Lactic acid bacteria as probiotics*

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Summary

Lactic acid bacteria (LAB) are commonly found on human and animal mucous membranes in dairy products and naturally on some plant surfaces. They are normal residents of the gastrointestinal tract of human beings. Several species are commercially used in production of fermented milks, yoghurt, other dairy and meat products, as well as other foods. The antimicrobial activities of LAB have been known long ago and recognised as important in food fermentations, food preservation and intestinal ecology. The general nutritious effect is scientifically well established and documented but a considerable amount of doubt or scepticism exists in relation to various prophylactic and therapeutic claims attributed to probiotic preparations. This article represents the species of LAB that have been used as human probiotics or in fermented dairy products claimed to promote health.

Additional index words: Lactic acid bacteria, species used in fermented milks production, Antimicrobial activities, Fermentations, Food preservation, Intestinal ecology.

Introduction

Lactic acid bacteria (LAB) are industrially important organisms due to their fermentative ability as well as to their health and nutritional benefits. Many studies have shown that they give fermented foods distinctive flavour and texture. The fermentation using LAB improves nutritional value of food products increasing the quantity, availability, digestibility and assimilability of nutrients. The essential role of LAB is growth inhibition of food spoilage and pathogenic bacteria and in this way improving safety and food products shelf life. Many beneficial aspects are atributed to LAB regarding human health, such as on gut microbial ecology, lactose digestion, mineral absorption and some other beneficial effects. Since the Metchnikow thesis (1908) LAB have been assumed to possess more or less probiotic properties.

The word »probiotic« is derived from Greek »for life« and had over the years several different meanings. The use of term »probiotic« in the context in which it's used today dated in 1974 when Parker defined probiotics as »Organisms and substances contributing to intestinal microbial balance«. Fuller in 1989 redefined probiotics as »A live microbial feed supplement which beneficially effects

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animal host improving its intestinal microbial balance (Fuller, 1992). This definition stresses the importance of viability and avoids the use of too broad term »substances«. Definitions for probiotics were mainly restricted to applications in animal feed. Huis and Havenaar (1991) proposed to broaden the definition and to include application humans as well. Their definition is: »A mono-or mixed culture of live micro-organisms which, applied to man or animal, affects beneficially the host improving the properties of indigenous microflora«. Probiotics generally refer to viable bacteria, cultured dairy products or food supplements containing viable LAB. The belief in beneficial effects of probiotics approach is based on the knowledge that intestinal microflora provides protection against various diseases. Although there seems no doubt that gut microflora is protective, in order to produce an effective probiotic one must know which micro-organisms are responsible for induced effect.

Probiotic Strains Requirements

Several basic properties are required to promote an effective probiotic LAB strain. Among the most important properties is ability to survive passage through the mounth, stomach, small intestine and large intestine. Thus, the strain must be stable in gastric conditions. In order to be able to influence functions of human intestinal tract, probiotic strains must have the capacity to adhere to intestinal mucosal cells and to grow in intestinal conditions.

Some properties of a good probiotic strain are summarised in Table 1. Other requirements of an effective probiotic strain include antagonism against pathogenic bacteria, production of antimicrobial substances and proven safety in human use.

Table 1: Some properties of a good probiotic strain (Salminen et al, 1993)
Tablica 1.: Neka svojstva dobrog probiotskog soja

Acid stability (especially gastric acid)
Bile stability
Human origin (species – specific properties)
Adherence to human intestinal cells
Colonisation of human intestinal tract
Production of antimicrobial substances
Antagonism against pathogenic bacteria
Good growth in vitro
Safety in human use

Besides all mentioned properties proposed probiotic strain needs to be suitable for industrial application in common dairy processes or in production of pharmaceutical preparations.

Composition of probiotic preparations

The purposes for application probiotic in human health promotion, and animal growth promotion seem to be different, and therefore different approaches are needed. Probiotic preparations for animals are generally food supplements in the form of paste, pellets or capsules. Preparations for humans are in the form of various fermented products or in pharmaceutical preparations (tablets or powders). The strains of *Lactobacillus* (L.), *Lactococcus* (Lac.), *Streptococcus* (Str.) and *Bifidobacterium* (B.) are important components of probiotics, currently available on the market (Table 2). Used LAB strains are mostly intestinal isolates. Yoghurt starter bacteria are also included as yoghurt was in the past associated to health benefits. However, yoghurt bacteria *L. delbrueckii* subsp. *bulgaricus* and *Str. salivarius* subsp. *thermophilus* are not typical human intestinal flora and the ability to colonise gut is extremely doubtful. There is lack of information not only regarding the true intestinal origin of some intestinal cultures but also about their behaviour in the intestine (Fuller, 1991).

Table 2: Intestinal bacteria for preparation of starter cultures (Kurmann, 1988)
Tablica 2.: Bakterije probavnog trakta za pripremu kultura

Genus Rod	Species Vrsta	Application Primjena
Bifidobacteria	*B. bifidum*	L, P, D
	B. breve	L
	B. infantis	L
	B. longum	D
	B. adolescentis	L
Lactobacillus	*L. acidophilus*	L, D
	L. casei	P, D
	L. casei subsp. rhamnosus	D, L
	L. kefir	L
	L. fermenturm	L
	L. helveticus	L
	L. plantarum	L
	L. reuteri	L
	L. salivarius	L
Streptococcus	*S. faecalis*	L
	S. faecium	P
	S. salivarius subsp. salivarius	L
Propionibacterium	*P. freudenreichii subsp. shermanii*	L

L = Laboratory preparation = laboratorijsko pripremanje
D = Industrial dairy preparation = industrijsko mljekarsko pripremanje
P = Industrial pharmaceutical preparation = industrijsko farmaceutsko pripremanje

Various strains of *Lactobacillus* have been used in studies on probiotics use in humans. Among all strains studied the most successful were two of human origin. *L. acidophilus* (NFCO 1748) and *Lactobacillus casei* strain GG. *Lactobacillus* GG were tested in several groups of patients in the form of fermented dairy products and freeze dried cultures. The strain was proved effective in treatment of acute gastroenteritis. It also helps in preserving intestinal integrity in various disordered states and helps the intestinal immune system in fighting against infection (Salminen and Isolauri, 1994). Some other studies investigated use of preparations containing *Enterococcus faecium* strain 68 (SF68) and in Japan *Lactobacillus casei* Shirota (Salminen et al, 1993). The results obtained with this bacteria are still questionable.

Industrial production of fermented milks with probiotic bacteria of intestinal origin initially encountered several problems (Marshall, 1991):
a) human originated strains in milk usually grow slowly,
b) during storage strains tend to lose viability,
c) flavour and aroma were lacking.

To achieve better growth, milk may be supplemented with growth-promoting compounds. To avoid lacking of aroma and flavour probiotic bacteria can be used in mixed starters. The advantages of cultivating intestinal bacteria in mixed cultures are: utilisation of species originating from the small and the large intestines with a more widespread action, cultivation of obligate anaerobes (oxygen tolerant strains making possible growth of oxygen sensitive strains in milk), better growth and acid production in milk, better taste and improved consistency, especially if using slime production strains. For a successful mixed culture combination the interactions of different bacteria have to be studied trough the growth tests, viability of microorganisms and decimal death rate during storage. Some examples of fermented milk products with selected intestinal bacteria are summarised in Table 3.

Action mechanisms of LAB as probiotics

Various studies data on probiotic strains of LAB suggest four ways of probable action: production of antimicrobial substances, competition for adhesion receptors, competition for nutrients and stimulation of immunity. Thus, proposed mechanisms include the suppression of harmful bacteria and viruses, stimulation of local and systemic immunity and alteration of gut microbial activity (Fuller, 1992; Salminen et al, 1993).

a) *Production of antimicrobial substances*

The ability of probiotic bacteria to suppress growth of pathogens was attributed to production of antibacterial substances such as lactic acid, peroxide, bacteriocins and bacteriocin-like inhibitory substances. Production of antimicrobial substances might have viable cells number reducing effect or affecting intestinal bacteria metabolism or toxin production.

Table 3: Some examples of fermented milk products containing selected intestinal bacteria (intestinal origin was not confirmed in all cases) (Tamime, 1988)
Tablica 3.: Neki uzorci fermentiranih mliječnih proizvoda koji sadrže selekcionirane bakterije probavnog trakta (intestinalno porijeklo nije potvrđeno u svim slučajevima) (Tamime, 1988)

Country Zemlja	Name Naziv	Bacteria
Australia	*Acidophilus*	*L. acidophilus*
Denmark	A-38 Fermented milk AB-fermented milk AB-Yogurt	*L. acidophilus, Lactococcus spp.* *L. acidophilus, B. bifidum* *S. thermophilus, L. acidophilus, L. bulgaricus, B. bifidum*
Germany	Biogarde Bioyogurt Bifighurt	*S. thermophilus, L. acidophilus* *S. thermophilus, B. bifidum* *B. bifidum*
Japan	Mil-Mil E Yakult Yakult Miru-Miru	*S. thermophilus, L. bulgaricus, B. bifidum* *L. casei* *L. acidophilus, L. casei, B. breve*
Switzerland	Aco-Yogurt	*S. thermophilus, L. acidophilus, L. bulgaricus*
USA	Di-gest	*L. acidophilus*
Former countries of USSR	Biolakt Moskovskii Acidophilin Acidophilus paste	*L. acidophilus* *L. acidophilus* *Lac. lactis, L. acidophilus,* kefir grain *L. acidophilus*

b) *Competition for adhesion receptors*

It is now accepted that many intestinal pathogens must be albe to adhere to gut wall, colonise gut and cause disease. Consequently, some probiotic strains were chosen due to their ability to adhere to epithelial wall and thus compete with pathogens for attachment sites.

c) *Competition for nutrients*

The gut is rich source of nutrients and it seems unlikely that competition for nutrients is the way in which probiotic bacteria could act. However, one should keep in mind that bacteria require only one limiting nutrient to make this mechanism operate successfully. In vitro results suggest that some probiotic bacteria compete more efficiently for monomeric glucose, N-acetyl-glucosamine and sialic acid found in the colon than *Clostridiumn difficile.*

d) *Stimulation of immunity*

Human intestinal microflora has a profound influence on the immunological host's status. Alive indigenous bacteria or their antigens can penetrate trough intestinal epithelial barrier, thus stimulating immunocompetent cells. They may favour production of suppressor or helper cells and stimulate lymphocyte differentiation. In recent years was found that lactobacilli administered through mounth can stimulate macrophage activity against several different bacteria species. Presumably the effect is brought about either by absorption of soluble antigen or by translocation of lactobacilli through the gut wall into the blood stream.

LAB and health

In spite of claims relative to beneficial effects of probiotics in constipation, cancer, heart disease, lactase deficiency, cholesterol metabolism, pseudomembranous colitis, enteric infections, infantile diarrhoea, travellers' diarrhoea and antibiotic-associated diarrhoea only in some examples the evidence for a positive beneficial effect in human was very good. The following is a bricf account covering some of them.

Deficienty of intestinal enzyme b-galactosidase (lactase) causes lactose intolerance, resulting as inability to digest lactose. The congenital lactose intolerance varies depending on ethnic origin. Acquired lactase deficiency may be that result of pelvic radiotherapy, or it may follow an intestinal infection such as rotavirus infection. Some time ago it is known that lactase deficient subjects could tolerate lactose in yoghurt better than equal ammount of it in milk. This was now confirmed using hydrogen breath analysis experiments. Several authors reported studies in which lactose-intolerant individuals were given equivalent amounts of lactose in water, milk, yoghurt or other fermented dairy products. In most experiments yoghurt group suffered significantly less gastrointestinal symptoms compared with all other control groups. Results suggest that bacterial lactase produced by *L. delbrueckii* subsp. *bulgaricus, Str. salivarius* subsp. *thermophilus* and *L. acidophilus,* contributes to improved degradation of lactose in lactose-intolerant individuals (Salminen et al., 1993).

Many different diseases may induce diarrhoea in infants and young children. Successful curing of infants from acute diarrhoea, mainly due to rotavirus, was reported by Isolauri and co-workers (1991). The results demonstrated significant reduction in duration of rotavirus diarrhoea by administration of human *Lactobacillus* GG. The study was confirmed by Kaila et al. (1992). They also showed that diarrhoea duration reduction was associated to potentiation of intestinal immune response and an increase in rotavirus-specific antibodies.

Diarrhoea is usually the most frequent travellers health problem specially travelling to developing countries. It is estimated that at least 6 million tourists visiting developing countries in 1985 got travellers' diarrhoea. Some travellers'

diarrhoea forms appear to be preventable when viable LAB are administered during the period of risk. In one experiment, a lyophilised mixture of 90% *L. acidophilus* and *Bifidobacterium bifidum* + 10% of *L. bulgaricus* and *Str. thermophilus* was given prophylactically to tourists on a two-week trip to Egypt. A dose of 3 × 109 cfu was commenced two days prior to travel and continued throught the trip. The probiotic preparation had no effect on duration of diarrhoea but significantly reduced the frequency from 71% to 43% (Salminen et al., 1993).

Diarrhoea and other gastrointestinal disturbances are frequent complications after antibiotics treatment. About one third of cases of antibiotic associated diarrhoea and virtually all cases of pseudomembraneous colitis are related to overgrowth of toxin-producing strains *Clostridium difficile* in large intestine. Probiotics have been proposed as prophylaxis for antibiotic associated diarrhoea. Effect of *Lactobacillus* GG Yoghurt in prevention of antibiotic associated diarrhoea was studied by Siitonen et al. (1990). Subjects receiving *Lactobacillus* GG yoghurt with erythromycin endured less diarrhoea than control group. Also these bacteria colonised subject during experiment even during erytromycin treatment.

Conclusions

Many beneficial aspects relative to human health are attributed to lactic acid bacteria in cultured dairy products and other probiotic preparations. Although there is apparent substantial progress in knowledge on these topics, it is obvious that all these claimed beneficial effects could not be considered as well established from a scientific point of view. The use of selected intestinal bacterial starter cultures leads to a new or »third« generation of fermented milk, that would be according to Kurmann (1988), widely in use around 2000. This is a great challenge and prosperous future for dairy industry.

BAKTERIJE MLIJEČNE KISELINE KAO PROBIOTICI

Sažetak

Bakterije mliječne kiseline (BMK) obično se nalaze u mukoznim membranama ljudi i životinja, u mliječnim proizvodima i, naravno, na površinama nekih biljaka. Normalno naseljavaju probavni trakt ljudi. Brojne se vrste upotrebljavaju komercijalno za proizvodnju fermentiranog mlijeka, jogurta, te drugih mliječnih i mesnih, kao i drugih prehrambenih proizvoda. Mikrobicidna aktivnost BMK dugo je već poznata i priznata kao važna u fermentaciji hrane, očuvanju hrane kao i u ekologiji probavnog trakta. Opći hranidbeni učinak znanstveno je dobro utvrđen i dokumentiran, no značajan je broj sumnjivih i postoji skepticizam o terapeutskim i profilaktičkim učincima probiotskih preparata. Članak prikazuje vrste BMK koje se upotrebljavaju kao humani probiotici ili u fermentiranim mliječnim proizvodima kojima se pripisuje koristan zdravstveni učinak.

Riječi natuknice: Bakterije mliječne kiseline, sojevi za proizvodnju fermentiranog mlijeka, konzerviranje hrane, ekologija probavnog trakta.

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