adaptive immunity, most studies comparing the two have favoured viable probiotics (5, 20, 24–26). However, one study suggested that both viable and killed *Lactobacillus* cells are able modulate the phenotype and functions of human myeloid dendritic cells (27).

In conclusion, many potential mechanisms of probiotic action are clearly dependent on cell viability and activity, but there is preclinical evidence suggesting that some mechanisms associated with probiotics may not be directly dependent on cell viability. These include adhesion to host tissues and modulation of innate immune responses. However, *in vivo* situation may be different and viability may be an indirect determinant of the health effect, since viable probiotics may be more likely to reach the site of action in the first place and remain at the site long enough to confer a health benefit.

Clinical benefits of probiotics – is viability essential?

Probiotic microbes have been linked with a range of beneficial effects on host health. By far, most of the health efficacy documentation has been generated using viable probiotics, and there are too few data to make firm conclusions on the clinical efficacy of non-viable probiotics. Nevertheless, some studies have been carried out using different non-viable probiotics.

Gut health is the most important target for probiotics. Prevention and treatment of different forms of diarrhoea is one of the most successful and best documented health benefits of viable probiotics, but efficacy studies with non-viable probiotics are rare. One study suggested that a treatment with heat-killed *Lactobacillus acidophilus* LB was effective, even more so than a treatment with viable non-specified strain of *L. acidophilus* (28). One study compared viable or heat-killed *Lactobacillus rhamnosus* GG and found no difference in their effect on diarrhoea duration, but the study lacked a proper placebo group (29). Ouwehand and Salminen (30) have earlier concluded that both viable and non-viable probiotics may be useful for short-term treatment or prophylactic treatment of diarrhoea, but viable probiotics are necessary for an enhanced immunological response. Irritable bowel syndrome is a popular target for probiotic research, but to date, the research has focused almost exclusively on viable probiotics. However, in one clinical study, heat-inactivated cells were used as controls for viable cells (31). The administration of the viable product resulted in subjective improvement of the symptoms in 80% of the

patients, compared to 40% in the control group, suggesting that viable probiotics may be more efficient in the treatment of irritable bowel syndrome. While no clinical data suggest that probiotics alone would be efficient in eradicating *Helicobacter pylori*, both viable and non-viable probiotics have been reported to increase the eradication rates of a standard anti-*H. pylori* regimen (32, 33). Some studies have concluded that both viable and non-viable probiotics are equally effective in the treatment of *H. pylori* infections (34), which others have highlighted the importance of viability (35, 36).

Improvement of lactose digestion by probiotics deserves special attention in the context of viability. Most studies comparing the efficacy of live and dead lactobacilli in improving lactose digestion have been performed using yoghurt starter cultures, not probiotics. Most of the clinical studies comparing live and pasteurized yoghurt suggest that viable cells are more effective in improving lactose digestion (37, 38). However, cell viability as such may not be the critical factor for the efficacy. In one study, it was concluded that, to improve lactose digestion, the bacteria need not to be alive, but intact cell walls are required to protect the active β-galactosidase during gastrointestinal passage; the efficacy of pasteurized bacteria was low, but the effect of bacteria killed with gamma irradiation was similar to the effect of viable bacteria (39).

Prevention and treatment of allergic disease has been a popular target of probiotic research, and some studies have also included non-viable probiotics. In one small trial comparing viable and heat-inactivated *L. rhamnosus* GG in the management of infant atopic eczema and cow's milk allergy, the latter were associated with increased gastrointestinal symptoms (40). Moreover, one study reported fewer subjective allergy symptoms in adults consuming yoghurt containing viable bacteria compared to subjects consuming heat-inactivated yoghurt (41). On the other hand, certain reports have suggested that both viable and non-viable probiotics may be useful in the treatment of allergic rhinitis (42, 43). It is possible that probiotic viability is more important in the management of eczema compared to the management of allergic rhinitis.

Efficacy of probiotics in prevention and supportive treatment of cancer is challenging and far from elucidated. Nevertheless, some early reports have suggested that heat-killed *Lactobacillus casei* Shirota could be useful in the treatment of carcinoma of the uterine cervix (44, 45) and secondary to lung cancer (46). In one preclinical study, viable *L. casei* was found to be more effective than heat-killed *L. casei* in the prevention of secondary tumours in preimmunized mice (47). On the other hand, heat-killed lactic acid bacteria are more effective than viable bacteria in the binding of aflatoxin, a potent dietary carcinogen (48).

Conclusions

Viability is an inherent property of probiotics, since the current definition of probiotics includes a requirement of viability. The definition of probiotics also includes a requirement of a health benefit. Probiotic viability has traditionally been thought to be a prerequisite for a health benefit. Indeed, in most cases, viable probiotics have proven to be more effective than inactivated probiotic products. Most importantly, the overwhelming majority of the clinical health efficacy research has been carried out with viable probiotics. Nevertheless, depending on the mechanism of action, there may be situations in which the health effects of probiotics are not dependent on the viability status of the cells, and there are some clinical reports suggesting efficacy of products containing inactivated probiotics. The research focussing on the importance of viability of probiotics is further complicated because – in a manner similar to other microbes – the viability of probiotics is not a simple on/off situation. For example, during storage in fermented probiotic products, part of the microbial population may become 'dormant', while other parts of the population may be already dead or still fully active and viable (49). The relevancy of these different subpopulations on the health efficacy of probiotics is unknown. There may also be a need to redefine the concept of viable in this context as several of the gut bacteria are viable but not culturable.

Conflict of interest and funding

The author is employed by DuPont Nutrition and Health, a manufacturer of probiotics.

References

1. Joint FAO/WHO Working Group Report on Drafting for the Evaluation of Probiotics in Food. Guidelines for the evaluation of probiotics in food. London, Ontario, Canada: FAO/WHO; 2002.
2. Ouwehand AC, Tölkkö S, Kulmala J, Salminen S, Salminen E. Adhesion of inactivated probiotic strains to intestinal mucus. Lett Appl Microbiol 2000; 31: 82–6.
3. Hood SK, Zottola EA. Effect of low pH on the ability of *Lactobacillus acidophilus* to survive and adhere to human intestinal cells. J Food Sci 1988; 53: 1514–6.
4. Tuomola EM, Ouwehand AC, Salminen SJ. Chemical, physical and enzymatic pre-treatments of probiotic lactobacilli alter their adhesion to human intestinal mucus glycoproteins. Int J Food Microbiol 2000; 60: 75–81.
5. Maldonado Galdeano C, Perdigon G. Role of viability of probiotic strains in their persistence in the gut and in mucosal immune stimulation. J Appl Microbiol 2004; 97: 673–81.
6. Chauviere G, Coconnier MH, Kerneis S, Darfeuille-Michaud A, Joly B, Servin AL. Competitive exclusion of diarrheagenic *Escherichia coli* (ETEC) from human enterocyte-like Caco-2 cells by heat-killed *Lactobacillus*. FEMS Microbiol Lett 1992; 70: 213–7.
7. Montalto M, Maggiano N, Ricci R, Curigliano V, Santoro L, Di Nicuolo F, et al. *Lactobacillus acidophilus* protects tight

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junctions from aspirin damage in HT-29 cells. Digestion 2004; 69: 225–8.
8. Yan F, Polk DB. Probiotic bacterium prevents cytokine-induced apoptosis in intestinal epithelial cells. J Biol Chem 2002; 277: 50959–65.
9. Solis Pereyra B, Lemonnier D. Induction of human cytokines by bacteria used in dairy foods. Nutr Res 1993; 13: 1127–40.
10. Matsuguchi T, Takagi A, Matsuzaki T, Nagaoka M, Ishikawa K, Yokokura T, et al. Lipoteichoic acids from *Lactobacillus* strains elicit strong tumor necrosis factor alpha-inducing activities in macrophages through Toll-like receptor 2. Clin Diagn Lab Immunol 2003; 10: 259–66.
11. Rachmilewitz D, Karmeli F, Takabayashi K, Hayashi T, Leider-Trejo L, Lee J, et al. Immunostimulatory DNA ameliorates experimental and spontaneous murine colitis. Gastroenterology 2002; 122: 1428–41.
12. Takahashi N, Kitazawa H, Iwabuchi N, Xiao JZ, Miyaji K, Iwatsuki K, et al. Immunostimulatory oligodeoxynucleotide from *Bifidobacterium longum* suppresses Th2 immune responses in a murine model. Clin Exp Immunol 2006; 145: 130–8.
13. Konstantinov SR, Smidt H, de Vos WM, Bruijns SC, Singh SK, Valence F, et al. S layer protein A of *Lactobacillus acidophilus* NCFM regulates immature dendritic cell and T cell functions. Proc Natl Acad Sci U S A 2008; 105: 19474–9.
14. Kotani Y, Shinkai S, Okamatsu H, Toba M, Ogawa K, Yoshida H, et al. Oral intake of *Lactobacillus pentosus* strain b240 accelerates salivary immunoglobulin A secretion in the elderly: a randomized, placebo-controlled, double-blind trial. Immun Ageing 2010; 7: 11.
15. Hirose Y, Murosaki S, Yamamoto Y, Yoshikai Y, Tsuru T. Daily intake of heat-killed *Lactobacillus plantarum* L-137 augments acquired immunity in healthy adults. J Nutr 2006; 136: 3069–73.
16. van Baarlen P, Troost FJ, van Hemert S, van der MC, de Vos WM, de Groot PJ, et al. Differential NF-kappaB pathways induction by *Lactobacillus plantarum* in the duodenum of healthy humans correlating with immune tolerance. Proc Natl Acad Sci U S A 2009; 106: 2371–6.
17. Haller D, Blum S, Bode C, Hammes WP, Schiffrin EJ. Activation of human peripheral blood mononuclear cells by nonpathogenic bacteria in vitro: evidence of NK cells as primary targets. Infect Immun 2000; 68: 752–9.
18. Korhonen R, Korpela R, Saxelin M, Maki M, Kankaanranta H, Moilanen E. Induction of nitric oxide synthesis by probiotic *Lactobacillus rhamnosus* GG in J774 macrophages and human T84 intestinal epithelial cells. Inflammation 2001; 25: 223–32.
19. Perdigón G, de Macias ME, Alvarez S, Oliver G, de Ruiz Holgado AA. Effect of perorally administered lactobacilli on macrophage activation in mice. Infect Immun 1986; 53: 404–10.
20. Gill HS, Rutherfurd KJ. Viability and dose-response studies on the effects of the immunoenhancing lactic acid bacterium *Lactobacillus rhamnosus* in mice. Br J Nutr 2001; 86: 285–9.
21. Miettinen M, Vuopio-Varkila J, Varkila K. Production of human tumor necrosis factor alpha, interleukin-6, and interleukin-10 is induced by lactic acid bacteria. Infect Immun 1996; 64: 5403–5.
22. Lee Y, Lee TS. Enhancement in ex vivo phagocytic capacity of peritoneal leukocytes in mice by oral delivery of various lactic-acid-producing bacteria. Curr Microbiol 2005; 50: 24–7.
23. Ma D, Forsythe P, Bienenstock J. Live *Lactobacillus reuteri* is essential for the inhibitory effect on tumor necrosis factor alpha-induced interleukin-8 expression. Infect Immun 2004; 72: 5308–14.
24. De Simone C, Vesely R, Negri R, Bianchi Salvadori B, Zanzoglu S, Cilli A, et al. Enhancement of immune response of murine Peyer's patches by a diet supplemented with yogurt. Immunopharmacol Immunotoxicol 1987; 9: 87–100.
25. Perdigon G, Alvarez S, Rachid M, Aguero G, Gobbato N. Immune system stimulation by probiotics. J Dairy Sci 1995; 78: 1597–606.
26. Ibnou-Zekri N, Blum S, Schiffrin EJ, von der Weid T. Divergent patterns of colonization and immune response elicited from two intestinal *Lactobacillus* strains that display similar properties in vitro. Infect Immun 2003; 71: 428–36.
27. Mohamadzadeh M, Olson S, Kalina WV, Ruthel G, Demmin GL, Warfield KL, et al. Lactobacilli activate human dendritic cells that skew T cells toward T helper 1 polarization. Proc Natl Acad Sci U S A 2005; 102: 2880–5.
28. Xiao SD, Zhang de Z, Lu H, Jiang SH, Liu HY, Wang GS, et al. Multicenter, randomized, controlled trial of heat-killed *Lactobacillus acidophilus* LB in patients with chronic diarrhea. Adv Ther 2003; 20: 253–60.
29. Kaila M, Isolauri E, Saxelin M, Arvilommi H, Vesikari T. Viable versus inactivated *Lactobacillus* strain GG in acute rotavirus diarrhoea. Arch Dis Child 1995; 72: 51–3.
30. Ouwehand AC, Salminen S. The health effects of cultured milk products with viable and non-viable bacteria. Int Dairy J 1998; 8: 749–58.
31. Tsuchiya J, Barreto R, Okura R, Kawakita S, Fesce E, Marotta F. Single-blind follow-up study on the effectiveness of a symbiotic preparation in irritable bowel syndrome. Chin J Dig Dis 2004; 5: 169–74.
32. Zou J, Dong J, Yu X. Meta-analysis: *Lactobacillus* containing quadruple therapy versus standard triple first-line therapy for *Helicobacter pylori* eradication. Helicobacter 2009; 14: 97–107.
33. Canducci F, Armuzzi A, Cremonini F, Cammarota G, Bartolozzi F, Pola P, et al. A lyophilized and inactivated culture of *Lactobacillus acidophilus* increases *Helicobacter pylori* eradication rates. Aliment Pharmacol Ther 2000; 14: 1625–9.
34. de Vrese M, Schrezenmeir J. Probiotics and non-intestinal infectious conditions. Br J Nutr 2002; 88: S59–66.
35. Cats A, Kuipers EJ, Bosschaert MA, Pot RG, Vandenbroucke-Grauls CM, Kusters JG. Effect of frequent consumption of a *Lactobacillus casei*-containing milk drink in *Helicobacter pylori*-colonized subjects. Aliment Pharmacol Ther 2003; 17: 429–35.
36. Cruchet S, Obregon MC, Salazar G, Diaz E, Gotteland M. Effect of the ingestion of a dietary product containing *Lactobacillus johnsonii* La1 on *Helicobacter pylori* colonization in children. Nutrition 2003; 19: 716–21.
37. Lerebours E, N'Djitoyap Ndam C, Lavoine A, Hellot MF, Antoine JM, Colin R. Yogurt and fermented-then-pasteurized milk: effects of short-term and long-term ingestion on lactose absorption and mucosal lactase activity in lactase-deficient subjects. Am J Clin Nutr 1989; 49: 823–7.
38. Pelletier X, Laure-Boussuge S, Donazzolo Y. Hydrogen excretion upon ingestion of dairy products in lactose-intolerant male subjects: importance of the live flora. Eur J Clin Nutr 2001; 55: 509–12.
39. de Vrese M, Stegelmann A, Richter B, Fenselau S, Laue C, Schrezenmeir J. Probiotics – compensation for lactase insufficiency. Am J Clin Nutr 2001; 73: 421S–9S.
40. Kirjavainen PV, Salminen SJ, Isolauri E. Probiotic bacteria in the management of atopic disease: underscoring the importance of viability. J Pediatr Gastroenterol Nutr 2003; 36: 223–7.
41. Van de Water J, Keen CL, Gershwin ME. The influence of chronic yogurt consumption on immunity. J Nutr 1999; 129: 1492S–5S.
42. Peng GC, Hsu CH. The efficacy and safety of heat-killed *Lactobacillus paracasei* for treatment of perennial allergic rhinitis induced by house-dust mite. Pediatr Allergy Immunol 2005; 16: 433–8.

43. Ishida Y, Nakamura F, Kanzato H, Sawada D, Yamamoto N, Kagata H, et al. Effect of milk fermented with *Lactobacillus acidophilus* strain L-92 on symptoms of Japanese cedar pollen allergy: a randomized placebo-controlled trial. Biosci Biotechnol Biochem 2005; 69: 1652–60.
44. Okawa T, Kita M, Arai T, Iida K, Dokiya T, Takegawa Y, et al. Phase II randomized clinical trial of LC9018 concurrently used with radiation in the treatment of carcinoma of the uterine cervix. Its effect on tumor reduction and histology. Cancer 1989; 64: 1769–76.
45. Okawa T, Niibe H, Arai T, Sekiba K, Noda K, Takeuchi S, et al. Effect of LC9018 combined with radiation therapy on carcinoma of the uterine cervix. A phase III, multicenter, randomized, controlled study. Cancer 1993; 72: 1949–54.
46. Masuno T, Kishimoto S, Ogura T, Honma T, Niitani H, Fukuoka M, et al. A comparative trial of LC9018 plus doxorubicin and doxorubicin alone for the treatment of malignant pleural effusion secondary to lung cancer. Cancer 1991; 68: 1495–500.
47. Kato I, Endo K, Yokokura T. Effects of oral administration of *Lactobacillus casei* on antitumor responses induced by tumor resection in mice. Int J Immunopharmacol 1994; 16: 29–36.
48. El-Nezami H, Kankaanpää P, Salminen S, Ahokas J. Physicochemical alterations enhance the ability of dairy strains of lactic acid bacteria to remove aflatoxin from contaminated media. J Food Prot 1998; 61: 466–8.
49. Lahtinen SJ, Gueimonde M, Ouwehand AC, Reinikainen JP, Salminen SJ. Probiotic bacteria may become dormant during storage. Appl Environ Microbiol 2005; 71: 1662–3.

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