Safety of probiotic bacteria

DC Donohue, S Salminen

Key Centre for Applied and Nutritional Toxicology, RMIT, Melbourne, Australia

In recent years interest has been renewed in health promotion and disease prevention by the incorporation of probiotic bacteria into foods to counteract harmful bacteria in the intestinal tract. There is considerable interest in extending the range of foods containing probiotic organisms from dairy foods to infant formulae, baby foods, fruit juice-based products, cereal-based products and pharmaceuticals. New and more specific strains of probiotic bacteria are being sought. Traditional probiotic dairy strains of lactic acid bacteria have a long history of safe use and most strains are considered commensal microorganisms with no pathogenic potential. It cannot be assumed that these novel probiotic organisms share the historical safety of the traditional strains. Before their incorporation into products new strains should be carefully assessed and tested for the safety and efficacy of their proposed use. As yet, no general guidelines exist for the safety testing of probiotics. Different aspects of the safety of probiotic bacteria can be assessed using a panel of *in vitro* methods, animal models and human subjects.

Introduction

Probiotic bacteria are commonly defined as viable bacteria, in single or mixed culture, that have a beneficial effect on the health of the host.

In the dairy industry the most widely used probiotic bacteria belong to the group of lactic acid bacteria, though some bifidobacteria and yeasts are also utilised. The term 'lactic acid bacteria' (LAB) currently includes the genera *Lactobacillus*, *Leuconostoc, Pediococcus* and *Lactococcus*. Although some strains of *Streptococcus* and *Enterococcus* share the properties of LAB, *Streptococcus thermophilus* is the only strain currently used in fermented dairy products.

The use of LAB in foods has a long history and most strains are considered commensal microorganisms with no pathogenic potential. Their ubiquitous presence in intestinal epithelium and the human gastrointestinal tract, and their traditional use in fermented foods and dairy products without significant problems attest to their safety. Members of the genus *Lactobacillus* are most commonly given safe or generally recognised as safe (GRAS) status, whilst members of the genera *Streptococcus* and *Enterococcus* contain many opportunistic pathogens (Table 1).

The safety of probiotics has been questioned in recent reviews and clinical reports which have drawn attention to cases of human bacteraemia associated with the presence of LAB[1-3].

A variety of strains of probiotic organisms have been used in the clinical treatment of gastrointestinal disorders in both children and adults. These include conditions where mucosal integrity is impaired by antibiotics or radiotherapy, acute diarrhoea of bacterial or viral origin, and in prevention of gut colonisation by pathogens[4]. No evidence of opportunistic infection by probiotics was seen in these studies.

Recent analyses by Saxelin *et al*[5,6] of clinical isolates of lactobacilli from bacteraemic patients and comparison with both starter strains and strains used in pharmaceutical preparations has confirmed that these LAB are not involved in human infections.

Table 1. Classification of probiotic organisms and their safety status

Organism	Infection potential
Lactobacillus	Mainly non-pathogens, some opportunistic infections (usually in immunocompromised patients),
Lactococcus	Mainly non-pathogens
Leuconostoc	Mainly non-pathogens, some isolated cases of infection
Streptococcus	Oral streptococci mainly non-pathogens (including *Streptococcus thermophilus*); some may cause opportunistic infections
Enterococcus	Some strains are opportunistic pathogens with haemolytic activity and antibiotic resistance
Bifidobacterium	Mainly non-pathogens, some isolated cases of human infection
Saccharomyces	Mainly non-pathogens, some isolated cases of human infection

In addition to these clinical studies and animal studies showing an absence of infectivity, toxicity studies have also been carried out[7-9] confirming the absence of acute toxicity of the studied strains of probiotic bacteria. Although acute toxicity tests were originally designed for chemicals they also give an indication of any harmful effects associated with extremely high doses of freeze-dried bacteria.

Correspondence address: Diana Donohue, Key Centre for Applied and Nutritional Toxicology, RMIT, GPO Box 2476V, Melbourne, Vic 3001, Australia
Tel: +61-3-9660-2650 Fax: +61-3-9663-6087

Studies on safety of probiotic bacteria

Different aspects of the safety of probiotic bacteria can be studied using *in vitro* methods, animal models and human subjects. As yet no general guidelines exist for the safety testing of probiotics. However, some recommendations are given in the review of Donohue *et al*[7] Many countries, including the European Community, are currently developing more detailed guidelines with respect to regulations for novel and functional foods and related probiotic preparations.

***In vitro* studies**

One of the most important requirements for a probiotic organism is that it be non-invasive.

In vitro studies are an initial means of assessing whether a test organism alters the integrity of the intestinal mucosa and its ability to penetrate the intestinal cells. The local effects of LAB on the intestine are commonly measured by their *in vitro* ability to adhere to human intestinal cell lines and to degrade protective intestinal mucus. These tests provide an indirect measure of the potential of LAB to invade intestinal cells and to damage the protective glycoproteins of the intestinal mucus.

A large number of adhesion studies have been conducted with different strains of LAB using Caco-2 cells as the most common cell line. Most strains of LAB have shown no invasive properties in this test system, even though the selection of new probiotics has favoured those strains that are strongly adherent to human intestinal cell lines[10,11].

Degradation of intestinal mucus has also been used as a marker of toxicity. It is thought a stable gastrointestinal microflora with normal patterns of fermentation and colonisation resistance and low pH are important in protecting the mucosal layer from injury[12]. Strains that do not degrade intestinal mucus or its glycoproteins are thought to be non-invasive. Strains which do not degrade intestinal mucosa are also thought to be therapeutic in the probiotic treatment of mucosal diseases such as pouchitis, ulcerative colitis and Crohn's disease[12]. In a recent study, commercial probiotic strains (*Lactobacillus* GG, *Lactobacillus acidophilus, Bifidobacterium bifidum*) were shown to be inactive in mucosal degradation[12]. In earlier studies, some faecal Bifidobacteria were found to take part in mucus degradation[13,14].

Production of antimicrobial compounds and inhibition of pathogen growth by LAB has been assessed *in vitro*. The competitive exclusion of pathogens altering the balance of the intestinal microflora has been studied in the Caco-2 cell line. Data from these tests support the safety of LAB and indicate that many strains decrease intestinal pH and reduce the numbers of pathogenic bacteria in the intestinal tract, thus protecting the host[10,11]. It has been shown by Australian researchers that LAB strains isolated from cases of infective endocarditis have some properties in common. These properties include platelet aggregation, binding of fibronectin and fibrinogen and the production of glycosidases and proteases which are postulated as factors in the pathogenesis of endocarditis[15,16]. However, comparative studies are needed to determine whether these are also properties of the strains of LAB normally found in the oral cavity and the intestinal tract of healthy humans.

Animal studies

Acute toxicity studies have been conducted with several strains of LAB and for reference they have also included *Bifidobacterium longum* strains. In general, no acute toxicity has been observed with any of the tested strains as indicated in Table 2.

Table 2. Acute toxicity of probiotic bacteria (Adapted from 7)

Probiotic strain	LD_{50} (g/kg body weight)
Streptococcus faecium AD1050[a]	>6.6
Streptococcus equinus[a]	>6.39
Lactobacillus fermentum AD002[a]	>6.62
Lactobacillus salivarius AD0001[a]	>6.47
Lactobacillus GG (ATCC 53103)	>6.00
Lactobacillus helveticus	>6.00
Lactobacillus bulgaricus	>6.00
Bifidobacterium longum	25

a: Heat-treated nonviable preparations

Recently, the association of LAB in germ free rodents has also been used as a criterion for safety. Ruseler-van Embden *et al*[12] studied the association or colonisation of germ free rodents with several probiotic lactobacilli and detected no adverse effects in these animals.

Studies by Goldin and Gorbach[17] of the promotion and induction of colon cancer in laboratory animals have indicated that adherent lactobacilli appear to delay and slow down the development of dimethyl hydrazine (DMH)-induced colon tumours. The strains tested were *Lactobacillus acidophilus* and *Lactobacillus casei.*

Clinical Studies

A large amount of data from clinical trials or studies in human volunteers also attest to the safety of LAB. These studies have included short-term trials in normal volunteers; prevention and treatment of acute diarrhoea in premature infants[18], infants[19,20], children with diarrhoea[20-22], studies on immune effects[23] and studies in patients with severe intestinal infections[24-26]. A study using *Lactobacillus acidophilus* preparations in the effective prevention of intestinal side-effects during pelvic radiotherapy has also been reported[27,28]. Aso *et al*[29] reported that the recurrence-free interval after resection of superficial bladder cancer in humans was extended by treatment with *Lactobacillus casei* Shirota strain. A summary from the literature of safety studies and reported effects for probiotic and yoghurt strains is shown in Table 3.

All available data indicate that no harmful effects have been observed in controlled clinical studies with lactobacilli and bifidobacteria. To the contrary, during treatment of intestinal infections beneficial effects have been observed including stabilization of gut mucosal barrier, prevention of diarrhoea and amelioration of infant and antibiotic-associated diarrhoea.

Table 3. Safety studies and reported effects of current successful probiotic strains and yoghurt strains.

Probiotic strain	Reported effects	Safety studies		
		In vitro	Animal studies	Human studies
Lactobacillus acidophilus NCFB 1748	Treatment of constipation, alleviation of radiotherapy related diarrhoea, lowering of faecal enzymes (4,6,12,27,28,31)	+	+	+
Lactobacillus casei Shirota	Balancing intestinal microflora, prevention of intestinal disturbances, treatment of superficial bladder cancer (4,30,31)	+	+	+
Lactobacillus GG (ATCC 53103)	Treatment of acute viral and bacterial diarrhoea in infants, prevention of antibiotic associated diarrhoea, immune enhancing, stabilisation of intestinal permeability (6,7,10,11,18,20,21,24,26)	+	+	+
Lactobacillus acidophilus LA1	Immune enhancing, vaccine adjuvant, balancing intestinal microflora (4,10,23)	+	+	+
Bifidobacterium bifidum	Prevention of rotavirus diarrhoea (4,12,22,31)	+	+	+

Epidemiological Data

Case reports from the literature of LAB in association with clinical infection in humans have recently been analysed in reviews by Gasser[2] and Aguirre and Collins[1]. Both reviews conclude that, considering their wide-spread consumption, LAB appear to have very low pathogenic potential. Two recent Finnish studies confirm that the number of infections associated with LAB is small. In the first study, genetic methods (16 SRNA) were used to characterise and identify LAB isolated from blood cultures of bacteraemic patients in Southern Finland[5]. The results showed that a newly introduced probiotic strain in fermented milks was not associated with infections and the total number of infections caused by lactobacilli was extremely low. In a further study, lactobacilli isolated from bacteraemic patients between 1989 and 1994 were compared to common dairy or pharmaceutical strains[6]. From a total of 5192 blood cultures 12 were positive for lactobacilli, an incidence of 0.23 per cent. None of the clinical cases could be related to lactobacilli strains used by the dairy industry. In both studies, patients with LAB bacteraemia had other severe underlying illnesses.

Safety of novel probiotics

Traditional probiotic dairy strains of LAB have a long history of safe use. In latter years interest has been renewed in preventing disease and promoting health by using probiotic bacteria to fight harmful bacteria in the intestinal tract[31]. There is considerable interest in extending the range of foods incorporating probiotic organisms from dairy foods to infant formulae, baby foods, fruit juice-based products, cereal-based products and pharmaceuticals[4]. New and more specific strains of probiotic bacteria are being sought. It cannot be assumed that these novel probiotic organisms share the historical safety of traditional strains. Before their incorporation into products new strains should be carefully assessed and tested for the safety and efficacy of their proposed use. The following suggestions and recommendations have been proposed as suitable models and methods to test the safety of probiotic bacteria[3,7].

1. Determine the intrinsic properties of bacteria and strains selected for probiotic use eg adhesion factors, antibiotic resistance, plasmid transfer, enzyme profile.
2. Assess the effects of the metabolic products of the bacteria.
3. Assess the acute and subacute toxicity of ingestion of extremely large amounts of the bacteria.
4. Estimate the *in vitro* infective properties of probiotic bacteria using cell lines and human intestinal mucus degradation. Assess infectivity in animal models eg immunocompromised animals or lethally irradiated animals.
5. Determine the efficacy of ingested probiotic bacteria as measured by dose-response (minimum and maximum dose required, consequent health effects); assess the effect of massive probiotic doses on the composition of human intestinal microflora.
6. Carefully assess side-effects during human volunteer studies and clinical studies in various disease-specific states.
7. Epidemiological surveillance of people ingesting large amounts of newly introduced probiotic bacteria for infections.
8. The most rigorous safety testing along the above lines to be undertaken for genetically modified strains and strains derived from animals.

Safety of probiotic bacteria

Donohue DC, Salminen S

Asia Pacific Journal of Clinical Nutrition (1996) Volume 5, Number 1: 25-28

原生菌（Probiotic bacteria）的安全性

摘要

最近幾年的興趣又重新回到將原生菌摻合進食物以對抗腸道中有害的細菌從而促進健康和預防疾病上。相當大的興趣集中在擴大含有原生菌（Probiotic）的食物範圍，從乳制品到嬰兒奶粉、兒童食品，以果汁、谷類為基礎的產品和葯品。新的和特異性更強的原生菌（Probiotic）菌株正在探索發展中。傳統原生菌（Probiotic）乳制品乳酸菌株在安全應用已有很長的歷史，大多數菌株被認為是沒有致病可能性的共生微生物體，但不能假定這些新的原生菌能分享傳統菌株歷史上的安全性。在他們摻合進產品前，新菌株的安全性以及計劃使用的功效必須經歷嚴格評估和試驗，至今未有原生菌安全性試驗的一般準則。原生菌在不同方面的安全性可通過體外方法，動物模型和人體實驗者幾方面來評估。

References

1. Aguirre M, Collins K. Lactic acid bacteria and human clinical infection. J Appl Bacteriol 1993; 75: 95-107.
2. Gasser F. Safety of lactic acid bacteria and their occurrence in human clinical infections. Bull Inst Pasteur 1994; 92: 45-67.
3. Adams MR, Marteau P. On the safety of lactic acid bacteria from food. Int J Food Microbiol. 1995; 27: 263-264.
4. Lee Y-K, Salminen S. The coming of age of probiotics. TIFST 1995; 6: 241-245.
5. Saxelin M, Rautelin H, Chassy B, Gorbach SL, Salminen S, Mäkelä P. Lactobacilli and septic infections in Southern Finland during 1989-1992. Clin Infect Dis 1996 (in press).
6. Saxelin M, Rautelin H, Salminen S, Mäkelä P. The safety of commercial products with viable *Lactobacillus* strains. Infectious Diseases in Clinical Practice 1996 (in press).
7. Donohue DC, Deighton M, Ahokas JT, Salminen, S. Toxicity of lactic acid bacteria. In: Salminen S, von Wright A, eds. Lactic acid bacteria. Marcel Dekker Inc, New York 1993: 307-313.
8. Ishikara K, Miyakawa H, Hasegawa A, Takazoe I, Kawai Y. Growth inhibition of *Streptococcus mutans* by cellular extracts of human intestinal lactic acid bacteria. Infect Immun 1985; 3: 692-694.
9. Momose H, Igarashi M, Era T, Fukuda Y, Yamada M, Ogasa K. Toxicological studies on *Bifidobacterium longum* BB-536. Pharmacometrics 1979; 17: 881-887.
10. Coconnier MH, Klaenhammer TR, Kerneis S, Bernet MF, Servin A. Protein mediated adhesion of *Lactobacillus acidophilus* BG2F04 on human enterocyte and mucus secreting cell lines in culture. Appl Environ Microbiol 1992; 58: 2034-2039.
11. Elo S, Saxelin M, Salminen S. Attachment of *Lactobacillus casei* strain GG to human colon carcinoma cell line Caco-2: comparison with other dairy strains. Lett Appl Microbiol 1991; 13: 154-156.
12. Ruseler-van Embden JGH, Liesholt LMC, Gosselink MJ, Marteau P. Inability of *Lactobacillus casei* strain GG, *L. acidophilus* and *Bifidobacterium bifidum* to degrade intestinal mucus glycoproteins. Scand J Gastroenterol 1995; 30: 675-680.
13. Hoskins LC, Augustines M, McKee WB, Boulding ET, Kriaris M, Niedermeyer G. Mucin degradation in human colon ecosystems. Isolation and properties of faecal strains that degrade ABH blood group antigens and oligosaccharides from mucin glycoproteins. J Clin Invest 1985; 75: 944-953.
14. Coley AM, Lee AJ, Hunter JO. *Lactobacillus casei* GG in the treatment of colonic disorders. Microb Ecol Health Dis 1992;5:ii.
15. Oakey HJ, Harty DWS, Knox KW. Enzyme production by lactobacilli and the potential link with infective endocarditis. J Appl Bacteriol 1995; 78: 142-148.
16. Harty DWS, Oakey HJ, Patrikakis M, Hume BBH, Knox KW. Pathogenic potential of lactobacilli. Int J Food Micr 1994; 24: 179-189.
17. Goldin BR, Gorbach SL. Alterations of the intestinal microflora by diet, oral antibiotics and *Lactobacillus*: decreased production of free amines from aromatic nitroso compounds, azo dyes and glucuronides. J Natl Cancer Inst 1984: 73: 689-695.
18. Millar MR, Bacon C, Smith SL, Walker V, Hall MA. Enteral feeding of premature infants with *Lactobacillus* GG. Arch Dis Child 1993; 69: 483-487.
19. Sepp E, Mikelsaar M, Salminen S. Effect of administration of *Lactobacillus casei* strain GG on the gastrointestinal microbiota of newborns. Microbial Ecol Health Dis 1993; 6: 309-314.
20. Majamaa H, Isolauri E, Saxelin M, Vesikari T. Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. J Pediatr Gastroenterol Nutr 1995; 20: 333-338.
21. Isolauri E, Juntunen M, Rautanen T, Sillanaukee P, Koivula T. A human *Lactobacillus* strain (*Lactobacillus casei* sp strain GG) promotes recovery from acute diarrhoea in children. Pediatrics, 1991; 88: 90-97.
22. Saavedra JM, Bauman NA, Oung I. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet 1994; 344: 1046-1049.
23. Link-Amster H, Rochat F, Saudan KY, Mignot O, Aeschliman JM. Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. FEMS Immunol Med Microbiol 1994; 10: 55-64.
24. Gorbach SL, Chang T, Goldin B. Succesful treatment of relapsing *Clostridium difficile* colitis with *Lactobacillus* GG. Lancet 1987; 2: 1519.
25. Aronsson B, Barany P, Nord CE, Nyström B, Stenvinkel P. *Clostridium difficile*-associated diarrhoea in uremic patients. Eur J Clin Microbiol 1987; 6: 352-356.
26. Biller JA, Katz AJ, Flores AF, Buie TM, Gorbach SL. Treatment of recurrent *Clostridium difficile* colitis with *Lactobacillus* GG. J Pediatr Gastroenterol Nutr 1995; 21: 224-226.
27. Salminen E, Elomaa I, Minkkinen J, Vapaatalo H, Salminen S. Preservation of intestinal integrity during radiotherapy using live *Lactobacillus acidophilus* cultures. Clin Radiol 1988; 39: 435-437.
28. Salminen E, Salminen S, Vapaatalo H. Adverse effects of pelvic radiotherapy. Progress in Radio-Oncology, Vienna, 1995:501-504.
29. Aso Y, Akazan H. Prophylactic effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer. Urol Int 1992; 49: 125-129.
30. Tanaka R, Ohwaki M. A controlled study of the effects of the ingestion of *Lactobacillus casei* fermented milk on the intestinal microflora, its micobiology and immune system of healthy humans. Proceedings of XII Riken Symposium on Intestinal Flora, Tokyo, Japan. 1994: 85-104.
31. Saavedra JM. Microbes to fight microbes: a not so novel approach to controlling disease. J Pediatr Gastroenterol Nutr 1995; 21: 125-129.