

The biotechnology of lactic acid bacteria with emphasis on applications in food safety and human health

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Fermentation of various foodstuffs by lactic acid bacteria (LAB) is one of the oldest forms of bio-preservation practised by mankind. In recent years, significant advances have been made in elucidating the genetic and physiological basis of key LAB traits involved in these industrially significant processes. One important attribute of many LAB is their ability to produce antimicrobial compounds called bacteriocins. Interest in these compounds has grown substantially due to their potential usefulness as natural substitutes for chemical food preservatives in the production of foods with enhanced shelflife and/or safety.

There is growing consumer awareness of the link between diet and health. Recent scientific evidence supports the role of probiotic LAB in mediating many positive health effects. In addition, some LAB are currently being assessed for their ability to act as live delivery vectors in the development of new oral vaccines.

Key words: biopreservation, functional foods, probiotics, LAB-vaccines

Introduction

Mankind throughout the ages has practised fermentations by lactic acid bacteria (LAB) as an effective means of improving the shelflife of otherwise perishable foodstuffs and as such they represent a long-standing application of biotechnology. Many substrates including milk, meats, cereals, vegetables and fruits have been fermented generating a wide range of nutritious end products with desirable flavours and attributes.

LAB are a phylogenetically diverse group of

bacteria. Members of the genera *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Pediococcus*, in particular, are involved in these fermentations. In addition, some LAB (mainly *Lactobacillus* spp.) as well as the functionally related, though non-LAB, *Bifidobacterium*, are known to form part of the normal human intestinal microflora and accumulating evidence suggests that these bacteria may exert a positive effect on human health.

Given the economic value of food fermentations and a growing acceptance that at least some of these products may contribute to improved

health, it is not surprising that LAB are attracting major attention at this time. This is reflected in the increased volume of fermented products available world-wide especially in the area of functional foods containing probiotic or health-promoting bacteria.

Through the BIOTECHNOLOGY and AGRI-INDUSTRIAL programmes, the European Commission (EC) has provided outstanding financial support for research on LAB and has fostered many high-quality transnational collaborations. Two projects within the current European Union (EU) Fourth Framework Programme, in particular, illustrate the integrated approach that has been taken. The STARLAB project within the BIOTECHNOLOGY programme has 56 participating laboratories, 13 of which are industry based (Mercenier et al. 1997). It has the following research themes:

1. Cell engineering of *Lactococcus lactis*
2. LAB with modified proteolytic properties in milk fermentation
3. Control of bacteriophage development in LAB: towards a rational solution to a major problem of food fermentation
4. The molecular biology and genetics of thermophilic LAB
5. LAB as cell factories for the production and delivery of mucosal immunogens
6. Carbon catabolite control in food grade lactobacilli to provide the tools for strain improvement.

As part of the AGRI-INDUSTRIAL programme, the PROBDEMO project supports the development of novel probiotic products in the European market by providing a sound assessment of their functionality and subsequently disseminating the information to relevant authorities, consumer organisations and participating industry partners. The PROBDEMO project encompasses nine groups including four major dairy industries (Mattila-Sandholm 1997). The project's research tasks include the following:

1. To establish a scientifically based selection

of probiotic bacterial strains currently available for functional foods

2. To demonstrate the beneficial value of probiotic products in human pilot testing both in children and in adults, applying molecular tools for identification of gastrointestinal flora
3. To demonstrate and meet the functional and technological requirements essential for the industrial production of probiotics as functional foods
4. To disseminate the knowledge and results to the extended audience consisting of the group of industrial users, authorities and consumer organisations.

These and other research efforts world-wide have significantly advanced our understanding of key functional processes in LAB. They have already been instrumental in providing well-characterised strains for use in large-scale food fermentations and they underpin the development of future genetic strategies aimed at constructing strains with superior performance characteristics. Many of these developments will, either directly or indirectly, have applications in improving the quality and safety of foods.

Research on the contribution of various lactobacilli and bifidobacteria to the normal healthy functioning of the human gastrointestinal system and their likely probiotic effects is evolving rapidly, driven by the eagerness of food companies to satisfy a growing consumer market. Already several products are being marketed with probiotic claims and a number of manufacturers have developed and licensed specific probiotic bacteria – *Lactobacillus johnsonii* LA1 from Nestlé, LA7 from Bauer, Causido culture from MD Foods, the Lactigel strain from Danone and *Lactobacillus* GG from Valio, Mona and other companies (Young, J. 1996).

One of the newer and very exciting areas of LAB research concerns their exploitation as live oral vaccine delivery vehicles. The improved ability to genetically manipulate these bacteria, their 'generally regarded as safe' (GRAS) status and the potential ease of production and admin-

istration of LAB-based vaccines make them very attractive candidates for such applications (Wells et al. 1996).

The aim of this paper is to review developments in LAB research that have already impacted, or are likely to impact, the production of foods that are safer and of better quality. In addition, it examines the research supporting the potential therapeutic applications of some of these bacteria.

Developments in the biotechnology of LAB

The past 20 years have seen a major impetus in LAB research. Although initially much of this research focused on dairy lactococci, investigations now encompass many different LAB involved in a wide variety of fermentation processes and, more recently, various lactobacilli and bifidobacteria belonging to the human microbiota. A very large number of genes have already been cloned, sequenced and subjected to intensive analyses regarding their genetic and molecular organisations, modes of action and regulation. Two of the most important functional properties, lactose utilisation and proteolytic activity, are particularly far advanced. However, significant developments in other areas such as bacteriophage biology and resistance mechanisms, pyruvate metabolism and the production of bacteriocins have also been made (Fitzgerald and Hill 1996, von Wright and Sibakov 1998). Progress in LAB genetics was greatly aided early on by the fact that many of the industrially significant properties of these bacteria were encoded by plasmids (Fitzgerald and Hill 1996). However, research on their chromosomal genetics is also progressing rapidly. Physical and genetic maps have been constructed for a number of strains and there are an increasing number of chromosomally located genetic loci under investigation (Davidson et al. 1996).

The development of tools that facilitated the genetic manipulation of LAB has been crucial to the success of these endeavours. In particular, electrotransformation, which mediates high frequency uptake of *in vitro* DNA, allowed classical recombinant DNA technologies to be applied across a wide range of LAB (Gasson and Fitzgerald 1994, Mercenier et al. 1994). Also conjugation, one of the natural processes of gene exchange common among lactococci, has played an important role in non-recombinant strategies of strain improvement (Gasson and Fitzgerald 1994). Since the early 1980s, the array of cloning vectors available to researchers has expanded enormously. In addition to general cloning vectors, there is a wide choice of vectors available with specialised functions (de Vos and Simons 1994). These include genetic signal screening vectors, high expression vectors and inducible expression systems. Two further systems are worthy of special note. First is the development of vectors suitable for use in food industry applications. These contain only LAB-derived DNA and use food grade selection markers such as bacteriocin resistance, lactose-fermenting ability, bacteriophage resistance etc (von Wright and Sibakov 1998). The second system of note concerns the development of vectors that facilitate heterologous gene expression and secretion (de Vos and Simons 1994). These are particularly relevant for the exploitation of LAB as vaccine delivery vehicles, an area of research that will be discussed in more detail in a later section.

The understanding and exploitation of industrial traits are not the only aspects of LAB research that have benefited from the development of more sophisticated technologies. Reliable methods of strain identification and classification are vitally important. Newer techniques such as the ability to sequence large tracts of 16S and 23S rRNA genes using polymerase chain reaction (RAPD-PCR) and the use of pulsed field gel electrophoresis (PFGE) to fingerprint genomic restriction patterns have contributed enormously to these efforts (Axelsson 1998). This relates very much to the field of probiotics where the

ability to monitor strains through clinical trials and to evaluate their effects on the gastrointestinal tract microflora as well as the protection of their proprietary value depends on exact and reproducible strain identification.

The following sections of this review will focus on some of the properties of LAB that contribute to their roles in biopreservation and in modulating the health of their hosts.

Bacteriocins of LAB – Roles in biopreservation

Despite improved manufacturing facilities and the implementation of effective process control procedures such as HACCP (Hazard Analysis and Critical Control Points) throughout much of the food industry, the number of reported food borne illnesses has continued to rise. Concomitantly, there is a strong trend on the part of consumers favouring less processed foods containing fewer chemical preservatives (Daeschel 1993). As a result, there is an increased interest in the preservative aspects of LAB particularly in view of their long and safe association with human fermented foods. Several metabolic compounds produced by these bacteria have antimicrobial effects, including organic acids, fatty

acids, hydrogen peroxide and diacetyl (Holzapfel et al. 1995, Ouwehand 1998). However, the majority of attention has focused on the ability of many LAB to produce specific proteinaceous inhibitory substances, bacteriocins, that inhibit the growth of other bacteria and can, therefore, enhance the shelf-life of foods in which they are present. Significantly, some bacteriocins inhibit serious food-borne pathogens such as *Listeria*, *Clostridium*, *Staphylococcus*, and certain *Bacillus* spp. and *Enterococcus* spp.

At present four classes of LAB bacteriocins have been defined (Table 1). Members of classes I and II are the most frequently characterised probably reflecting the well-established isolation procedures for these bacteriocins and their potential for industrial application.

Nisin, which is produced by some *L. lactis* subsp. *lactis* strains, belongs to the class I lantibiotics and is by far the most extensively studied bacteriocin of the LAB (Dodd and Gasson 1994, Jack et al. 1995). It was discovered as far back as 1928 and has a broad spectrum of activity against many Gram-positive bacteria including *Listeria* spp. It prevents the outgrowth of germinating bacillus and clostridial spores and, through the addition of a calcium chelator, it is possible to broaden its activity to include some Gram negative bacteria (Stevens et al. 1991). The mature nisin molecule is just 34 amino acids long and undergoes extensive post-translational mod-

Table 1. Classes of bacteriocins produced by LAB.

Class	Subclass	Description
I		Lantibiotics – small, heat stable, containing unusual amino acids
II		Small (30–100 amino acids), heat stable, non-lantibiotic
	IIa	Pediocin-like bacteriocins, with anti-listerial effects
	IIb	Two peptide bacteriocins
	IIc	Sec-dependent secretion of bacteriocins
III		Large (> 30 kDa) heat-labile proteins
IV		Complex bacteriocins with glyco- and/or lipid moieties, heat stable

Adapted from Nes et al. 1996, Ouwehand 1998

ifications in which serine and threonine residues are dehydrated and several thio-ether bridges are formed. These modifications result in the formation of the five ring structures that are characteristic of the molecule. The primary target of nisin's antimicrobial action is the cell membrane. It is thought that nisin interferes with the energy supply of the cell by creating pores in the membrane and dissipating its potential (Sahl et al. 1995). Owing to its extensive genetic and molecular characterisation, nisin has been the target of several protein engineering studies aimed at broadening its functional attributes. Modified nisins containing specific amino acid substitutions have been generated some of which exhibit enhanced practical features such as increased activity against food pathogens and improved stability and/or solubility under various food-processing conditions (Kuipers et al. 1991, 1995, Rollema et al. 1995).

Other class I lantibiotic type compounds apart from nisin have been isolated from a wide variety of LAB sources. One, lacticin 3147, was recently identified from a lactococcal isolate of Irish kefir grains (used in the manufacture of buttermilk) during a collaborative study between the Teagasc Research Centre, Moorepark, Ireland and the Microbiology Department at University College, Cork, Ireland (Ryan et al. 1996). This bacteriocin is particularly attractive as it inhibits a wide spectrum of Gram-positive bacteria including potential food-borne pathogens such as *Staphylococcus*, *Clostridium* and *Listeria* spp. as well as several mastitic staphylococci and streptococci (Meaney et al. 1997). Lacticin 3147 requires two peptides for activity. Both peptides are produced in a precursor form and are subjected to post-translational modifications involving the dehydration and linkage of a number of amino acids producing typical lanthionine rings and the cleavage of prepeptide sequences. The genetic determinants of lacticin 3147 are located on a large 60 kb conjugative plasmid, pMRC01, flanked by two iso-*ISS1*-like elements (Dougherty et al. in press). The intervening region contains 13 open reading frames (ORFs), eleven of which, arranged in two operon struc-

tures, are thought to be associated with the bacteriocin functions. Six of the ORFs showed significant sequence homology with genes known to be involved in the production, immunity and transport of other recognised lantibiotics. It has not yet been possible to conclusively identify the structural genes of the bacteriocin. As with nisin, lacticin 3147 acts on susceptible cell membranes by introducing ion-specific pores that disrupt the membrane potential and rapidly cause cell death (McAuliffe et al. 1998).

Class II bacteriocins contain a wide variety of bacteriocins and, therefore, are categorised into three further subclasses. In general, however, they are all relatively small cationic peptides (30–100 amino acids) exhibiting a high degree of heat stability. Like the lantibiotics, class II bacteriocins also target the cell membrane as their active site forming oligomeric pores. However, unlike lantibiotics, their bacteriocidal activity is independent of the membrane's energisation state and appears to require a cell membrane receptor molecule. Lactococcin A, whose mode of action has been studied in some detail, is thought to insert itself as an α -helical structure across the cell membrane. Several lactococcin A molecules subsequently combine to form pores in the membrane. These pores cause an efflux of small cytoplasmic molecules and ions resulting in dissipation of the membrane potential (Venema et al. 1995).

The genetic determinants of several class II bacteriocins have been cloned and sequenced. Many have been linked to plasmids and, in some cases, an individual host may produce multiple bacteriocins (Dodd and Gasson 1994). In the case of lactococcins A, B and M, all three were located on the same plasmid (van Belkum et al. 1991, 1992).

Less is known about the classes III and IV bacteriocins. Members of the *Lactobacillus* genera produce all of the class III bacteriocins isolated to date. Helveticin J is the best known compound of this class. The legitimacy of the fourth bacteriocin class is somewhat controversial. The requirement of the glyco and/or lipid component for the action of these bacteriocins is not well

established and may be a consequence of incomplete purification procedures. The modes of action for both these classes are poorly understood (Klaenhammer 1993, Venema et al. 1995).

Clearly, many bacteriocins of the LAB, especially those with broad spectra of activity, have tremendous potential to be exploited as safe and effective 'natural' inhibitors of potential pathogenic and food spoilage bacteria in various food systems. Nisin is the classic example with a particularly long and successful history in food applications. Some of its commercial applications include: preventing clostridial spoilage of processed and natural cheeses, inhibiting the growth of some psychrotrophic bacteria in cottage cheeses, extending the shelf-life of milk in warm countries, preventing the growth of spoilage lactobacilli in beer and wine fermentations and providing additional protection against bacillus and clostridial spores in canned foods. Nisin is a permitted food additive in more than 50 countries including the US and Europe where it is commercially available through Aplin and Barrett (UK) under the trade name Nisaplin® (Vandenberg 1993, Delves-Broughton et al. 1996).

The emergence of *Listeria*, specifically *Listeria monocytogenes*, as a serious food-borne pathogen is of major concern in the food industry especially in light of the fact that these bacteria are common contaminants of many raw food materials such as milk, meat and vegetables (Ryser and Marth 1991). Consequently, bacteriocins belonging to the subclass IIa, which demonstrate antilisterial activity, have received significant research attention. Pediocin PA-1/AcH produced by *Pediococcus acidilactici* is regarded as the prototype bacteriocin of this subclass and various studies have demonstrated its ability to control *Listeria* in cheese, vegetable and meat systems. The application of pediocin in the biopreservation of meats is particularly relevant, as nisin is not very effective in this environment. It is also significant to note that *Pediococcus acidilactici* is a common starter culture used in the production of most fermented meats (Vandenberg 1993, Stiles 1996).

The bacteriocin, lacticin 3147, has also been

the subject of food application studies. Ryan et al. (1996) developed a range of lacticin 3147-producing starter strains suitable for use in commercial cheese making. When incorporated, these strains effectively controlled the growth of any non-starter LAB in Cheddar cheese and completely eliminated deliberately inoculated *Listeria monocytogenes* from cottage cheese. Lacticin 3147 has a number of advantages over nisin. It is effective at neutral pH and starter cultures producing this bacteriocin have good acid producing and bacteriophage resistance properties unlike their counterparts producing nisin. Significantly, C. Hill (University College, Cork), W.J. Meaney and P. Ross (Teagasc, Moorepark) are seeking approval from the European Agency for the Evaluation of Medicinal Products (Veterinary Medicines Evaluation Unit) for the use of lacticin 3147 as a mastitis-controlling therapy in dry cows (C. Hill, pers. comm.).

Unfortunately, a major drawback associated with LAB bacteriocins lies in the fact that gram negative bacteria as well as yeasts and moulds are normally refractive to their bactericidal action. As a result, the usefulness of these compounds in commercial practice has been somewhat limited as many important food borne pathogens and food spoilage microorganisms belong to these resistant categories. This has sparked several recent studies aimed at broadening the bactericidal activity of LAB bacteriocins to encompass these normally resistant groups. In general, these studies have focused on the synergistic effects of bacteriocins, most notably nisin, with other antibacterial factors such as the lactoperoxidase system present in milk, hydrolytic enzymes, various chelating agents (including siderophores) and other bacteriocins (Helander et al. 1997).

To date, nisin remains the only LAB bacteriocin to be legally permitted as a food additive. This has had major implications for the many other bacteriocins that, in recent years, have demonstrated commercial potential. Two products, ALTA™2431 and Microgard®, have been developed as shelf-life extenders based on crude LAB fermentation products and, therefore, do not re-

quire a food additive label. ALTA™2341 is produced from a *Pediococcus acidilactici* fermentation and is assumed to rely on the inhibitory effects of Pediocin PA-1/AcH. It is commonly added to Mexican soft cheeses which are particularly susceptible to listerial contamination (Glass et al. 1995). Microgard® is the result of a *Propionibacterium* fermentation. It is active against Gram negative bacteria such as *Pseudomonas*, *Salmonella*, and *Yersinia*, as well as yeasts and moulds. Microgard®'s protective action is probably due to the presence of propionic acid as a metabolic end-product. However, a role for a bacteriocin in this product has also been proposed (Al-Zoreky et al. 1991). Microgard® has been approved by the FDA for use in food applications such as cottage cheese and fruit-flavoured yoghurts. Approximately 30% of the cottage cheese produced in the US contains this product as a preservative. In another product, Bioprofit®, a combination of specific *Lactobacillus* and *Propionibacterium* strains is used as a protective adjunct to normal starter cultures to inhibit the growth of yeasts, moulds, *Bacillus* spp. *Clostridium* spp. and heterofermentative lactobacilli during some dairy fermentations (Mäyrä- Mäkinen and Suomalainen 1995).

It must be emphasised that the use of bacteriocins either exogenously or by the adventitious use of bacteriocin-producing cultures should not be regarded as a panacea for poor-quality raw materials or manufacturing practices. Instead, it is proposed that bacteriocins be used in combination with other physical, chemical and microbial preservation factors as an additional 'hurdle' against potential pathogenic or food spoilage bacteria.

LAB and health: Probiotic studies

As we approach a new millennium, there is a growing appreciation world-wide that a healthy lifestyle, including diet, can play a major role in preventing diseases and promoting human health.

Functional foods containing probiotic cultures are a well-established concept in Japan and, in recent years, such products comprise a rapidly expanding, lucrative, internal and export market for the EU. Several factors have fuelled this interest in functional foods. Today, consumers are better informed than ever and are keen to take proactive decisions with regard to maintaining their health. Changing population dynamics towards older societies and the increased prevalence of chronic illnesses such as cardiovascular disease and cancer are placing heavy demands on already stretched and expensive healthcare services. In addition, there is serious concern at the dramatic increase in microbial resistance to antibiotics as a result of widespread overprescription and misuse. In this context, the World Health Organisation (WHO) has advocated moving towards alternative disease control strategies including the use of probiotic bacteria in the prevention and treatment of certain infections (Bengmark 1998).

The human gastrointestinal (GI) tract supports a rich and dynamic microbial population of more than 500 bacterial species. Maintaining this delicately balanced ecosystem is important for the normal functioning of the gut, particularly with regard to preventing GI infections and stimulating the host's immune response. Modern antibiotic treatments, radiation therapy, stress and Western dietary preferences can significantly affect the gut microflora predisposing the host to various diseases (Salminen et al. 1995, 1998a, Schaafsma 1995).

Probiotic cultures are generally defined as live, non-pathogenic bacteria which when ingested exert a positive influence on the host's health. *Lactobacillus* spp. and *Bifidobacterium* spp. are prominent members of the commensal intestinal flora of most healthy individuals and are the most commonly studied probiotic bacteria. Their probable and theoretical benefits have been outlined in several recent reviews and include reduced lactose intolerance, alleviation of some diarrhoeas, lowered blood cholesterol, increased immune responses and prevention of cancer (Marteau and Rambaud 1993, 1996, Gilliland

1996, Salminen et al. 1996, 1998a). The concept of probiotics is not new, having been proposed originally by Metchnikoff in 1907. However, despite numerous studies in the past, there has been very little convincing scientific evidence to substantiate their health claims until recently. This has been due to difficulties in unequivocally identifying strains, differences in experimental systems and data interpretation and a general lack of coordination between clinicians and microbiologists (Sanders 1994). Considerable efforts have been made recently to redress this situation. Modern taxonomic methods have improved the identification of test strains and emphasis is being placed on performing random double-blind placebo-controlled clinical trials to demonstrate the efficacy of potential probiotic strains and products. Table 2 outlines the agreed criteria that should be fulfilled by such studies.

Many different strains of both *Lactobacillus* and *Bifidobacterium* have been used in probiotic preparations. Few, however, have well documented beneficial properties. Salminen et al. (1998a) presented a comprehensive list of successful probiotic strains and their reported clinical effects. *Lb. acidophilus* NCFB 1478, *Lb. johnsonii* LA1, *Lb. casei* Shirota strain and *Lb. rhamnosus* GG are among the best studied and have consistently demonstrated their effectiveness in carefully designed trials that fulfil the requirements in Table 2. Selection criteria for probiotic LAB include: human origin, safety, viability/activity in delivery vehicles, resistance to acid and bile, adherence to gut epithelial tissue, ability to colonise the GI tract, production of antimicrobial substances, ability to stimulate a host immune response and the ability to influence metabolic activities such as vitamin production, cholesterol assimilation and lactase activity (Huis in't Veld and Shortt 1996, Salminen et al. 1996). Of course, it is unlikely that any individual strain will be able to present all of these credentials and a blend of strains with complementary attributes may be required to deliver optimum probiotic performance.

At University College, Cork, Ireland, Collins and co-workers applied stringent *in vitro* selec-

Table 2. Requirements for clinical studies of probiotic foods for functional and clinical use.

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- Each strain documented and tested independently
 - Extrapolation of data from closely related strains not acceptable
 - Well defined probiotic strains, study products, and study populations
 - Double-blind, placebo-controlled, and randomised human studies
 - Result confirmed by several independent research groups
 - Publication in peer-reviewed journals
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(Salminen et al. 1996, 1998a)

tion criteria to a bank of human *Lactobacillus* isolates in an effort to identify a range of new strains with potential probiotic characteristics. Eight candidates survived the selection process and one, *Lb. salivarius* UCC118 producing a broad spectrum anti-microbial protein, was chosen for further clinical trials. *Lb. salivarius* UCC118 was demonstrated to be efficiently delivered to the gut following oral administration in milk or yoghurt carriers. In addition, in a proportion of volunteers (<10%) significant numbers were still present in faeces 3 weeks after the cessation of its administration, indicating that this strain was capable of colonising the human GI tract *in vivo*. *Lb. salivarius* UCC118 did not disturb the numbers of other lactobacilli in the gut, but there was a statistically significant reduction in the numbers of excreted clostridia. Although it is recognised that further work is required to build up the medical dossier on *Lb. salivarius* UCC118, these preliminary trials strongly support this strain as an effective probiotic culture (K. Collins, pers. comm.).

Most of the disease states that benefit from LAB therapy are characterised to a greater or lesser extent by a disturbed intestinal microflora, intestinal inflammation and increased gut permeability. For example, there is clear evidence that lactose intolerant individuals tolerate fermented dairy products better than their unfermented counterparts even when they contain significant amounts of lactose. Milks fermented by various LAB, including the common yoghurt

cultures, *Lb. bulgaricus* and *S. thermophilus*, are effective and viable cultures are important to achieve maximum benefits. At least three mechanisms, or a combination thereof, are thought to contribute to the effect: the reduced lactose concentration in the fermented product, breakdown of lactose in the gut lumen by residual LAB lactase activity and a slower transit time in the intestines of the fermented products compared to liquid milk (Gilliland 1996, Salminen et al. 1996, 1998a).

Probiotic preparations have also been found to be beneficial in the prevention and treatment of certain GI infections including infantile rotavirus diarrhoea and diarrhoeas associated with antibiotic and pelvic radiation treatments. Strongest evidence has been presented for *Lactobacillus* GG but positive results were also achieved with *Lb. johnsonii* LA1 and NCFB 1748, *Lb. casei* Shirota strain and more recently with *Lb. reuterii* (Lee and Salminen 1995, Salminen et al. 1998a). The mechanisms by which these effects were achieved are not well defined. However, the ability of the lactobacilli to adhere to and potentially modify host mucosal surfaces is thought to be important. It is likely that the lactobacilli suppress the growth of pathogens at the mucosal surface probably by out-competing them for nutrients or by producing antibacterial compounds (Salminen et al. 1998a, Isolauri et al. 1998).

Cardiovascular disease is responsible for approximately half of the Western world's deaths and high serum cholesterol levels are usually indicative of an increased risk of this disease. Consequently, claims regarding the potential cholesterol-lowering properties of probiotic cultures have attracted much research attention. The results to date are inconclusive. Several studies demonstrated that various strains could assimilate cholesterol *in vitro*. However, reliable data regarding an *in vivo* function have not yet been reported (Lichtenstein and Goldin 1998).

The ability of GI microflora to enzymatically convert precursors naturally present in the diet to carcinogenic forms is well documented and is likely to contribute to the aetiology of colonic

cancer. Significantly, LAB and bifidobacteria tend to have low levels of such activities in comparison to other gut bacteria. Several studies in both animals and humans have demonstrated the ability of these bacteria to reduce the toxicity of intestinal contents by suppressing the levels of bacterial enzymes such as β -glucuronidase, nitroreductase, azo-reductase and urease, all of which have been implicated in activating procarcinogens (Salminen et al. 1996, 1998a, Isolauri et al. 1998). In addition, many LAB produce metabolic end-products (butyrate/butyric acid) that have anti-tumorigenic activities *in vitro* (Young, G. 1996). There are also a number of *in vitro* and *in vivo* animal studies that demonstrate more directly tumour inhibition by LAB. In humans, the evidence for such activities is still largely circumstantial. Recently, however, Aso and co-workers (Aso and Akazan 1992, Aso et al. 1995) reported the first clinical instances in which oral administration of *Lb. casei* Shirota strain was shown to reduce the recurrence of superficial bladder carcinoma in humans.

It has also been documented that various LAB can modulate the host immune response. Reports have described increased production of immunoglobulins, interleukins 6 and 10, gamma interferon, tumour necrosis factor- α and increased phagocytic activity. Notably, *Lactobacillus* GG was able to stimulate local and systemic IgA to rotavirus during infection of children with this agent (Kaila et al. 1992). This effect was thought to contribute to protection against reinfection. *Lb. salivarius* UCC118 also exhibited a strong mucosal IgA immune response in human volunteers during clinical trials (Mattila-Sandholm 1997). Both *Lactobacillus* GG and *Lb. johnsonii* LA1 have been successfully used as adjuvants to oral vaccines (Isolauri et al. 1998).

Another approach to the maintenance of a healthy gut microflora is the provision of substrates that preferentially select for the growth of desirable bacteria in the host. These substrates, called prebiotics, are based on non- or slowly absorbable complex carbohydrates that can be assimilated by beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* but in contrast

are hardly ever utilised by potentially pathogenic Gram-negative organisms. Examples of prebiotic substrates include inulin, lactulose, various galacto-, fructo-, xylo-oligosaccharides and sugar alcohols such as lactitol and xylitol (Salminen et al. 1998b). Many of the functional foods recently launched in Europe contain a combination of a probiotic culture with a prebiotic substrate that favours its growth. One such 'synbiotic' product is the fermented drink Fyos (Nutricia), which combines the probiotic culture *Lb. casei* with the prebiotic oligofructose, inulin, LA7 (Bauer), Vifit (Mona) and Actimel (Danone) employ similar strategies (Young, J. 1996).

Although in comparison with Japan, the European and US markets for functional foods are still relatively underdeveloped, there are definite indications that this situation is changing. It has been estimated that by the year 2000, the global market for these products will be in the region of \$17 billion (Young, J. 1996). In Europe especially, there is a growing number of dairy-based products available that contain probiotic cultures and/or prebiotic substrates (Table 3). Currently most companies are adopting a prudent approach to marketing their probiotic products relying on general health claims such as 'helps boost the body's natural defences' or 'restores the body's natural balance'. In light of the high R+D costs and in order for these products to achieve a maximum return on investment, it is essential that consumers are presented with clear and substantiated health claims. The PROBDEMO project has an important role to play in this respect and underlines the EU's commitment to supporting this market segment.

LAB as live vaccine delivery vehicles

In recent years there has been increasing interest in exploiting some LAB as live vaccine delivery vehicles. LAB present a number of advantages that make them attractive for this function. They have a long history of safe use in foods, there is extensive knowledge already available

regarding their production on a large scale and, through fermented products, they are easily administered orally. Furthermore, it is recognised that the gut is an important site for antigen immune education.

Different approaches have been adopted in the development of LAB-based vaccines. One relies on colonising lactobacilli that are capable of remaining in the gut or genital tract for a period of time during which an immune response may be elicited to an expressed antigen. *Lactobacillus* vectors are chosen on the basis of their potential for genetic manipulation and the ability to express foreign antigens as well as for their capacity to stimulate a host immune response. Several lactobacilli including *Lb. casei* and *Lb. plantarum* strains have been targeted for research. In another approach, the oral commensal bacterium *S. gordonii* has been exploited. This strain is particularly advantageous, as it is easily transformable at high frequencies by natural competence. Also, the strain colonises the oral cavity very efficiently and has been demonstrated to colonise mice vaginal tracts for up to 8 weeks. A third strategy has focused on the use of non-colonising *L. lactis* strains. In this instance, antigens expressed by these bacteria are generally retained intracellularly and, therefore, are not as susceptible to degradation in the gut and, in addition, there is evidence that protein antigens are more immunogenic when they are contained either within or associated with the recombinant bacteria.

Results to date with all three approaches are encouraging. Several antigenic epitopes have been expressed in all three host types using various cellular locations (intracellular, cell surface, extracellular) and have demonstrated an ability to elicit local and systemic immune responses (Wells et al. 1996).

Concluding remarks

Without doubt, advances in biotechnology over the last two to three decades have significantly

Table 3. Examples of fermented milk products containing probiotic bacteria available in food retail outlets in Europe, the UK and Ireland.

Product	Brand name	Company (Organism – 10^7 – 10^8 viable LAB/ml)	Countries
Yoghurt	LC1	Nestlé (<i>Lb. johnsonii</i> LA1)	France, Belgium, Spain, Switzerland, Portugal, Italy, Germany, UK.
Yoghurt	Gefilus	Valio (<i>Lb. rhamnosus</i> GG)	Finland
Yoghurt	Vifit	Mona (<i>Lb. rhamnosus</i> GG)	Netherlands, Ireland
Yoghurt	Vifit	Sudmilch (<i>Lb. rhamnosus</i> GG)	Germany
Yoghurt drink	Yo-Plus	Waterford Foods (<i>Lb. acidophilus</i>)	Ireland
Yoghurt	Bio-Pot	Onken (Biogarde cultures)	Europe
Yoghurt	LA7	Bauer (<i>Lb. acidophilus</i>)	Germany
Fermented milk drink	Yakult	Yakult (<i>Lb. casei</i> Shirota strain)	Netherlands, UK, Germany
Cultured yoghurt-style product	Gaio	MD Foods (<i>E. faecium</i>)	Denmark
Yoghurt	SNO	Dairygold (<i>Lb. acidophilus</i>)	Ireland
Yoghurt	Actimel Cholesterol Control	Danone (<i>Lb. acidophilus</i>)	Belgium
Fermented milk drink	Actimel	Danone (<i>Lb. casei</i>)	Europe
Yoghurt	Yoplait	Waterford Foods (<i>Lb. acidophilus</i>)	Ireland
Fermented milk drink	Bra-Mjolk	Arla (<i>Bifidus</i> , <i>Lb. reuterii</i> , <i>Lb. acidophilus</i>)	Sweden
Fermented milk drink	Fyos	Nutricia (<i>Lb. casei</i>)	Netherlands
Yoghurt	Symbalance	Tonilait (<i>Lb. reuterii</i> , <i>Lb. casei</i> , <i>Lb. acidophilus</i>)	Switzerland
Yoghurt	Shape	St. Ivel (<i>Lb. acidophilus</i>)	Ireland, UK

(Young, J. 1996 and various sources)

expanded our ability to produce high quality, nutritious and tasteful foods that remain fresher for longer, are completely safe and that are less reliant on artificial additives. The potential applications of bacteriocins as 'consumer friend-

ly' biopreservatives either in the form of protective cultures or as additives are significant. Disappointingly, with the exception of nisin and to a much lesser extent Pediocin PA-1/AcH, very little of this potential has been realised in an in-

dustrial context. Despite strong arguments in favour of their efficacy and safety, the process of obtaining regulatory approval for the more widespread use of these compounds (other than nisin) in various foodstuffs is lengthy and expensive. Progress in this regard will be necessary to unblock a major bottleneck facing the practical application of these important but, as yet, underexploited proteins of LAB.

In contrast, the development of the functional foods market, particularly with regard to the use of probiotic cultures, has been exceptional over the last few years and is poised to grow considerably more. In order to support this growth, several fundamental issues need to be addressed. A major challenge for scientists will be unravelling the complex probiotic-host interactions and activities that dictate the *in vivo* functionality of these bacteria. Obviously this is quite a daunting task given the complexity of the human microbiota and the multiplicity of interdependent reactions that are likely to be involved. Essential to these efforts, however, will be a thorough understanding of the genetics and molecular biology of these probiotic strains. Unfortunately, many of the strains that show the most probiotic potential are very difficult to manipu-

late technically, a factor that is sometimes overlooked in initial selection procedures. While the benefits of probiotic cultures appear to be many and wide-ranging, at present very few have real scientific backing. It is important that in the rush to expand the market for these products, unsubstantiated claims or adverse publicity do not damage consumer confidence. The public in general, and especially those involved in consumer affairs and in policy decision-making bodies, must be carefully educated regarding their potential benefits. In addition, important consumer requirements such as taste and convenience should not be compromised in the development of effective probiotic products.

Extending the traditional fermentation roles of LAB is an important goal of scientific research and new product development. In this respect, the use of certain LAB as potential vaccine delivery vehicles has opened up a completely new avenue for the exploitation of these bacteria. Although this area is in the early stages of development as yet, the initial successes in eliciting immune responses to heterologous antigens bode well for the future development of new oral vaccines.

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SELOSTUS

Terveyttä ja ruoan turvallisuutta edistävät maitohappobakteerien biotekniset sovellukset

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Ruoka-aineiden käyttäminen maitohappobakteerien avulla on yksi vanhimmista säilöntämenetelmistä. Näiden teollisuustuotannossakin merkittävien maitohappobakteerien geneettisten ja fysiologisten ominaisuuksien tutkimuksessa on viime vuosina edistytty merkittävästi. Yksi maitohappobakteerien tärkeä ominaisuus on niiden kyky tuottaa mikrobeille vastustuskykyisiä yhdisteitä. Mikrobeille vastustuskykyiset yhdisteet kiinnostavat aiempaa enemmän, koska nii-

tä voidaan käyttää elintarviketeollisuudessa kemiallisten säilöntäaineiden sijaan.

Kuluttajat ovat yhä enemmän tietoisia ruoan ja terveyden välisestä yhteydestä. Viime aikaiset tutkimustulokset tukevat oletuksia, joiden mukaan maitohappobakteereilla on probioottisia, terveyttä edistäviä, ominaisuuksia. Lisäksi tällä hetkellä selvitetään eräiden maitohappobakteerien kykyä toimia suun kautta nautittavien rokotteiden elävinä kuljetusvektoreina.