Review article

Probiotic bacteria: safety, functional and technological properties

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Abstract

During the past two decades probiotic (health promoting) micro-organisms have been increasingly included in various types of food products, especially in fermented milks. Several aspects, including safety, functional and technological characteristics, have to be taken into consideration in the selection process of probiotic micro-organisms. Safety aspects include specifications such as origin (healthy human GI-tract), non-pathogenicity and antibiotic resistance characteristics. Functional aspects include viability and persistence in the GI-tract, immunomodulation, antagonistic and antimitagenic properties. Before probiotic strains, chosen on the basis of their good safety and functional characteristics, can benefit the consumer, they must first be able to be manufactured under industrial conditions. Furthermore, they have to survive and retain their functionality during storage, and also in the foods into which they are incorporated without producing off-flavours. Factors related to the technological and sensory aspects of probiotic food production are of utmost importance since only by satisfying the demands of the consumer can the food industry succeed in promoting the consumption of functional probiotic products in the future. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Probiotic bacteria; Health; Safety

1. Introduction

Probiotics are live microbial food supplements which benefit the health of consumers by maintaining or improving their intestinal microbial balance (Fuller, 1989). Due to their perceived health benefits probiotic bacteria have been in-
creasingly included in yoghurts and fermented milks during the past two decades. Most commonly they have been lactobacilli such as *Lactobacillus acidophilus*, and bifidobacteria often referred to as ‘bifidus’ (Daly and Davis, 1998). A major development in functional foods pertain to foods containing probiotics and prebiotics which enhance health promoting microbial flora in the intestine. There is growing scientific evidence to support the concept that the maintenance of healthy gut microflora may provide protection against gastrointestinal disorders including gastrointestinal infections, inflammatory bowel diseases, and even cancer (Haenel and Bendig, 1975; Mitsuoka, 1982; Salminen et al., 1998a). The use of probiotic bacterial cultures stimulates the growth of preferred micro-organisms, crowds out potentially harmful bacteria, and reinforces the body’s natural defence mechanisms. Today, plenty of evidence exists on the positive effects of probiotics on human health. However, this has usually been demonstrated in diseased human populations only (Salminen et al., 1998a). Thus there is an urgent need for evidence for probiotic health benefits in average (generally healthy) populations.

Before a probiotic can benefit human health it must fulfil several criteria: It must have good technological properties so that it can be manufactured and incorporated into food products without loosing viability and functionality or creating unpleasant flavours or textures; it must survive passage through the upper gastrointestinal (GI) tract and arrive alive at its site of action; and it must be able to function in the gut environment. To study the probiotic strain in the GI-tract, molecular techniques must be established for distinguishing the ingested probiotic strain from the potentially thousands of other bacterial strains that make up the gastrointestinal ecosystem. Additionally, techniques are required to establish the effect of the probiotic strain on other members of the intestinal microbiota and importantly on the host. This includes not only positive health benefits, but also demonstration that probiotic strains do not have any deleterious effects. Armed with this knowledge, the probiotics can then enter human pilot studies that attempt to assess their health benefits to consumers (Mattila-Sandholm and Salminen, 1998; Mattila-Sandholm et al., 1999).

2. Selecting probiotic strains: important aspects

The theoretical basis for the selection of probiotic micro-organisms including safety, functional and technological aspects is illustrated in Fig. 1.

Current safety criteria for successful probiotics have been defined in several reviews (Lee and Salminen, 1995; Donohue and Salminen, 1996; Salminen et al., 1996b, 1998b; Adams, 1999). The significance of human origin has been debated recently, but most current successful strains are indicated to be of human origin. It can also be argued that a probiotic strain can function better in a similar environment (e.g. human GI-tract) to where it was originally isolated from. Safety aspects include the following specifications:

1. Strains for human use are preferably of human origin.

![Fig. 1. The theoretical basis for selection of probiotic microorganisms includes safety, functional (survival, adherence, colonisation, antimicrobial production, immune stimulation, antigenotoxic activity and prevention of pathogens) and technological aspects (growth in milk, sensory properties, stability, phage resistance, viability in processes).](image-url)
2. They are isolated from healthy human GI-tract.
3. They have a history of being non-pathogenic.
4. They have no history of association with diseases such as infective endocarditis or GI-disorders.
5. They do not deconjugate bile salts (bile salt deconjugation or dehydroxylation would be a negative trait in the small bowel; Marteau et al., 1995).
6. They do not carry transmissible antibiotic resistance genes.

The functional requirements of probiotics should be established by using in vitro methods and the results of these studies should be reflected in controlled human studies. While selecting a preferable probiotic strain several aspects of functionality have to be considered:
1. Acid tolerance and tolerance to human gastric juice.
2. Bile tolerance (an important property for survival in the small bowel).
3. Adherence to epithelial surfaces and persistence in the human GI-tract.
4. Immunostimulation, but no proinflammatory effect.
5. Antagonistic activity against pathogens such as *Helicobacter pylori*, *Salmonella* sp., *Listeria monocytogenes* and *Clostridium difficile*.
6. Antimutagenic and antigarcinogenic properties.

Feeding trials with different probiotic strains have shown that the probiotic strain usually disappears from the GI-tract within a couple of weeks after the ingestion is discontinued (Fukushima et al., 1998; Johansson et al., 1998; Alander et al., 1999; Donnet-Hughes et al., 1999).

The role of the probiotic persistence in the human GI-tract has therefore been questioned. However, even temporary persistence, which has been noted for several ingested probiotic strains, may enhance their chances for beneficial functions in the GI-tract, and is therefore considered a desirable trait.

Even though a probiotic strain fulfils the necessary safety and functional criteria the aspects related to probiotic production and processing are also of utmost importance. Several technological aspects have to be considered in probiotic selection. These include the following:
1. Good sensory properties.
2. Phage resistance.
3. Viability during processing.
4. Stability in the product and during storage.

Good viability and activity of probiotics are considered prerequisites for optimal functionality. However, several studies have shown that non-viable probiotics can have beneficial effects such as immune modulation and carcinogen binding in the host (for a review see Ouwehand and Salminen, 1998; Salminen et al., 1999). Thus, for certain probiotic strains it might be sufficient that they grow well during initial production steps (to obtain high enough numbers in the product) but they do not necessarily need to retain good viability during storage.

Safety, functional and technological aspects of probiotics are discussed in more detail below. The review is limited to *Lactobacillus* and *Bifidobacterium*, since these genera are by far the most important with probiotic strains for human use.

### 3. Safety aspects of probiotics

The safety of probiotic strains is of prime importance and guidelines for the safety assessment can be found in several articles (Lee and Salminen, 1995; Donohue and Salminen, 1996; Salminen et al., 1996b, 1998b; Adams, 1999) (Fig. 2). Several approaches are possible in the assessment of the probiotic safety: (a) studies on the intrinsic properties of the probiotic strain; (b) studies on...
the pharmacokinetics of the probiotic strain; and (c) studies on interactions between the probiotic strain and the host.

Knowledge on survival of the probiotics within the GI-tract, their translocation and colonization properties, and the fate of probiotic-derived active components is important for the evaluation of possible positive and negative effects of probiotic consumption. The survival of different probiotic strains in different parts of the GI-tract varies: Some strains are rapidly killed in the stomach while others can pass through the whole gut in high numbers (Marteau et al., 1993). The pharmacokinetics of probiotics has been studied in vivo using intubation, perfusion and biopsy techniques (Pochart et al., 1992; Johansson et al., 1993; Nielsen et al., 1994; Alander et al., 1997; Marteau et al., 1997). Some enzymatic properties such as excessive deconjugation of bile salts or degradation of mucus has been postulated to be potentially detrimental (Marteau et al., 1995; Ruseler-van Embden et al., 1995). Such properties can be studied in vitro. Platelet aggregation properties (Korpela et al., 1997), enzymes which seem to favour cardiac valve colonization (Pelletier et al., 1996) and formation of unwanted metabolites can also be studied in vitro.

Assessing the risks of probiotic consumption could be a very expensive and time-consuming task. A low risk may have to be accepted when recommended to immunocompromised individuals, but the risk to benefit ratio needs to be clearly established in such cases. This requires relevant information on the efficacy and safety of the products. At present, the available information on current probiotic lactic acid bacteria provides good safety back-up. To our knowledge there are up to date two reports of probiotic bacterium causing infection. A L. rhamnosus strain indistinguishable from L. rhamnosus GG has been isolated from a liver abscess from an elderly lady with a history of hypertension and diabetes mellitus (Rautio et al., 1999). In another case a probiotic L. rhamnosus strain (strain or product specifications were not given) was suggested to have caused endocarditis in an elderly male (Mackay et al., 1999). However, unlike in the liver abscess case where strain identification was based on thorough genomic fingerprinting of the L. rhamnosus isolates, the endocarditis case study relied on conventional phenotypic strain identification methods and on pyrolysis mass spectrometry identification. The discriminatory power of conventional phenotypic identification methods in strain differentiation is usually poor and there is no data on the ability of pyrolysis mass spectrometry to differentiate between different lactobacillar strains. Therefore the data on the endocarditis case must be considered insufficient for proving that the ingested probiotic L. rhamnosus strain and not one of the indigenous L. rhamnosus strains is the causative agent.

These two findings (one proved and one yet presumptive) indicate that although probiotic products have been safely consumed in large quantities over the years throughout Europe and Japan, occasional severe infections, especially in immunocompromised patients, may occur.

3.1. Safety considerations of antibiotic resistances in lactobacilli and bifidobacteria

Due to the indiscriminate use of antibiotics in human and veterinary medicine and in animal growth promoters antibiotic resistance has become an increasingly common characteristic in micro-organisms (Austin et al., 1999; Robredo et al., 2000), thus causing serious problems in treatment of microbial infections. Antibiotic resistance in bacteria may be intrinsic or acquired. Intrinsic resistance is a naturally occurring trait and may be considered as a species characteristic, whereas acquired resistance derives either from genetic mutations or acquisition of foreign DNA from other bacteria.

Lactobacilli display a wide range of antibiotic resistances naturally (Charteris et al., 1998b), but in most cases antibiotic resistance is not of the transmissible type. Lactobacillus strains with non-transmissible antibiotic resistances do not usually form a safety concern. Several species of lactobacilli including L. rhamnosus and L. casei are intrinsically resistant to vancomycin (Nicas et al., 1989; Swenson et al., 1990; Charteris et al., 1998b). These species have peptidoglycan precursors terminating with D-lactate instead of the
target precursor for vancomycin activity terminating with D-alanine (Billot-Klein et al., 1994). Many intrinsically vancomycin resistant strains of lactobacilli have a long history of safe use as probiotics and there is no indication that vancomycin resistant lactobacilli could transfer the resistance to other bacteria. Tynkkynen et al. (1998) have demonstrated that the vancomycin resistance factor of the probiotic strain *L. rhamnosus* GG is not closely related to those of enterococci, and they could not observe the transfer of antibiotic resistances between *L. rhamnosus* GG and enterococci.

Although plasmid-linked antibiotic resistances are not very common among lactobacilli, they do occur (Ishiwa and Iwata, 1980; Vescovo et al., 1982; Rinckel and Savage, 1990), and their safety implications should be taken into consideration. Since transfer of antibiotic resistant genes may occur between phylogenetically distant bacteria (Courvalin, 1994), strains harbouring mobile elements carrying resistance genes should not be used either as human or animal probiotics. Checking the ability of a proposed probioticstrain to act as a donor of conjugative antibiotic resistance genes may be a prudent precaution especially when probiotics are administered during antibiotic therapy, and at least in the case of animal feeding, where the use of antibiotics as growth promoters apparently creates selective advantage for spreading of the resistance factors. Antibiotic susceptibility patterns vary greatly between different species of lactobacilli, and strains with atypical resistance to some clinically important antibiotics have been detected among lactobacilli (Charteris et al., 1998b; Felten et al., 1999), indicating the necessity for susceptibility testing of each probiotic strain.

Most bifidobacteria are intrinsically resistant to nalidixic acid, neomycin, polymyxin B, kanamycin, gentamycin, streptomycin and metronidazole (Miller and Finegold, 1967; Matteuzzi et al., 1983; Charteris et al., 1998a). In earlier studies vancomycin has been found highly inhibitory against bifidobacteria (Miller and Finegold, 1967; Lim et al., 1993), whereas in a recent study by Charteris et al. (1998a) vancomycin resistance was suggested to be a general characteris- tic of bifidobacteria. However, differences in the techniques used in susceptibility testing hinders the comparison of data. Suppression of fecal counts of bifidobacteria during vancomycin therapy would suggest susceptibility of intestinal bifidobacteria to this agent (Edlund et al., 1997). Studies on the genetics of the antibiotic resistance of bifidobacteria are warranted for understanding of the mechanisms of resistance in this genus.

4. Functional aspects of probiotics

Clinical effects of some probiotic strains in humans are shown in Table 1. These include, for example, immunomodulation, modulation of intestinal flora, prevention of diarrhoeas, and lowering of fecal enzyme activities. Some of the functional aspects of probiotics are discussed in more detail below.

4.1. Adhesion properties

Adhesion of probiotic strains to the intestinal surface and the subsequent colonization of the human GI-tract has been suggested as an important prerequisite for probiotic action. Adherent strains of probiotic bacteria are likely to persist longer in the intestinal tract and thus have better possibilities of showing metabolic and immunomodulatory effects than non-adhering strains. Adhesion provides an interaction with the mucosal surface facilitating the contact with gut associated lymphoid tissue mediating local and systemic immune effects. Thus, only adherent probiotics have been thought to effectively induce immune effects and to stabilise the intestinal mucosal barrier (Salminen et al., 1996a). Adhesion may also provide means of competitive exclusion of pathogenic bacteria from the intestinal epithelium: Exclusion of pathogens by lactic acid bacteria and bifidobacteria has been shown in vitro using Caco-2 and HT-29-MTX cell lines (Bernet et al., 1993, 1994; Coconnier et al., 1993a,b). In the inhibition of pathogen adhesion in vitro both living and heat-killed *L. acidophilus* cells have been effective (Coconnier et al., 1993a).
<table>
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<tr>
<th>Strain</th>
<th>Clinical effects in humans</th>
<th>References</th>
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<tr>
<td><em>Lactobacillus casei</em> Shiroti</td>
<td>Modulation of intestinal flora, lowering faecal enzyme activities, positive effects on superficial bladder cancer and cervical cancer, no influence on the immune system of healthy subjects</td>
<td>Aso and Akazan (1992), Okawa et al. (1993), Tanaka and Ohwaki (1994), Aso et al. (1995), Spanhaak et al. (1998)</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em> DSM9843 (299v)</td>
<td>Modulation of intestinal flora, increase in faecal short-chain fatty acid content</td>
<td>Johansson et al. (1993, 1998)</td>
</tr>
<tr>
<td><em>Saccharomyces boulardii</em></td>
<td>Prevention of antibiotic-associated diarrhoea, treatment of <em>Clostridium difficile</em> colitis, prevention of diarrhoea in critically ill tube-fed patients</td>
<td>Surawicz et al. (1989), Buts et al. (1993), McFarland et al. (1994), Bleichner et al. (1997)</td>
</tr>
<tr>
<td>Yoghurt strains (<em>Streptococcus thermophilus</em> and/or <em>L. delbrueckii</em> subsp. <em>bulgaricus</em>)</td>
<td>No effect on rotavirus diarrhoea, no immune enhancing effect during rotavirus diarrhoea, no effect on faecal enzymes, weak effect on respiratory burst activity of blood leucocytes but not on overall phagocytic activity in healthy adults</td>
<td>Goldin et al. (1992), Majamaa et al. (1995), Domnet-Hughes et al. (1999)</td>
</tr>
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Controlled comparable studies on in vitro model systems, such as the human colon carcinoma cell lines HT-29 and Caco-2, are important in the assessment of adhesion properties (Chauviere et al., 1992; Lehto and Salminen, 1997; Tuomola and Salminen, 1998). HT-29 and Caco-2 cell lines differentiate into enterocytes and can thus be used as a model for the small intestine epithelium. A mucus (gel covering the intestinal epithelial surface) secreting variant of the HT-29 cell line, HT-29-MTX, has also been used in adhesion studies (Tuomola, 1999). In vitro adhesion experiments have indicated differences in the adhesion potential of different probiotic strains (Lehto and Salminen, 1997; Tuomola and Salminen, 1998). Lately, glycoproteins obtained from human ileostomy effluent or from faecal material have been used as a model for small intestinal mucus in probiotic adhesion assays (Kirjavainen et al., 1998; Tuomola et al., 1999). The mucus layer is the first contact between ingested bacteria and intestinal mucous membrane, and therefore important additional data on probiotic adhesion properties can be obtained by supplementing the data obtained with cell lines without mucus-secreting ability (Caco-2 and HT-29) with results of mucus adhesion assays. Already it has been shown that probiotic strains vary in their ability to adhere to mucus, and also that strains adhering to Caco-2 cells do not necessarily adhere to mucus as efficiently (Tuomola et al., 1999). Furthermore, it has been shown that bifidobacteria adhere differently to mucus isolated from subjects representing different age groups (being poorest to mucus isolated from elderly) (Ouweland et al., 1999).

In vivo human adhesion of probiotic strains can be studied by obtaining biopsy material from a subject after a period of probiotic consumption. Since biopsy sampling raises ethical issues, sampling is usually limited to subjects going through routine diagnostic colonoscopy. From these subjects tissue samples can be obtained, not only from the rectal-sigmoidal region, but also from other parts of large intestine (ascending, transverse, and descending colon). The preferential adhesion of a commercial probiotic strain (L. rhamnosus GG) to the descending part of large colon was detected by using biopsy material (Alander et al., 1997). Johansson et al. (1993) have also demonstrated the adhesion of different Lactobacillus strains to rectal mucosal biopsy samples obtained from volunteers who had consumed fermented oatmeal soup. Biopsy sampling probably gives the most accurate information on the adhesion ability of probiotic strains. However, there are severe limitations in the technique: first and most importantly, ethical considerations limit the use of the technique. Secondly, the technique is very laborious and therefore only few individuals can be included in trials. Thirdly, the evacuation of the colon prior to colonoscopy probably leads to a loss of large number of adhering bacteria, leaving only the bacteria with the strongest adhering ability attached.

Although a lot of research effort has been expended on probiotic adhesion studies, the role of adhesion in successful probiotic function remains speculative. It could also be argued that strong adhesion ability may increase the risk of infection in the host. Some probiotic strains are poorly adhering in vitro and/or in vivo and still they can show positive effects in the hosts. In addition, the reproducibility of in vitro adhesion studies can be poor (especially between different laboratories), which also complicates the interpretation of the results.

4.2. Immunomodulatory properties

Gut associated lymphoid tissue may have contact with adhesive probiotic strains and their components and therefore adhesion is one way of provoking immune effects. Human studies have shown that probiotic bacteria can have positive effects on the immune system of their host. However, differences between probiotic bacteria in respect to their immunomodulatory effects have been observed (Isolauri et al., 1999). In two separate trials, L. johnsonii LJ-1 (previously L. acidophilus LA1) and L. salivarius UCC 118 stimulated a mucosal IgA response and increased phagocytic activity. The immunomodulation mediated by these strains was not linked to an inflammatory response or general modification of immune responsiveness that could potentially have harmful effects, but was rather associated
with transient alterations beneficial to the consumer (Schiffrin et al., 1997; Mattila-Sandholm and Kauppila, 1998). L. rhamnosus GG and Bifidobacterium lactis Bb-12 derived extracts have been shown to suppress lymphocyte proliferation in vitro (Mattila-Sandholm and Kauppila, 1998). Further evidence for immunomodulation by these two strains was provided by a trial involving children with severe atopic eczema resulting from food allergy. Children fed L. rhamnosus GG and B. lactis Bb-12 showed a significant improvement in clinical symptoms compared to the placebo group (Mattila-Sandholm and Kauppila, 1998; Alander and Mattila-Sandholm, 2000). L. rhamnosus GG has been shown to down-regulate the milk-induced inflammatory response in milk-hypersensitive subjects, whereas it had an immunostimulatory effect in healthy subjects (Pelto et al., 1998). Furthermore, L. rhamnosus GG was shown to promote IgA immune response in Crohn’s disease patients (Malin et al., 1996) and enhanced the circulating antibody secreting cell response in children with rotavirus diarrhoea (Kaila et al., 1992).

4.3. Antagonistic properties

To have an impact on the colonic flora it is important for probiotic strains to show antagonism against pathogenic bacteria via antimicrobial substance production or competitive exclusion. Enormous research efforts have focused on bacteriocins. Although probiotic strains may produce bacteriocins, their role in the pathogen inhibition in vivo can only be limited, since traditional bacteriocins have an inhibitory effect only against closely related species such as other Lactobacillus or on sporeformers such as Bacillus or Clostridium (Holzapfel et al., 1995). However, low molecular weight metabolites (such as hydrogen peroxide, lactic and acetic acid, and other aroma compounds) and secondary metabolites may be more important since they show wide inhibitory spectrum against many harmful organism like Salmonella, Escherichia coli, Clostridium, and Helicobacter (Skyttä et al., 1992; Helander et al., 1997; Niku-Paavola et al., 1999). L. rhamnosus strain GG produces in vitro low molecular weight antimicrobial(s), possibly short chain fatty acid(s) but distinct from lactic and acetic acid, with inhibitory activity against anaerobes such as Clostridium, Bacteroides and Bifidobacterium, against Enterobacteriaceae, Pseudomonas, Staphylococcus and Streptococcus, but not against other lactobacilli (Silva et al., 1987). The antagonistic activity of L. rhamnosus GG against enteropathogenic bacteria has also been shown in vivo in S. typhimurium infected mice (Hudault et al., 1997). The spent culture supernatant (SCS) of L. acidophilus strain LB decreased the viability of Staphylococcus aureus, Listeria monocytogenes, S. typhimurium, Shigella flexneri, Escherichia coli, Klebsiella pneumoniae, Bacillus cereus, Pseudomonas aeruginosa, and Enterobacter spp. in vitro. The unidentified low molecular weight antimicrobial substance(s) was independent of lactic acid production and did not effect Lactobacillus or Bifidobacterium strains tested. The antibacterial activity of L. acidophilus LB SCS towards S. typhimurium was also maintained in vivo in the infected-mouse model (Cocconier et al., 1997). L. acidophilus (johnsonii) strain LA1 (LJ-1) produces nonbacteriocin antibacterial substance(s) (unidentified but distinct from lactic acid) that inhibits in vitro a wide range of gram-negative and gram-positive pathogens, such as S. aureus, L. monocytogenes, S. typhimurium, S. flexneri, K. pneumoniae, P. aeruginosa and Enterobacter cloacae. However, inhibition of lactobacilli and bifidobacteria could not be detected. Inhibitory activity of the strain LA1 towards S. typhimurium was also shown in vivo in mouse model (Bernet-Camard et al., 1997). Furthermore, L. acidophilus LA1 shows antimicrobial effect against H. pylori, both in vitro and in humans (Michetti et al., 1999).

4.4. Antimutagenic and anticarcinogenic properties

Antimutagenic and anticarcinogenic properties of bacteria ingested in foods and/or representing the microflora of gastrointestinal tract have been widely studied for a number of years. Bacterial anticarcinogenic properties are considered to represent one or more of the following types: binding and degradation of (pro)carcinogens, production
of antimutagenic compounds, modulation of procarcinogenic enzymes in the gut, and suppression of tumours by an immune response mechanism (Fernandes et al., 1987; Lidbeck et al., 1992a; Hirayama and Rafter, 1999). Special emphasis has been put on in vitro antimutagenic (especially mutagen binding) and ant carcino genic properties of lactic acid bacterial strains (representing Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, and Pediococcus species) isolated from fermented dairy and non-dairy products (Hosono et al., 1986, 1990a,b; Fernandes and Shahani, 1990; Zhang et al., 1990; Thyagaraja and Hosono, 1993, 1994; El-Nezami et al., 1998a,b). However, according to the current criteria, only few of these strains can be considered to represent probiotics. In addition, antimutagenic property is by no means typical for lactic acid bacteria (or probiotics) only: This property seems to be distributed among bacteria representing both gram-positive and gram-negative species (for a review see Fernandes and Shahani, 1990). A wide range of bacteria (and also yeasts) are capable of mutagen binding in vitro (Morotomi and Mutai, 1986; Zhang and Ohta, 1993; Orrhage et al., 1994). Furthermore, also non-viable bacterial cells are able to bind mutagens and carcinogens (Zhang et al., 1990; Thyagaraja and Hosono, 1994; El-Nezami et al., 1998a). In addition to in vitro studies antigenotoxic and antitumor activities of some Bifidobacterium and Lactobacillus strains has also been shown using rat and mice models (Reddy and Rivenson, 1993; Pool-Zobel et al., 1993, 1996; Singh et al., 1997; Takagi et al., 1999).

In human trials probiotic strains have been associated with the reduction of fecal mutagenicity or fecal enzymatic activities involved in mutagen or carcinogen activation. Faecal enzymes such as nitroreductase and β-glucuronidase have the ability to convert procarcinogens to carcinogens in the colon. In human studies L. acidophilus NCFB 1748 consumption was shown to decrease fecal and urinary mutagenicity (Lidbeck et al., 1992b). Suppression of urinary mutagenicity has also been shown after the consumption of L. casei strain Shirota (Hayatsu and Hayatsu, 1993). L. rhamnosus GG supplementation decreased fecal β-glucuronidase, nitroreductase and glycocholic acid hydrolase activities in healthy females (Ling et al., 1994). Reduction of faecal enzyme activities has also been shown after L. gasseri strain ADH and L. casei strain Shirota consumption in humans (Pedrosa et al., 1995; Spanhaak et al., 1998). L. casei strain Shirota consumption has further proved beneficial for some cancer patients by reducing the recurrence of superficial bladder cancer and by prolonging survival and relapse-free interval in cervical cancer (Aso and Akazan, 1992; Aso et al., 1995; Okawa et al., 1993). However, although there is evidence that probiotic bacteria may show antimutagenic and ant carcinogenic properties in vitro and in animal models, the possible role of probiotics in the cancer prevention in humans still remains highly controversial.

5. Technological aspects of probiotics

Functional foods with probiotics are now well established on the European market. Starting about 20 years ago this product range has increased (Young, 1998) and is presently known to most consumers. To succeed in promoting the consumption of functional probiotic products the food industry has to satisfy the demands of the consumer. All probiotic foods should be safe and have good sensory properties. The probiotic foods should also include specific probiotic strains at a suitable level during the storage time. By examining existing products it has been suggested that this is not always the case (Hamilton-Miller et al., 1999).

Before probiotic strains can be delivered to consumers, they must first be able to be manufactured under industrial conditions, and then survive and retain their functionality during storage as frozen or freeze dried cultures, and also in the food products into which they are finally formulated. Additionally, they must be able to be incorporated into foods without producing off-flavours or textures. The packaging materials used and the storage conditions under which the products are stored, are important for the quality of products containing probiotic bacteria.

Foods used for dissemination of probiotics are usually fermented foods even if probiotics also
could be present in infant formula, fruit drinks, whey drinks and sweet milk. Fermented milk and cheese are the most common foods with probiotics (Svensson, 1999). Fermented foods are produced by a microbial fermentation in which fermentable carbohydrates are transformed into ethanol and/or organic acids mainly acetic, lactic and propionic acid. Yeast and lactic acid bacteria are the microbes commonly used in food fermentation. In fermented dairy products mainly Lactobacillus and Lactococcus species, but also yeast and propionic acid bacteria, are used (Driessen and Loones, 1992).

5.1. Manufacturing processes for fermented probiotic products

Most leading starter culture manufacturers today produce common GI lactic acid bacteria and bifidobacteria commercially (Spork, 1994; Mogensen and Friis, 1997). Commercially available probiotic cultures may consist of a single strain or a mixture of several strains. In most cases the probiotic properties are affected by the way of which the strain or culture has been produced (for a review see German et al., 1999). Therefore specific information on strain-specific properties should be available for the process optimisation. Probiotic cultures may be used in special formulations like capsules or tablets, or they may be used in the production of a large variety of fermented food products. In some cases the cultures may be added to a food to contribute specific probiotic or functional properties.

Most commercial probiotic culture preparations are supplied in highly concentrated form, and most of them are constructed for DVS (direct vat set) application (Honer, 1995). Use of these highly concentrated DVS cultures is common due to the difficulties involved in propagating probiotic micro-organisms at the production site. The DVS cultures are supplied either as highly concentrated frozen cultures or as freeze-dried cultures. The cultures should be filled in gas and light proof containers to protect the cultures against light and humidity. Most often alu-foil coated cartons or pouches are used. As the cultures are sensitive, it is important to handle and store the cultures according to the manufacturers’ instructions. Usually deep-frozen cultures contain more than $10^{10}$ cfu g$^{-1}$, whereas freeze-dried cultures typically contain more than $10^{11}$ cfu g$^{-1}$ (Oberman and Libudzisz, 1998). The cell concentration per gram of product varies with the culture and the type of organisms used.

In fermented probiotic products it is important that the probiotic culture used contributes to good sensory properties. Therefore it is quite common to use probiotic bacteria mixed together with other types of bacteria suited for the fermentation of the specific product. For milk-based products the probiotic strains are often mixed with S. thermophilus and L. delbrueckii to achieve the desired flavour and texture. The main flavour components of species often used in probiotic formulations are as shown in the Table 2. In many cases the consumers find products fermented with L. delbrueckii too acidic and with too heavy acetaldehyde flavour (yoghurt flavour). Therefore probiotic cultures have been developed to bring out the preferred flavours in the products in which they are used. Examples of such cultures are the so called ABT cultures (ABT standing for L. acidophilus, Bifidobacterium and S. thermophilus). The manufacturing technology for producing fermented milks containing Bifidobacterium strains has been extensively reviewed by Tamime et al. (1995).

In selecting starter micro-organisms reliable acid-forming ability is the most important characteristics. However, when selecting probiotics the

<table>
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<th>Culture</th>
<th>Main flavour components</th>
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<tr>
<td>Lactobacillus acidophilus$^a$</td>
<td>Lactate (DL)</td>
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<tr>
<td>Bifidobacterium spp.$^a$</td>
<td>Lactate (L$^+$), acetate</td>
</tr>
<tr>
<td>Streptococcus thermophilus$^b$</td>
<td>Lactate (L$^+$), acetaldehyde, diacetyl</td>
</tr>
<tr>
<td>Lactobacillus delbrueckii subsp. bulgaricus$^b$</td>
<td>Lactate (D$^-$), acetaldehyde, diacetyl</td>
</tr>
</tbody>
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$^a$ Probiotic strains.

$^b$ Non-probiotic strains.
criteria should be connected to the impact on human health and well-being. As the environment within the GI-tract and within the food might be quite different the probiotic is often not suitable as a starter organism (Oberman and Libudzisz, 1998; German et al., 1999). The growth rate might be too slow and they might give off-flavours (Svensson, 1999). This could partly be overcome by using specific aseptic processes as used in producing fermented acidophilus milk with levels of probiotic reaching $10^9$ cfu g$^{-1}$ (Fonde´n, 1989). Another possibility is to improve the suitability of the food as a substrate for the probiotic by adding energy sources (e.g. glucose), growth factors (e.g. yeast extract and protein hydrolysates) or suitable antioxidants, minerals or vitamins (Kurmann, 1988; Ishibashi and Shimamura, 1993; Dave and Shah, 1998; Gomes et al., 1998). However, even if such an adjustment may improve the performance of the probiotic as a starter, it is often not enough. By use of a help starter in addition to a probiotic preparation this problem can usually be solved (Fonde´n et al., 2000).

When producing probiotic-containing fermented products a fermentation temperature of 37–40°C is usually recommended since these temperatures span the range in which most probiotic strains multiply well. Probiotic strains can be an integral part of the starter and grow in a symbiotic relation with the other strains composing the culture (Saxelin et al., 2000). There are examples where the probiotic strain or strains are added to the fermented milk after fermentation (e.g. the Danish product ‘Cultura’). In special milk products (e.g. sweet products) probiotics are added to the product in such a way that they will retain their viability but are prevented from multiplying by cooling the product.

5.1.1. Probiotic interaction with starter bacteria

The interactions between probiotic and starter might have an impact on the quality of the product. It has been shown that it is possible to produce fermented dairy products with excellent sensory properties and good survival of the bacteria by using starter and probiotic organisms together (Fondén et al., 2000). Even if several negative interactions have been proposed it seems to be possible to find a suitable starter for most probiotics by testing the presently available starters. Suitable starters might be $S$. thermophilus, yoghurt cultures and mesophilic starters with different combinations of $L$. lactis strains. The most suitable combination of starter and a specific probiotic bacteria has to be determined by a screening process evaluating the impact of different starters on the sensory properties and on the survival of the probiotic strain (Ishibashi and Shimamura, 1993; Samona et al., 1996).

In this screening process some general principles should be followed. If possible, the probiotic should be able to grow during the fermentation. It will increase the total number of the probiotic resulting in a lower process cost and increased adaptation of the probiotic to the fermented food. As most probiotics grow well at 37°C a thermophilic starter might be preferable to a mesophilic one (Svensson, 1999). The growth rate of the starter should be moderate allowing some growth of the probiotic bacteria. It is also important to add the probiotic before or at the same time as the starter (Reddy, 1989; Kailasapathy and Rybka, 1997). Addition of the probiotic after fermentation does not allow any growth but instead might result to a reduced viability as shown when $L$. acidophilus was mixed with yoghurt (Hull et al., 1984). The starter might improve the growth conditions of probiotic by producing substances favourable to the growth of the probiotic or by reducing the oxygen pressure. Bifidobacteria are quite sensitive to oxygen but by selection of specific strains of $S$. thermophilus it was possible to increase survival of $B$. longum (Ishibashi and Shimamura, 1993). $L$. delbrueckii subsp. bulgaricus strains might also increase growth of bifidobacteria by their proteolytic activity resulting in increased availability of valine, glycine and histidine (Misra and Kulia, 1994).

In selecting a suitable starter the negative impact on probiotic survival in vitro and in vivo should also be taken in consideration. The survival of the probiotic bacteria might be influenced by the metabolites formed by the starter such as lactic acid, hydrogen peroxide and bacteriocins. $L$. delbrueckii subsp. bulgaricus, a part of an
Fig. 3. Changes in CFU (10^5 log) of seven probiotic strains (A–G) in fermented milk products after 14 days storage at +4°C. Milks were fermented either with the probiotic strain only (None), or together with a supporter culture St 20 (S. thermophilus), YC 380 (S. thermophilus and L. delbrueckii subsp. bulgaricus) or YC 280 (S. thermophilus and L. delbrueckii subsp. bulgaricus). The figure shows the strain to strain variation between probiotics in regard to their survival in the presence of different supporter cultures.

ordinary yoghurt culture, produces high levels of D-lactic acid also when the product is stored after fermentation at low temperature. The impact of D-lactate on the probiotic varies from strain to strain (Dave and Shah, 1997; Kaïlasapathy and Rybka, 1997; Rybka and Fleet, 1997). Samona et al. (1996) showed that bifidobacterial strains could not grow in the presence of youghurt cultures but by choosing the right combination of probiotic and starter strains the decline in bifidobacterial counts could be prevented. Starters with only S. thermophilus might function better together with probiotics sensitive to acids and low pH. They are forming less acid both during fermentation and storage (Okongi et al., 1984; Ishibashi and Shimamura, 1993) (Fig. 3).

The impact of the metabolites formed by the starter might be enhanced in vivo. When the functional food reaches the stomach the pH decreases and the level of undissociated lactic acid increases. As the concentration of the unionised acid has the greatest impact on survival of the probiotic strain, the viability might be influenced by the amount of hydrochloric acid from the gastric juice and the amount of lactic acid from the product. The importance of this is indicated by the difference in survival of L. acidophilus NCFB 1748 through the GI-tract when comparing sweet milk with fermented milk. Survival rate was ten times higher with the sweet milk (Pettersson et al., 1983).

5.1.2. Interactions between probiotics and prebiotics

Functional foods with both probiotics and prebiotics are called synbiotics (Roberfroid, 1998). Most prebiotics as known today are fermentable, non-digestible carbohydrates with different number of sugar moieties from two up to several hundreds. Some examples are lactulose, galacto- and fructooligosaccharides and resistant starch. As the concept of synbiotics is quite new there are not many specific studies of interactions between pro- and prebiotics. Due to their general properties prebiotics might influence the growth and survival of the probiotic by influencing the growth and metabolites of both the probiotic and the starter. This has to be kept in mind while considering interactions between probiotics and starters.

Interaction in vivo might be favoured by an adaptation of the probiotic to the prebiotic. By adapting its metabolism to a substrate given simultaneously with the probiotic might result in a competitive advantage for the probiotic. However, as far as we know most studies have been done with probiotic grown without such an adaptation.

5.2. Manufacturing of non-dairy probiotic foods

Application of probiotic cultures in non-dairy products and environments represents a challenge (Andersen, 1998). Probiotic viability in the food matrix depends on factors such as pH, storage temperature, presence of competing micro-organisms and inhibitors (e.g. NaCl). In products like probiotic-containing baby foods or confectionery (e.g. chocolate) it is important that the formulation maintains the activity and viability of the probiotic for extended periods of time. Since the probiotic cultures are added as additives to these kind of products, they do not usually multiply, which sets great demands for the probiotic stability. Factors like water activity, oxygen tension and temperature becomes increasingly important when dealing with these kinds of products. Storage at room temperature, which is common for
several types of non-dairy products such as cereal products, drinks, chocolate etc. can create an overwhelming challenge for probiotic stability. This problem can sometimes be solved by using probiotic encapsulation technology to ensure the viability and stability of probiotic cultures (Mylärinen et al., 1998). Stable probiotic-containing baby food formulations and confectioneries have been developed and are currently on the market (Langhendries et al., 1995; Fukushima et al., 1997).

6. Future trends

Diet is a major focus of public health strategy aimed at maintaining optimum health throughout life, preventing early onset of chronic diseases such as gastrointestinal disorders, cardiovascular disease, cancer, osteoporosis, as well as promoting healthier ageing. Although the highly complex relationship of food and health is still poorly understood, recent research advances in different disciplines provide promising new approaches to improve our understanding. The growing demand for ‘healthy’ foods is stimulating innovation and new product development in the food industry internationally. The food industry has a central role in facilitating healthier eating practices through the provision and promotion of healthy foods.

Continuously increasing consumer health consciousness and expenditure are socio-economic factors responsible for the expanding European and world-wide interest in functional foods. Considerable confusion and scepticism, however, exists between consumers, consumer organisations, scientific communities and media about the claims associated with probiotic products. Recent EU projects have demonstrated that, with co-ordinated efforts towards communication and a scientific approach to selecting and applying probiotics, functional food products can be developed with measurable health benefits for consumers. Probiotic strains can be successfully manufactured and incorporated into highly acceptable food products where they can retain their viability and functionality. There are many strain variations, not only in their technological properties, but also in their effects on human health (Alander and Mattila-Sandholm, 2000).

The probiotic concept is today widely spread in the scientific and industrial fields. However, further scientific input is required. Important target research areas, including GI-tract diagnostics and immunology, methodology, biomarkers, and functionality, will lead to tools and scientifically sound methods for well-designed informative human studies. Controlled human studies are essential for the socio-economic success of probiotic functional foods, and they should be tailored for specific population groups such as the elderly and babies. Future research on probiotic bacteria will centre on selecting new and more specific strains for the well-being of the host (age groups, healthy populations, disease specific).

The future scientific and technological research trends will be:

- to inter-link the expertise and scientific knowledge on food, GI-tract functionality and human health.
- to study the mechanisms of action of probiotics in the GI-tract, and develop diagnostic tools and biomarkers for their assessment.
- to evaluate the role of probiotics in healthy consumer groups.
- to examine the effects of probiotics on GI-diseases, GI-infections, and allergies.
- to address the consumer aspects and trade-offs.
- to ensure the stability and viability of probiotic products by developing technologies (e.g. bioencapsulation).
- to develop technology for non-dairy probiotic applications (i.e. cereal based materials).

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References


McFarland, L.V., Surawicz, C.M., Greenberg, R.N., 1994. A randomised placebo-controlled trial of Saccharomyces bou- 
lardii in combination with standard antibiotics for Clostridium difficile disease. JAMA 271, 1913–1918.
Michetti, P., Dorta, G., Wiesel, P.H., Brassart, D., Verdu, E., Herranz, M., Felley, C., Porta, N., Rouvet, M., Blum, 
A.L., Corthesy-Theulaz, I., 1999. Effect of whey-based culture supernatant of Lactobacillus acidophilus 
(Johnson) L1l on Helicobacter pylori infection in humans. Digestion 60, 203–209.
Miller, L.G., Finegold, S.M., 1967. Antimicrobial sensitivity of 
Bifidobacterium bifidum
Misra, A., Kulia, R., 1994. Use of
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Misra, A., Kulia, R., 1994. Use of


McFarland, L.V., Surawicz, C.M., Greenberg, R.N., 1994. A randomised placebo-controlled trial of Saccharomyces bou- 
lardii in combination with standard antibiotics for Clostridium difficile disease. JAMA 271, 1913–1918.
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McFarland, L.V., Surawicz, C.M., Greenberg, R.N., 1994. A randomised placebo-controlled trial of Saccharomyces bou- 
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(Johnson) L1l on Helicobacter pylori infection in humans. Digestion 60, 203–209.
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McFarland, L.V., Surawicz, C.M., Greenberg, R.N., 1994. A randomised placebo-controlled trial of Saccharomyces bou- 
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McFarland, L.V., Surawicz, C.M., Greenberg, R.N., 1994. A randomised placebo-controlled trial of Saccharomyces bou- 
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(Johnson) L1l on Helicobacter pylori infection in humans. Digestion 60, 203–209.
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(Johnson) L1l on Helicobacter pylori infection in humans. Digestion 60, 203–209.
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lardii in combination with standard antibiotics for Clostridium difficile disease. JAMA 271, 1913–1918.


