REVIEW ARTICLE

Potential use of probiotics

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Abstract

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Probiotics (Greek: for life) are commonly defined as mono- or mixed cultures of live microbes that, when applied to animal or human, possess a beneficial effect on health of the host. These beneficial effects include disease treatment and prevention as well as improvement of nutrients' digestion and absorption. Probiotic microorganisms are generally, albeit not exclusively, lactic acid bacteria (LAB) including *Lactobacillus acidophilus, L. bulgaricus, L. casei, L. plantarum,* and *L. rhamnosus*. However, use of other bacterial species such as *Bacillus* and *Bifidobacterium* spp. as probiotic strains has also been described in several commercial products. This article intends to present an up-to-date version regarding probiotics, strains currently used and health benefit obtained from their consumption.

Key words : probiotics, lactobacilli, lactic acid bacteria, beneficial effect

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ศักยภาพของการใช้ probiotics

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โพรไบโอติก หมายถึง กลุ่มจุลินทรีย์ที่เป็นประโยชน์ต่อผู้บริโภค คุณประโยชน์เหล่านี้รวมไปถึงการรักษาและการป้องกันโรค อีกทั้งยังช่วยให้ระบบการย่อยและดูดซึมอาหารทำงานได้ดีขึ้น โดยทั่วไปจุลินทรีย์ที่มีสมบัติเป็นโพรไบโอติกจัดอยู่ในกลุ่มของแลคติกแอซิดแบคทีเรีย อย่างไรก็ตามมีรายงานถึงการใช้แบคทีเรียชนิดอื่น ๆ เช่น บาซิลลัส และบิฟิโดแบคทีเรียม ในผลิตภัณฑ์โพรไบโอติกด้วย บทความนี้ผู้เขียนต้องการนำเสนอความรู้เกี่ยวกับโพรไบโอติก สายพันธุ์ของจุลินทรีย์ที่ใช้ ตลอดจนประโยชน์ทางด้านสุขภาพของผู้บริโภค

ภาควิชาเทคโนโลยีชีวภาพ สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง อำเภอเมือง จังหวัดเชียงราย 57100

The idea of using microbes to promote a good health and to prevent disease is not new. Initially, several microbes have been used unintentionally in food production such as dairy products and fermented vegetables. Such fermented foods are popular due to their distinct characteristic in terms of taste and aroma. In recent years, there has been a renewed interest in microbial uses due to, apart from improving food flavour, their beneficial aspect in health restoration and disease treatment. Several microorganisms, under the name of "probiotics", have been proposed and used in a wide range of clinical trials, ranging from diarrheal disease to cancer prevention (Fuller, 1994; Kaur *et al*., 2001).

The term "probiotics" was originally used by Lilley and Stillwell (1965) to mean a substance(s) that stimulates growth of other microorganisms. The meaning of this term has now been redefined and restricted to "a viable microbial agent(s) which, when used in animal or man, beneficially affects the host possibly by improving the balance of the indigenous microflora (Fuller, 1991; Salminen *et al*., 1999). Based on this meaning, several terms such as "friendly", "beneficial", or "healthy" bacteria are also commonly known for probiotics.

The first study regarding beneficial effect of probiotics was carried out by Metchnikoff in the early 1900's (Metchnikoff, 1908). He reported the favourable effects of soured milk in human and suggested that consumption of live microbes (possibly LAB) in such fermented milk may help improve the balance of the gut microflora. Since then, microbial probiotics have gained an increasing interest and their use is now widely accepted. The purpose of this review is to describe probiotics in terms of strains availability and their beneficial effect.

Probiotic strains

Ideally, microbial probiotics should have a beneficial effect and not cause any harm to the host. Therefore, all strains must have been studied comprehensively prior to use in humans or animals and thus are given GRAS (Generally Regarded As Safe) status (see Table 1). Typically, safety evaluation of a proposed or accepted probiotic strain(s) includes i) ability of cells to produce metabolites and enzymes, ii) colonisation (or adhesion) properties, iii) factors that influence the strain survival and, last but not least, iv) interactions with host, particularly in terms of pathogenicity.

As previously mentioned, the predominant species used as probiotic agents belong to the group of LAB. Due to their long history of safe use in foods, most species of LAB are considered as commensal microorganisms with no pathogenic potential. The LAB, a group of Gram-positive bacteria, consist of several species including the genera *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Aerococcus, Bifidobacterium,* and *Weissela*. Within the LAB group, the genus

Table 1. Microbial probiotics and their safety status

Organism	Infection potential
Lactobacillus	Mainly nonpathogens; a few opportunistics reported in AIDS patients
Lactococcus	Mainly nonpathogens
Streptococcus	Opportunistics; only *S. thermophilus* is used in dairy products.
Enterococcus	Opportunistics; some strains exhibit antibiotic resistance.
Bacillus	Only *B. subtilis*, GRAS status, is reported in probiotics use.
Bifidobacterium	Mainly nonpathogens; some strains are isolated from human infection.
Propionibacterium	Dairy propionibacterial group is a potential candidate for probiotics.
Saccharomyces	Mainly nonpathogens; some strains are isolated from human infection.

Source: Adapted from Donohue and Salminen, 1996.

Lactobacillus is the most widely encountered for probiotics (Havenarr *et al*., 1992; Greene and Klaenhammer, 1994; Reid, 1999); these include *L. acidophilus, L. bulgaricus, L. casei, L. fermentum, L. plantarum, L. reuteri, L. rhamnosus,* and *L. salivarius*. These *Lactobacillus* species possess several important properties and therefore can be used as effective probiotic organisms. These features are as follows: i) efficient adherence to intestinal epithelial cells to reduce or prevent colonisation of pathogens (Reid *et al*., 1993; Bernet *et al*., 1994; Sarem-Damerdjii *et al*., 1995; Kirjavainen *et al*., 1998; Ouwehand *et al*., 1999); ii) competitive growth (Casas and Dobrogosz, 1997; Holzapfel *et al*., 1998; Netherwood *et al*., 1999); iii) production of metabolites to inhibit or kill pathogen (Jack *et al*., 1994; Reid and Burton, 2002); and iv) nonpathogen (Gonzalez *et al*., 1995; Reid, 1999). Recently, use of genetically modified LAB (GM-LAB) has been introduced to enhance quality, texture, flavour and aroma for the food products such as buttermilk and yoghurt (Wymer, 1998).

In addition, use of other bacterial groups such as *Bacillus* spp. (Adami and Cavazzoni, 1999; Casula and Cutting, 2002), *Bifidobacterium* spp. (Charteris *et al*., 1998; Gill *et al*., 2001a), and *Propionibacterium* spp. (Glatz, 1992; Zarate *et al*., 2002) for probiotics has currently been recognised and accepted. *B. subtilis*, in particular, is now being used for oral bacteriotherapy; ingestion of appropriate quantities of *B. subtilis* is anticipated to revive the normal microflora after antibiotic use or critical illness (Green *et al*., 1999). *Propionibacterium* species is also a potential candidate because of its historical use as dairy starters such as cheese (Dupuis *et al*., 1995). Propionic acid, produced by this species, is thought to play a key role by inhibiting certain bacteria and fungi.

However, due to various applications of probiotics, there is an attempt to screen and identify new probiotic strains with specific characteristic(s) to be appropriate for specific work. For example, in fish farming, there is a need to seek alternative probiotics that are bile-tolerant and stable at low pH (Joborn, 1998). New probiotic candidates that are acid resistant would be an ideal for this approach; these include *Vibrio alginolyticus* and *Carnobacterium* spp. (Austin *et al*., 1995; Robertson *et al*., 2000; Nikoskelainen *et al*., 2001).

Health improvement by probiotics

For centuries, microbial probiotics have been used, without specific knowledge of how they function, as supplementary diets to promote the good health. It was Metchnikoff (1908) who first pointed out that some small living organisms or substances produced in fermented food products (such as yoghurt) might influence the balance of intestinal microflora. Since Metchnikoff's discovery, a number of studies have been carried out in this area and an increasing amount of information gained regarding the beneficial effects of probiotic use. Probiotics have currently used to improve the health of humans and animals in

various aspects (see Table 2). These topics will be discussed as follows:

Improvement of intestinal microflora balance

It is estimated that almost 100 different species (with a total bacterial population between 10^{10} - 10^{12}) are present in human intestinal tract (Simon and Gorbach, 1984). There are differences in microbial composition of the gastrointestinal (GI) tract among individuals, and also within the same individual during life. Microbes present in the GI tract are composed of both 'friendly' bacteria and pathogens forming a complex symbiosis. Various kinds of factors such as diet, climate, ageing, medication (particularly antibiotic consumption), illness, stress, and lifestyle, can upset this balance (Sanderson and Walker, 1993) leading to diarrhea, mucosal inflammation or other serious illness. For optimum gut flora balance, the so-called "eubiosis", the friendly bacteria such as the Gram-positive lactobacilli and bifidobacteria should dominate (> 85% of total bacteria) representing a barrier to pathogenic bacteria. As a result, use of probiotics is likely to be the most natural and safe means for maintaining this balance. 'Friendly' bacteria that are predominant will prevent coloni-sation of bacterial pathogens by competing for essential nutrients or attachment sites. For example, *L. casei* Shirota in Yakult (Japanese soured milk) appears to colonise efficiently with the epithelial cells of the intestine (Spanhaak *et al*., 1998) and thus limiting the GI area for other undesired microbes. In addition, several strains of lactobacilli contained in probiotics also inhibit, by producing antimicrobial compounds (i.e., bacteriocins), the growth of enteric pathogens such as *Salmonella* species (Gill *et al*., 2001b). For instance, *Lacto-bacillus* strain GG, when orally given at greater doses (~ 10^9 CFU/day), is able to adhere to the intestine and its use is reported to alleviate diarrhoea symptoms from Enterobacteria (Reid *et al*., 1993; Greene and Klaenhammer, 1994).

Enhancement of immune system

Microbial probiotics (especially those of LAB) can influence the systemic immune systems in various ways (Perdigon *et al*., 1995; Fang *et al*., 2000). Firstly, they can enhance the defensive property of intestinal mucosa whose function is

Table 2. Probiotic bacteria and their effects

Strain	Beneficial effect
Lactobacillus acidophilus LA1	Adherence to human intestinal cells Balances intestinal microflora Immune enhancement
Lactobacillus GG	Prevention of antibiotic-associated diarrhea Treatment of rotavirus diarrhea Treatment of diarrhea caused by *Clostridium difficile* Stabilisation of Crohn's disease
Lactobacillus casei Shirota	Prevention of intestinal microbiota disturbance Positive effects on bladder cancer
Lactobacillus gasseri	Carcinogenic-associated enzyme reduction
Bacillus subtilis	Use for oral bacteriotherapy Restoration of normal microbial flora Immunostimulatory agent
Bifidobacterium bifidum	Prevention of viral diarrhoea
Propionibacterium freudenreichii	Growth stimulation of other 'friendly' bacteria

Source: For detailed references see Salminen *et al*., 1998.

like a barrier against the antigens (i.e, bacterial pathogens) (Salminen *et al*., 1996a; 1996b). For example, Crohn's disease, associated with impairment of the barrier function, can be cured by including probiotic supplement in patient's diets (Isolauri *et al*., 1998). In addition, some certain strain of LAB and bifidobacteria are able to induce tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) as well as to stimulate other nonspecific immune system (Isolauri *et al*., 1998). Potential use of *L. casei* Shirota has also been described as probiotic agent for stimulating immune responses, preventing enterobacterial infections (Matsuzaki, 1998). *Lactobacillus* GG is also used as an effective oral vaccine for rotavirus (Isolauri *et al*., 1995). Application of probiotic use in this aspect is challenging and hence further clinical trials in humans are certainly warranted.

Prevention of cancer

In *in vitro* experiments, it is found that LAB in dairy products can reduce mutagenicity of some known mutagens such as 1, 2-dimethylhydrazine (Salminen *et al*., 1998). Besides, several studies reported that growth of the tumour cells can be inhibited by LAB (reviewed in Reddy, 1998; Burns and Rowland, 2000). For both phenomena, it is assumed that LAB may be indirectly involved by lowering carcinogenicity of carcinogen-activating bacterial enzymes in the GI tract. *L. acidophilus* NCFM strain exhibits ability to reduce levels of free amines in the intestine, leading to a low risk of colon cancer (Goldin and Gorbach, 1984). Furthermore, Ling *et al*. (1994) reported the decrease of enzyme activity (i.e., β-glucoronidase, azoreductase, and glycocholic acid hydrolase) in human volunteers following the intake of yoghurt containing *Lactobacillus* strain GG. These enzymes are thought to be involved in pathogenesis of bowel cancer.

Commercially available probiotics

Currently, there is a wide range of probiotic products commercially available to consumers. Such products are as follows: animal feeds, dairy foods, infant & baby foods, fruit juice-based products, cereal-based products, and pharmaceuticals. Before these products are sold, the probiotic strains used must have been studied and characterised extensively to ensure their safe and effective use. Among these commercial products, use of *Lactobacillus* species is predominant and there is probably no shortage of *Lactobacillus* products in health food products. Incorporation of *B. subtilis* in probiotic products is also found on the market

Table 3. Commercial probiotic products available to date

Brand product	Strain	Distributor
Yakult	*L. casei* Shirota	Yakult, Tokyo, Japan
Adult formula CP-1	Mixtures of *L. acidophilus, L. rhamnosus, L. plantarum, Bifidobacterium longum, and B. bifidum*	Custom Probiotics, Glendale, CA
KE-99 LACTO	*L. casei*	ProbioHealth, Beverly Hills, CA
Trenev Trio Capsules	*L. acidophilus, L. bulgaricus,* and *B. bifidum*	Probiotics New Zealand
Multibionta	*L. acidophilus, B. bifidum,* and *B. longum*	Seven Seas Health Care, Hull, UK
Enterogermina	*Bacillus subtilis*	Sanofi Winthrop, Milan, Italy
Biosubtyl	*B. subtilis*	Biophar Co. Ltd., Nha Trnag, Vietnam

Source: Adapted from Reid, 1999.

(Green *et al*., 1999). Table 3 summarises commercial probiotic products currently available.

Concluding remarks

Use of microbial probiotics to promote health maintenance and disease prevention is now widely accepted. Viable microbes possibly play a key role in balancing the intestinal microflora naturally although their modes of action have not been completely understood and further investigation is needed to understand the mechanism of these microbe-microbe interactions. Beneficial effects of use of microbial probiotics are now well-documented. However, questions regarding reliability, quality of many commercial products as well as safety issues have been raised (Saarela *et al*., 2000; Ishibashi and Yamazaki, 2001). The latter topic is of great importance due to its use in humans. Traditional probiotic dairy strains such as LAB are considered as major sources due to their long history of safe use in foods. However, with a broad range of applications, several probiotic strains are now being investigated and proposed for an alternative. It is therefore necessary to ensure that novel isolated probiotics are being screened and subjected to appropriate field tests with animals or humans.

References

Adami, A. and Cavazzoni, V. 1999. Occurrence of selected bacterial groups in the faeces of piglets fed with *Bacillus coagulans* as probiotic. J. Basic Microbiol., 39: 3-9.

Austin, B., Stuckey, L.F., Robertson, P.A.W., Effendi, I. and Griffith, D.R.W. 1995. A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida, V. anguillarum* and *V. ordalii*. J. Fish Dis., 18: 93-96.

Bernet, M.F., Brassart, D., Neeser, J.R. and Servin, A.L. 1994. *Lactobacillus acidophilus* LA1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. Gut, 35: 483-489.

Burns, A.J. and Rowland, I.R. 2000. Anit-carcinogenicity of probiotics and prebiotics. Curr. Issues Intest. Microbiol., 1: 13-24.

Casas, I.A. and Dobrogosz, W.J. 1997. *Lactobacillus reuteri*: overview of a new probiotic for humans and animals. Microecol. Ther., 26: 221-231.

Casula, G. and Cutting, S.M. 2002. *Bacillus* probiotics: spore germination in the gastrointestinal tract. App. Environ. Microbiol., 68: 2344-2352.

Charteris, W.P., Kelly, P.M., Morelli, L. and Collins, J.K. 1998. Antibiotic susceptibility of potentially probiotic *Bifidobacterium* isolates from the human gastrointestinal tract. Lett. Appl. Microbiol., 26: 333-337.

Donohue, D.C. and Salminen, S. 1996. Safety of probiotic bacteria. Asia Pac. J. Clin. Nutr., 5: 25-28.

Dupuis, C., Corr, C. and Boyaval, P. 1995. Proteinase activity of dairy propionibacteria. Appl. Microbiol. Biotechnol., 42: 750-755.

Fang, H., Elina, T., Heikki, A. and Seppo, S. 2000. Modulation of humoral immune response through probiotic intake. FEMS Immunol. Med. Microbiol., 29: 47-52.

Fuller, R. 1994. Probiotics: an overview. In: Human health: the contribution of microorganisms (Ed. by S.A.W. Gibson), pp. 63-73. New York: Springer-Verlag.

Fuller, R. 1991. Probiotics in human medicine. Gut, 32: 439-442.

Gill, H.S., Rutherfurd, K.J., Cross, M.L. and Gopal, P.K. 2001a. Enhancement of immunity in the elderly by dietary supplementation with the probiotic *Bifidobacterium lactis* HN019. Am. J. Clin. Nutr., 74: 833-839.

Gill, H.S., Shu, Q., Lin, H., Rutherfurd, K.J. and Cross, M.L. 2001b. Protection against translocating Salmonella typhimurium infection in mice by feeding the immuno-enhancing probiotic Lactobacillus rhamnosus strain HN001. Med. Microbiol. Immunol., 190: 97-104.

Glatz, B. 1992. The classical propionibacteria: their past, present, and future as industrial organisms. ASM News, 58: 197-201.

Goldin, B.R. and Gorbach, S.L. 1984. The effect of milk and *Lactobacillus* feeding on human intestinal bacterial enzyme activity. Am. J. Clin. Nutr., 39: 756-761.

Gonzalez, S.N., Cardoza, R., Apella, M.C. and Oliver,

G. 1995. Biotherapeutic role of fermented milk. Biotherapy, 8: 129-134.

Green, D.H., Wakeley, P.R., Page, A., Barnes, A., Baccigalupi, L., Ricca, E. and Cutting, S.M. 1999. Characterisation of two *Bacillus* probiotics. Appl. Environ. Microbiol., 65: 4288-4291.

Greene, J.D. and Klaenhammer, T.R. 1994. Factors involved in adherence of lactobacilli to human Caco-2 cells. Appl. Environ. Microbiol., 60: 4487-4494.

Havenaar, R., ten Brink, B. and Huis in't Veld, J.H.J. 1992. Selection of strains for probiotic use. In: Probiotics, the scientific basis (Ed. by R. Fuller), pp. 209-224. London: Chapman and Hall.

Holzapfel, W.H., Haberer, P., Snel, J., Schillinger, U. and Huis in't Veld, J.H. 1998. Overview of gut flora and probiotics. Int. J. Food Microbiol., 41: 85-101.

Ishibashi, N. and Yamazaki, S. 2001. Probiotics and safety. Am. J. Clin. Nutr., 73: 465S-470S.

Isolauri, E., Joensuu, J., Suomalainen, H., Luomala, M. and Vesikari, T. 1995. Improved immunogenicity of oral DxRRV reassortant rotavirus vaccine by *Lactobacillus casei* GG. Vaccine, 13: 310-312.

Isolauri, E., Salminen, E. and Salminen, S. 1998. Lactic acid bacteria and immune modulation. In: Lactic acid bacteria: microbiology and functional aspects (Ed. by S. Salminen and A. von Wright), pp. 255-268. New York: Marcel Dekker, Inc.

Jack, R.W., Tagg, J.R. and Ray, B. 1994. Bacteriocins of Gram-positive bacteria. Microbiol. Rev., 59: 171-200.

Joborn, A. 1998. The role of the gastrointestinal microbiota in the prevention of bacterial infections in fish. Ph.D. Thesis, Gothenburg University.

Kaneko, T., Mori, H., Iwata, M. and Meguro, S. 1994. Growth stimulator for bifidobacteria produced by *Propionibacterium freudenreichii* and several intestinal bacteria. J. Dairy Sci., 77: 393-404.

Kaur, I.P., Chopra, K. and Saini, A. 2001. Probiotics: potential pharmaceutical applications. Eur. J. Pharm. Sci., 15: 1-9.

Kirjavainen, P.E., Ouwehand, A.C., Isolauri, E. and Salminen, S.J. 1998. The ability of probiotic bacteria to bind to human intestinal mucus. FEMS Microbiol. Lett., 167: 185-189.

Ling, W.H., Korpela, R., Mykkanen, H., Salminen, S. and Hanninen, O. 1994. *Lactobacillus* strain GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults. J. Nutr., 124: 18-23.

Lilley, D.M. and Stillwell, R.H. 1965. Probiotics: growth promoting factors produced by microorganisms. *Science*, 147: 747-748.

Matsuzaki, T. 1998. Immunomodulation by treatment with *Lactobacillus casei* strain Shirota. Int. J. Food Microbiol., 41: 133-140.

Mazza, P., Zani, F. and Martelli, P. 1992. Studies on the antibiotic resistance of *Bacillus subtilis* strains used in oral bacteriotherapy. Boll. Chim. Farm., 131: 401-408.

Metchnikoff, E. 1908. The prolongation of life. New York: CP Putnam's Sons.

Netherwood, T., Gilbert, H.J., Parker, D.S. and O' Donnell, A.G. 1999. Probiotics shown to change bacterial community structure in the avian gastrointestinal tract. Appl. Environ. Microbiol., 65: 5134-5138.

Nikoskelainen, S., Salminen, S., Bylund, G. and Ouwehand, A.C. 2001. Characterisation of the properties of human- and dairy-derived probiotics for prevention of infectious diseases in fish. Appl. Environ. Microbiol., 67: 2430-2435.

Ouwehand, A.C., Kirjavainen, P.V., Gronlund, M.M., Isolauri, E. and Salminen, S. 1999. Adhesion of probiotic micro-organisms to intestinal mucus. Int. Dairy J., 9: 623-630.

Perdigon, G., Alvarez, S., Rachid, M., Aquero, G. and Gobbato, N. 1995. Immune system stimulation by probiotics. J. Dairy Sci., 78: 1597-1606.

Reddy, B.S. 1998. Prevention of colon cancer by pre- and probiotics: evidence from laboratory studies. Br. J. Nutr., 80: S219-S223.

Reid, G. 1999. The scientific basis for probiotic strains of *Lactobacillus*. Appl. Environ. Microbiol., 65: 3763-3766.

Reid, G. and Burton, J. 2002. Use of *Lactobacillus* to prevent infection by pathogenic bacteria. Microbes Infect., 4: 319-324.

Reid, G., Servin, A., Bruce, A.W. and Busscher, H.J. 1993. Adhesion of three *Lactobacillus* strains to human urinary and intestinal epithelial cells.

Microbios, 75: 57-65.

Robertson, P.A.W., O'Dowd, C., Burrells, C., Williams, P. and Austin, B. 2000. Use of *Carnobacterium* sp. As a probiotic for Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*, Walbaum). Aquaculture, 185: 235-243.

Saarela, M., Mogensen, G., Fonden, R., Matto, J. and Mattila-Sandholm, T. 2000. Probiotic bacteria: safety, functional and technological properties. J. Biotechnol., 84: 197-215.

Salminen, S., Deighton, M.A., Benno, Y. and Gorbach, S.L. 1998. Lactic acid bacteria in health and disease. In: Lactic acid bacteria: microbiology and functional aspects (Ed. by S. Salminen and A. von Wright), pp. 211-253. New York: Marcel Dekker, Inc.

Salminen, S., Isolauri, E. and Salminen, E. 1996a. Clinical uses of probiotics for stabilising the gut mucosal barrier: successful strains and future challenges. Antonie Leeuwenhoek, 70: 347-358.

Salminen, S., Isolauri, E. and Salminen, E. 1996b. Probiotics and stabilisation of the gut mucosal barrier. Asia Pac. J. Clin. Nutr., 5: 53-56.

Salminen, S., Ouwehand, A.C., Benno, Y. and Lee, Y.K. 1999. Probiotics: how should they be defined? Trends Food Sci. Technol., 10: 107-110.

Sanderson, I.R. and Walker, W.A. 1993. Uptake and transport of macromolecules by the intestine: possible role in clinical disorders (an update). Gastroenterology, 104: 622-639.

Sarem-Damerdjii, L.O., Sarem, F., Marchal, L. and Nicolas, J.P. 1995. *In vitro* colonisation ability of human colon mucosa by exogenous *Lactobacillus* strains. FEMS Micorbiol. Lett., 131: 133-137.

Simon, G.L. and Gorbach, S.L. 1984. Intestinal flora in health and disease. Gastroenterology, 86: 174-193.

Spanhaak, S., Havenaar, R. and Schaafsma, G. 1998. The effect of consumption of milk fermented by *Lactobacillus casei* strain Shirota on the intestinal microflora and immune parameters in humans. Eur. J. Clin. Nutr., 52: 899-907.

Wymer, P. 1998. The application of genetically modified lactic acid bacteria in food products in Europe. A workshop organised by the Lactic Acid Bacteria Industrial Platform, Geneva, Switzerland, Nov. 16-18, 1998.

Zarate, G., Morata De Ambrosini, V., Perez Chaia, A. and Gonzales, S. 2002. Some factors affecting the adherence of probiotic *Propionibacterium acidipropionici* CRL1198 to intestinal epithelial cells. Can. J. Microbiol., 48: 449-457.