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review

The Potential of Probiotics: A Review

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Summary

Probiotics, live cells with different beneficiary characteristics, have been extensivelly studied and explored commercially in many different products in the world. Their benefits to human and animal health have been proven in hundreds of scientific research. *Lactobacillus* and *Bifidobacterium* are the main probiotic groups; however, there are reports on the probiotic potential of *Pediococcus, Lactococcus, Bacillus* and yeasts. Some of the identified probiotic strains exhibit powerful anti-inflammatory, antiallergic and other important properties. Apart from that, the consumption of dairy and non-dairy products stimulates the immunity in different ways. Various food matrices have been used with probiotics, which are briefly documented. In this review, the history of probiotics, their application in the health and food areas and new trends in probiotic products and processes are presented.

Key words: probiotics, intestinal microflora, *Bifidobacterium*, *Lactobacillus*, immune stimulation, probiotic production, food carriers, dairy products, non-dairy products

Introduction

Probiotic is a relatively new word meaning 'for life', which is used to name microorganisms that are associated with the benefical effects for humans and animals. These microorganisms contribute to intestinal microbial balance and play a role in maintaining health. The probiotic microorganisms consist mostly of the strains of the genera *Lactobacillus* and *Bifidobacterium*, but strains of *Bacillus, Pediococcus* and some yeasts have also been found as suitable candidates. Together they play an important role in the protection of the organism against harmful microrganisms and also strengthen the host's immune system. Probiotics can be found in dairy and non dairy products. They are usually consumed after the antibiotic therapy (for some illnesses), which destroys the microbial flora present in the digestive tract (both the useful and the targeted harmful microbes). Regular consumption of food containing probiotic microorganisms is recommended to establish a positive balance of the population of useful or beneficial microbes in the intestinal flora.

The global market of probiotic ingredients, supplements and food was worth $14.9 billion in 2007 and it was expected to reach 15.9 billion in 2008, and 19.6 billion in 2013, representing a compound annual growth

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rate of 4.3 % (*1*). Extensive investigations of probiotics have been greatly enhanced by the research of new microbes for future probiotic bacteriotherapy applications. The scope of this manuscript is to review the definition, history, applications, production, technology and future trends of probiotics.

Probiotics: What Are They?

The name probiotic comes from the Greek 'pro bios' which means 'for life'. The history of probiotics began with the history of man; cheese and fermented milk were well known to the Greeks and Romans, who recommended their consumption, especially for children and convalescents. Probiotics are defined as the living microorganisms administered in a sufficient number to survive in the intestinal ecosystem. They must have a positive effect on the host (*2*). The term 'probiotic' was first used by Lilly and Stillwell (*3*) in 1965 to describe the 'substances secreted by one microorganism that stimulate the growth of another'. A powerful evolution of this definition was coined by Parker in 1974 (*4*), who proposed that probiotics are 'organisms and substances which contribute to intestinal microbial balance' (*5*). In more modern definitions, the concept of an action on the gut microflora, and even that of live microorganisms disappeared. Salminen *et al*. (*6*) defined probiotics as the 'food which contains live bacteria beneficial to health', whereas Marteau *et al*. (*7*) defined them as 'microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being'. Some modern definitions include more precisely a preventive or therapeutic action of probiotics. Charteris *et al*. (*8*), for example, defined probiotics as 'microorganisms which, when ingested, may have a positive effect in the prevention and treatment of a specific pathologic condition'. Finally, since probiotics have been found to be effective in the treatment of some gastrointestinal diseases (*7*), they can be considered to be therapeutic agents. It is clear that a number of definitions of the term 'probiotic' have been used over the years but the one derived by the Food and Agriculture Organization of the United Nations/World Health Organization (*9*) and endorsed by the International Scientific Association for Probiotics and Prebiotics (*10*) best exemplifies the breadth and scope of probiotics as they are known today: 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host'. This definition retains historical elements of the use of living organisms for health purposes but does not restrict the application of the term only to oral probiotics with intestinal outcomes (*11*).

Despite these numerous theoretical definitions, however, the practical question arises whether a given microorganism can be considered to be a probiotic or not. Some strict criteria have been proposed. Havenaar *et al.* (*12*), for example, proposed the following parameters to select a probiotic: total safety for the host, resistance to gastric acidity and pancreatic secretions, adhesion to epithelial cells, antimicrobial activity, inhibition of adhesion of pathogenic bacteria, evaluation of resistance to antibiotics, tolerance to food additives and stability in the food matrix. The probiotics in use today have not been selected on the basis of all these criteria, but the most commonly used probiotics are the strains of lactic acid bacteria such as *Lactobacillus*, *Bifidobacterium* and *Streptococcus* (*S. thermophilus*); the first two are known to resist gastric acid, bile salts and pancreatic enzymes, to adhere to colonic mucosa and readily colonize the intestinal tract (*5*).

The history of probiotics

The origin of cultured dairy products dates back to the dawn of civilization; they are mentioned in the Bible and the sacred books of Hinduism. Climatic conditions for sure favoured the development of many of the traditional soured milk or cultured dairy products such as kefir, koumiss, leben and dahi (*13*). These products, many of which are still widely consumed, had often been used therapeutically before the existence of bacteria was recognized (*14*).

At the beginning of the 20th century the main functions of gut flora were completely unknown. Ilya Ilyich Metchnikoff, the Nobel prize winner in Medicine in 1908, at the Pasteur Institute linked the health and longevity to ingestion of bacteria present in yoghurt (*15,16*). He believed that the constitution of the human body presented several disharmonies inherited from primitive mammals, such as body hair, wisdom teeth, stomach, vermiform appendix, caecum, and large intestine. In 1907, he postulated that the bacteria involved in yoghurt fermentation, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, suppress the putrefactive-type fermentations of the intestinal flora and that consumption of these yoghurts played a role in maintaining health. Indeed, he attributed the long life of Bulgarian peasants to their intake of yoghurt containing *Lactobacillus* species (*16*). In particular, he reported that the large intestine, useful to mammals in managing rough food composed of bulky vegetables, is useless in humans. Moreover, it is the site of dangerous intestinal putrefaction processes which can be opposed by introducing lactobacilli into the body, displacing toxin-producing bacteria, promoting health, and prolonging life (*17*).

Tissier's discovery of bifidobacteria in breast-fed infants also played a key role in establising the concept that specific bacteria take part in maintaining health. In 1906, Tissier reported clinical benefits from modulating the flora in infants with intestinal infections (*18*). At the time, many others were sceptical about the concept of bacterial therapy and questioned in particular whether the yoghurt bacteria (*L. bulgaricus*) were able to survive intestinal transit, colonize and convey benefits (*19*). In the early 1920s, *L. acidophilus* milk was documented to have therapeutic effects, in particular, a settling effect on digestion (*20*). It was believed that colonization and growth of these microorganisms in the gut were essential for their efficacy, and therefore, the use of intestinal isolates was advocated. In Japan in the early 1930s, Shirota focused his research on selecting the strains of intestinal bacteria that could survive passage through the gut and on the use of such strains to develop fermented milk for distribution in his clinic. His first product containing *L. acidophilus* Shirota (subsequently named *L. casei* Shirota) was the basis for the establishment of the Yakult Honsha company (*21*).

Only at the end of the century, it became clear that intestinal microflora had several functions, including metabolic, trophic and protective ones (*22*). Metabolic functions are primarily characterized by the fermentation of non-digestible dietary residue and endogenous mucus, savings of energy as short-chain fatty acids, production of vitamin K, and absorption of ions. Trophic functions are based on the control of epithelial cell proliferation and differentiation, and development and homeostasis of the immune system. Finally, protective functions are connected with the barrier effect and protection against pathogens (*17*). The health benefits derived from the consumption of foods containing *Lactobacillus acidophilus*, *Bifidobacterium* and *L. casei* are now well documented. *Streptococcus thermophilus* and *L. delbrueckii* ssp. *bulgaricus* are yoghurt starter cultures, which offer some health benefits; however, they are not natural inhabitants of the intestine. Therefore, for yoghurt to be considered as a probiotic product, *L. acidophilus*, *Bifidobacterium* and *L. casei* are incorporated as dietary adjuncts. Thus, the normal practice is to make a product with both starter organisms, *e.g. S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*, and one or more species of probiotic bacteria (*23*).

The guidelines that stipulate what is required for a product to be called a probiotic were published by FAO/WHO in 2002 (*24*). They require that strains be designated individually, speciated appropriately and retain a viable count at the end of their shelf life in the designated product formulation that confers a proven clinical end-point. The probiotic definition requires that the efficacy and safety of probiotics be verified and thus, assessment of this constitutes an important part of their characterization for human use (*25*).

Probiotic Microorganisms

The probiotic potential of different bacterial strains, even within the same species, differs. Different strains of the same species are always unique, and may have differing areas of adherence (site-specific), specific immunological effects, and actions on a healthy *vs.* an inflamed mucosal milieu may be distinct from each other. Current probiotic research aims at the characterization of the normal, healthy gut microbiota in each individual, assessing the species composition as well as the concentrations of different bacteria in each part of the intestine. The target is to learn to understand host–microbe interactions within the gut, microbe–microbe interactions within the microbiota and the combined health effects of these interactions. The goal is to define and characterize the microbiota both as a tool for nutritional management of specific gut-related diseases and as a source of new microbes for future probiotic bacteriotherapy applications. This may eventually include organisms specifically isolated to provide site-specific actions in disorders such as the irritable bowel syndrome (*25*).

According to Shah (*23*) and Chow (*26*) the most popular strains are represented by the following genera: *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* (Table 1, *27–38*), but other organisms including enterococci and yeasts have also been used as probiotics. Some of these strains have been chosen based on selection criteria (*12*)

Table 1. Commercial probiotic microorganisms

Microorganism	Strain	Company (product)	Ref.
Bifidobacterium adolescentis	ATCC 15703		*27–30*
Bifidobacterium animalis	Bb-12	Chr. Hansen	*27–30*
Bifidobacterium bifidum	Bb-11	Chr. Hansen	*27–30*
Bifidobacterium breve			*27–30*
Bifidobacterium essencis		Danone® (Activia)	*27–30*
Bifidobacterium infantis	Shirota Immunitas®	Yakult Danone®	*27–31*
Bifidobacterium lactis	Bb-02, Lafti™ B94	DSM	*27–30*
Bifidobacterium	CRL 431		*27–30*
Bifidobacterium longum	BB536 SBT-2928 UCC 35624	Morinaga Milk Industry Snow Brand Milk Products UCCork	*27–30*
Bacillus lactis	DR10	Danisco (Howaru™)	*27–30*
Enterococcus faecium			*32*
Lactobacillus acidophilus	LA-1/LA-5 NCFM DDS-1 SBT-2062	Chr. Hansen Rhodia Nebraska Cultures Snow Brand Milk Products	*27–30*
Lactobacillus bulgaricus	Lb12		*27–30*
Lactobacillus casei	Shirota	Yakult (Yakult®)	*27–30*
Lactobacillus casei	Immunitas®	Danone®	*27–30*
Lactobacillus delbrueckii ssp. *bulgaricus*			*27–30*
Lactobacillus fermentum	RC-14	Urex Biotech	*27–30*
Lactobacillus GG			*27–30, 33,34*
Lactobacillus helveticus	B02		*27–30*
Lactobacillus lactis	L1A	Essum AB	*27–30*
Lactobacillus paracasei	CRL 431	Chr. Hansen	*27–30*
Lactobacillus rhamnosus	GG GR-1 LB21 271	Valio Urex Biotech Essum AB Probi AB	*27–30*
Lactobacillus plantarum	299v Lp01	Probi AB	*27–30*
Lactobacillus reuteri	SD2112/MM2	Biogaia	*27–30*
Lactobacillus salivarius			*35*
Saccharomyces boulardii			*36–38*

Adapted from Gismondo *et al.* (*2*), Shah (*23*) and Chow (*26*)

that are believed to be important for their efficacy such as origin of strain, *in vitro* adherence to intestinal cells (*39–41*) and survival during passage through the gastrointestinal tract (*42–45*).

The genus Bifidobacterium

Bifidobacteria were first isolated and described in 1899–1900 by Tissier, who described rod-shaped, non-gas-producing, anaerobic microorganisms with bifidobacterial morphology, present in the faeces of breast-fed infants, which he termed *Bacillus bifidus*. Bifidobacteria are generally characterized as Gram-positive, non-spore-forming, non-motile and catalase-negative anaerobes (*46*). They have various shapes including short, curved rods, club-shaped rods and bifurcated Y-shaped rods. Presently, 30 species are included in the genus *Bifidobacterium*, 10 of which are from human sources (dental caries, faeces and vagina), 17 from animal intestinal tracts or rumen, two from wastewater and one from fermented milk (*47*).

Bifidobacteria are microorganisms of paramount importance in the active and complex ecosystem of the intestinal tract of humans and other warm-blooded animals, as well as of honeybees (*46*). They are distributed in various ecological niches in the human gastrointestinal and genitourinary tracts, the exact ratio of which is determined mainly by the age and diet. The indigenous microflora of infants is dominated by bifidobacteria, which are established shortly after birth. Their proliferation is stimulated by the glycoprotein components of κ-casein in human colostrum and, to a lesser extent, human milk. The number of bifidobacteria decreases with increasing age of an individual and eventually becomes the third most abundant genus (accounting for approx. 25 % of the total adult gut flora) after the genera *Bacteroides* and *Eubacterium* (*48*).

The genus Lactobacillus

In 1990, Moro was the first researcher to isolate a strain which he typified as *Bacillus acidophilus*, a generic name for intestinal lactobacilli. Lactobacilli are in general characterized as Gram-positive, non-spore-forming and non-flagellated rods or coccobacilli (*49*). They are either aerotolerant or anaerobic and strictly fermentative. Glucose is fermented predominantly to lactic acid in the homofermentative case, or equimolar amounts of lactic acid, CO_2 and ethanol (and/or acetic acid) in the heterofermentative counterpart. Gomes and Malcata (*47*) reported that 56 species of the genus *Lactobacillus* have been recognized.

Lactobacilli are distributed in various ecological niches throughout the gastrointestinal and genital tracts and constitute an important part of the indigenous microflora of man and higher animals. Their distribution is affected by several environmental factors, which include pH, oxygen availability, level of specific substrates, presence of secretions and bacterial interactions. They are rarely associated with cases of gastrointestinal and extra-intestinal infection, and strains employed technologically are regarded as non-pathogenic and safe microorganisms. Furthermore, they have the reputation of health promoters, especially in the human gastrointestinal and genitourinary tracts (*50*).

Other Probiotic Microorganisms

Although the term probiotic is more related to lactic acid bacteria as *Lactobacillus* and *Bifidobacterium*, it can be extended to other microorganisms which have not been explored. For example, *Bacillus* species have been used as probiotics for at least 50 years in an Italian product commercialized as Enterogermina® ($2 \cdot 10^9$ spores). Among this group some species that have been evaluated are *Bacillus subtilis*, *Bacillus clausii*, *Bacillus cereus*, *Bacillus coagulans* and *Bacillus licheniformis* (*51*). Some advantages of the bacterial spores are their resistance to heat, allowing the storage at room temperature and in a dried form. Also, these bacteria are able to reach small intestine since they survive the gastric pH of the stomach (*52*). The application of probiotic bacterial spores ranges from dietary supplements to growth promoters and uses in aquaculture (*e.g.* shrimp) (*51*).

Probiotic formulations involving some *Bacillus* species are recommended for use with antibiotics since these strains are resistant to them (*e.g. B. clausii*) (*53*). *B. coagulans* has been used as adjunct therapy for relieving rheumatoid arthritis (*54*). *B. subtilis* has been researched genetically and physiologically, and is strongly associated with a Japanese product known as natto. The consumption of this product can lead to stimulation of the immune system and reduction of blood coagulation by fybrinolysis (*55,56*). The secretion of antimicrobials such as coagulin, amicoumacin and subtilisin is also verified in *Bacillus*.

The proposed mechanisms for probiotic effects of the *Bacillus* spores are based on immunomodulation, which occurs through the stimulation of the gut-associated lymphoid tissue (GALT) by production of cytokines, competitive exclusion of gastrointestinal pathogens (*e.g.* competition for adhesion sites) and secretion of antimicrobial substances (*57*).

Several studies have been performed to assure the safety of *Bacillus* species using animal models and *in vitro* tests to evaluate the toxicity or adverse effects of the strains. The use of *B. subtilis* is approved for use as a supplement in Italy and the UK. However, the designation 'probiotic' should only be allowed if the microorganism presents the characteristics inherent to probiotic strains. On the other hand, studies of competitive exclusion of *Escherichia coli* 078:K80 by *Bacillus subtilis* (*58*) and the suppression of *Vibrio harveyi* in shrimp by several *Bacillus* spore formers (*59*) have strongly shown the probiotic potential of these strains.

Probiotic microorganisms used in animal preparations are *Enterococcus*, *Bacillus*, *Streptococcus*, *Lactobacillus*, *Aspergillus* and *Saccharomyces* (*60*). Vitacanis® is a probiotic formulation which can be used in preventing intestinal disorders in dogs and cats. Among *Enterococcus* species, *Enterococcus faecium* is the most used in commercial probiotics. The presence of *Enterococcus faecium* is important in preventing infection by *Salmonella enterica* ssp. *enterica* ser. Typhimurium (*61*). Additionally, a probiotic product known as Causido®, which contains *S. thermophilus* and *E. faecium*, has been proposed for a short-term hypocholesterolaemic effect (*62*). Interesting characteristics of the *Enterococcus* group are the survival

on dry surfaces for prolonged periods and the resistance to antibiotics (*63*).

Among the probiotic yeasts, the most common genus is *Saccharomyces*, which has been employed in livestock feed. *S. cerevisiae* has shown a beneficial effect when administrated in the Nile tilapia as growth promoter (*64*). The potential probiotic effect of *S. cerevisiae* and *S. cerevisiae* var. *boulardii* has been demonstrated since they are able to tolerate low pH and bile and protect against bacterial infections through the reduction of the intestinal pro-inflammatory response (*65*). However, the adhesion properties of these yeasts should be better investigated.

Safety

In theory, probiotics may be responsible for four types of side effects in susceptible individuals: systemic infections, deleterious metabolic activities, excessive immune stimulation, and gene transfer (*66*,*67*). In practice, however, lactobacilli and bifidobacteria (and probiotics based on these organisms) are extremely rare causes of infections in humans (*6*,*68*,*69*). This lack of pathogenicity extends across all age groups and also to immunocompromised individuals (*70*).

Traditional dairy strains of lactic acid bacteria (LAB) have a long history of safe use. LAB, including different species of *Lactobacillus* and *Enterococcus*, have been consumed daily since humans started to use fermented milk as food. Probiotic species such as *Lactobacillus acidophilus* have been safely used for more than 70 years. However, the safety aspects always have to be considered and possible adverse effects should be continuously evaluated, as illustrated by recent literature. Members of the genera *Lactococcus* and *Lactobacillus* are most commonly given the GRAS status, whilst members of the genera *Streptococcus*, *Enterococcus* and some other genera of LAB are considered opportunistic pathogens.

The safety of probiotics has been considered in reviews and clinical reports which have drawn attention to isolate cases of human bacteraemia (*71–73*). Surveillance studies support the safety of commercial LAB (*71*,*73*,*74*). Available data indicate that no harmful effects have been observed in controlled clinical studies with lactobacilli and bifidobacteria (*6*).

Three approaches can be used to assess the safety of a probiotic strain: studies on the intrinsic properties of the strain, studies on the pharmacokinetics of the strain (survival, activity in the intestine, dose–response relationships, faecal and mucosal recovery) and studies searching for interactions between the strain and the host.

The Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria recognized the need for guidelines to set out a systematic approach for the evaluation of probiotics in food in order to substantiate the health claims. Consequently, a Working Group was convened by FAO/WHO to generate guidelines and recommend criteria and methodology for the evaluation of probiotics, and to identify and define what data need to be available to accurately substantiate health claims. The aims of the Working Group were to identify and outline the minimum requirements needed for probiotic status. Then, guidelines were prepared in 2002 to meet this objective. These guidelines are available in: Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food (*24*).

Quantification of probiotics

Traditionally, appropriate dilutions of faecal samples have been cultured on selective media. However, the selectivity of any medium is at best relative and these methods are prone to both false-positive and false-negative results (*75*). More importantly, not all microbes can be cultured by the currently available techniques. With the advent of molecular biology, culture-independent techniques have been developed. In particular, methods using the variable and conserved regions of the 16S rRNA have proved successful in characterizing the gut microbiota. The use of 16S rRNA enables enumeration of microbes which are either unculturable by the current cultivation techniques or have died during transport and storage (*75*). Fluorescent *in situ* hybridization (FISH) is commonly used and employs species-, genus- or domain-specific fluorescently labelled 16S rRNA probes (*76*). Enumeration of the labelled microbes can be done microscopically by visual counting (*77*), which is, however, laborious. On the other hand, although image analysis of the microscopic view makes it possible to process a relatively large number of samples, this is expensive (*78*). Alternatively, enumeration of fluorescent microbes can be done by flow cytometry, which similarly allows the analysis of large numbers of samples, but is also expensive (*79*). Techniques based on the polymerase chain reaction (PCR) are also commonly used and provide rapid quantitative and qualitative information on the composition of the intestinal microbiota (*25*).

Mechanisms of action

The mechanisms by which probiotics exert biological effects are still poorly understood, but the nonspecific terms such as colonization resistance or competitive exclusion are often used to explain their mode of action (*39*). Colonization resistance or competitive exclusion describes a phenomenon whereby the indigenous anaerobic flora limits the concentration of potentially pathogenic (mostly aerobic) flora in the digestive tract (*80*). The concept of competitive exclusion was first developed during the early 1970s when it was discovered that the administration of mixed adult intestinal microorganisms conferred adult-type resistance against *Salmonella* infection to newly hatched chicks (*81*).

Oelschlaeger (*82*) reported that the effects of probiotics may be classified in three modes of action: (*i*) Probiotics might be able to modulate the host's defences including the innate as well as the acquired immune system. This mode of action is most likely important for the prevention and therapy of infectious diseases but also for the treatment of (chronic) inflammation of the digestive tract or parts thereof. In addition, this probiotic action could be important for the eradication of neoplastic host cells; (*ii*) Probiotics can also have a direct effect on other microorganisms, commensal and/or pathogenic ones. This principle is in many cases of importance for the preven-

tion and therapy of infections and restoration of the microbial equilibrium in the gut; (*iii*) Finally, probiotic effects may be based on actions affecting microbial products like toxins and host products, *e.g.* bile salts and food ingredients. Such actions may result in inactivation of toxins and detoxification of host and food components in the gut. The same author also stated that the kind of effect(s) a certain probiotic executes depends on its metabolic properties, the molecules presented at its surface or on the components secreted. Even integral parts of the bacterial cell such as DNA or peptidoglycan might be of importance for its probiotic effectiveness. The individual combination of such properties in a certain probiotic strain determines a specific probiotic action and as a consequence its effective application for the prevention and/or treatment of a certain disease.

Probiotics and Human Health

Nowadays, consumers are aware of the link among lifestyle, diet and good health, which explains the emerging demand for products that are able to enhance health beyond providing basic nutrition. The list of health benefits accredited to functional food continues to increase and the probiotics are one of the fastest growing categories within food for which scientific researches have demonstrated therapeutic evidence. Among several therapeutic applications of the probiotics can be cited the prevention of urogenital diseases, alleviation of constipation, protection against traveller's diarrhoea, reduction of hypercholesterolaemia, protection against colon and bladder cancer, prevention of osteoporosis and food allergy (*83*). One of the most studied strains, *Bifidobacterium lactis*, has been used in several types of studies to demonstrate its probiotic ability, and scientific evidence for this strain has been cited in many reviews (*84–88*).

Ingestion of LAB has been suggested to confer a range of health benefits including immune system modulation (*89,90*), increased resistance to malignancy (*91*) and infectious illness (*92*). Maldonado Galdeano *et al*. (*93*) studied the effect of fermented milk containing *Lactobacillus casei* DN114001, which induced mucosal immune stimulation reinforcing the non-specific barrier and modulating the innate immune response in the gut, maintaining the intestinal homeostasis.

Host immune modulation is one of the suggested benefits of the consumption of probiotic functional food. However, comparative studies on the immunological properties that support the selection of strains of the same species for specific health benefits are limited. Medina *et al*. (*94*) evaluated the ability of different strains of *Bifidobacterium longum* to induce cytokine production by peripheral blood mononuclear cells. *B. longum* live cells of all strains induced specific cytokine patterns, suggesting that they could drive immune responses in different directions. Kelly *et al*. (*95*) demonstrated the ability of species within the commensal microflora to modulate immune function. Arunachalam *et al*. (*96*) studied the dietary consumption of *B. lactis* HN019 and concluded that a relatively short-term dietary regime (6 weeks) is sufficient to impart measurable improvements in immunity. Chiang *et al*. (*97*) demonstrated that dietary consumption of probiotics in oligosaccharide-rich substrate enhanced immune function by *B. lactis* HN019 in a different range for two types of leucocytes. *In vivo* and *in vitro* indices of immunity in healthy mice fed with *Lactobacillus rhamnosus* (HN001, DR20), *L. acidophilus* (HN017) and *B. lactis* (HN019, DR10) were examined by Gill *et al*. (*98*) and the results suggested that supplementation of the diet with these strains was able to enhance several indices of natural and acquired immunity.

Infectious diseases are still the biggest human health problem for the world to solve. Intestinal infection caused by the intake of pathogenic microorganisms with the contaminated water and food are the main causes of death. Under this circumstance, probiotics can assist in part the foodborne problematic situation, as it is demonstrated in several studies. Shu and Gill (*99*) demonstrated that *B. lactis* HN019 can reduce the severity of infection caused by the enterohemolytic pathogen *Escherichia coli* O157: H7 and suggested that this reduction may be associated with enhanced immune protection conferred by the probiotic. *B. lactis* HN019 also demonstrated the ability to provide a significant degree of protection against *Salmonella* infection by enhancing various parameters of immune function that are relevant to the immunological control of salmonellosis (*100*). Moreover, the same authors suggested that dietary treatment using *B. lactis* HN019 could reduce the severity of weanling diarrhoea associated with rotavirus and *E. coli*, possibly *via* a mechanism of enhanced immune-mediated protection. As a consequence, probiotic treatment might be an effective dietary means of preventing or limiting diarrhoea in human infants (*101*).

The intestinal barrier maintains the epithelial integrity protecting the organism against bacterial or food antigens that could induce inflammatory processes leading to intestinal disorders such as inflammatory bowel diseases (IBD) (*102*). Probiotic microorganisms compete with pathogenic bacteria for epithelial binding sites, avoiding the colonization by *Salmonella* sp. and *E. coli* strains (*103,104*). In order to investigate the host-microbe interactions, the co-cultivation of intestinal bacteria with immune and/or intestinal epithelial cells (IEC) has been performed. This technique allows the evaluation of the importance of these interactions on barrier function, cytokine expression, bacterial recognition and pathogen invasion. Ukena *et al*. (*105*) demonstrated that *E. coli* Nissle 1917 (EcN 1917) strain was capable of inducing pro-inflammatory cell responses, since the co-incubation of Caco-2 cells with *E. coli* EcN 1917 resulted in the up-regulation of 126 genes, including the monocyte chemoattractant protein-1 ligand 2 (MCP-1), macrophage inflammatory protein-2α (MIP-2α) and macrophage inflammatory protein-2β (MIP-2β). Recent data have shown that EcN 1917 prevented the disruption of the mucosal barrier by enteropathogenic *E. coli* and restored the mucosal integrity in T84 epithelial cells (*106*).

L. casei ssp. *rhamnosus* has shown to be a promising probiotic in preventing the colonization of the gastrointestinal tract by pathogenic bacteria such as enteropathogenic *E. coli*, enterotoxigenic *E. coli*, and *Klebsiella pneumoniae* using *in vitro* model with Caco-2 cell line (*107*).

There are several reports about the action of the probiotics against *Helicobacter pylori* (*108–110*), a Gram-

-negative bacterium associated with the development of chronic gastritis, peptic ulcers and gastric cancer. It was reported that *L. salivarius* inhibited the colonization and the release of interleukin-8 in gnotobiotic mice inoculated with *H. pylori* (*111*).

Clinical studies have suggested the efficacy of the administration of probiotics in maintaining the remission of the pouchitis (*112*), ulcerative colitis (*113*), and Crohn's disease (*114*). Patients suffering from ulcerative colitis (UC) were treated with *Escherichia coli* Nissle 1917 (*115,116*) and *Lactobacillus rhamnosus* GG (*117*) and the results were similar to that of the standard medication (5-aminosalicylic acid – Mesalazine). When infants were fed with a supplemented formula containing *Lactobacillus reuteri* 55730 or *Bifidobacterium lactis*, there was a decrease in the cases of diarrhoea (*118*). *In vivo* assays with probiotic bacteria evaluated the effects of an acute or chronic gut inflammation using dextran sulphate sodium (DSS), which induces colitis in mice, presenting positive results (*119*).

Anti-carcinogenic effect of probiotics coming from *in vivo* studies in both men and animals was evaluated. Furthermore, *in vitro* studies with carcinoma cell lines and anti-mutagenicity assays also supported this effect. The anti-carcinogenic effect may be attributable to a combination of mechanisms such as the induction of pro-inflammatory, anti-inflammatory or secretory responses that might inhibit carcinogenesis. The strain-dependent variability, such as the immune modulation effect, complicates the understanding of the role of immunity in probiotic-mediated anti-carcinogenesis. Further work is needed to assess the long-term effects of probiotics on the host's immunity in relation to anti-carcinogenesis. Immune-based anticancer therapies have not yet demonstrated their efficacy because few clinical trials have been done (*120*).

Lactobacillus and *Bifidobacteria* strains and *E. coli* strain Nissle 1917 have shown anti-mutagenic activities *in vitro*, probably due to their capacity to metabolize and inactivate mutagenic compounds (*121*). Cytoplasmic fractions of *L. casei* YIT9029 and *B. longum* HY8001 showed the ability to suppress the proliferation of tumor cells when administrated orally to mice as dietary supplement (*122*). Other bacteria stimulated the immune system. It was found that *L. casei* strain Shirota, when administered intranasally in mice, stimulated the cell immune response by induction of interleukin-12, interferon-gamma and tumour necrosis factor alpha, which all have an important role in excluding influenza virus (*123*). Roller *et al.* (*91*) correlated the inhibition of carcinogenesis in rats with changes in the immune activity, in response to probiotic consumption. Furthermore, the protective role of probiotics in rodent models of colon carcinogenesis can be found in some other studies (*124–126*). Studies in animal models also suggest that increasing natural killer cell activity by probiotic consumption may have potential effects on delayed tumour development. For example, Takagi *et al.* (*127*) used dietary *Lactobacillus casei* strain Shirota to inhibit methylcholanthracene-induced tumour devolopment in mice and Varcoe *et al.* (*128*) investigated the efficacy of the *Lactobacillus acidophilus* NCFM in preventing gastrointestinal disease like overt colonic hyperplasia in mice.

Studies have suggested that probiotics could protect against allergies. Isolauri *et al.* (*129*) evaluated the effect of the hydrolysed whey formulas supplemented with probiotics (*B. lactis* Bb12 and *L. rhamnosus* GG) in 27 breast-fed infants that suffered from atopic eczema. These authors found that clinical signs and symptoms of atopic eczema were diminished after two months in both groups. Further, Kalliomäki *et al.* (*130*) performed a randomised controlled-placebo trial with 132 pregnant women with any degree of occurrence of an atopic disease such as atopic eczema, allergic rhinitis or asthma. These mothers received two capsules of a formulation containing *Lactobacillus* GG for 2–4 weeks and the newborn were administered the same formulation for 6 months postnatally. The children were examined during the first 2 years. The results showed that the frequency of the atopic eczema was only 23 % in the probiotic group against 46 % in the placebo group. These works reported great perspectives in using probiotic products in the prevention of different allergies.

Some bacterial species are recognised for their capacity to prevent or limit mycotoxinogenic mould growth such as *Lactobacillus* (*131,132*), *Lactococcus* (*133*), *Pediococcus* (*134*) and *Leuconostoc* (*135*). The probiotic strain *Saccharomyces boulardii* confers protection against toxin A produced by *Clostridium difficile* and prevents intestinal injury and inflammation. This is possible because *S. boulardii* inhibits the activation of extracellular signal-regulated ½ (ERK ½) and mitogen-activated protein (MAP) kinases, thus modulating host signalling pathways (*136*). In addition, mice that were fed with *S. boulardii* and immunized with *C. difficile* toxin A showed an increase in specific intestinal anti-toxin A levels (*137*), which could lead to protection against diarrhoeal diseases.

It has been reported that lactic acid bacteria are able to bind aflatoxin B_1 *in vitro* and *in vivo* (*138*), but this property seems to depend on bacterial strain (*139*). Compared to *L. plantarum* and *L. fermentum*, *L. casei* was reported to be the strongest binder of aflatoxin (*140*). Also, microorganisms such as *Saccharomyces cerevisiae* demonstrated good ability to bind this aflatoxin (*141*). In the study of Gratz *et al.* (*142*) rats received doses of aflatoxin B_1 and were fed with oral gavage containing *Lactobacillus rhamnosus* strain GG (ATCC 53013). After administration, an increase in the aflatoxin B_1 in fecal excretion was observed due to bacterial binding. The probiotic treatment prevented weight loss and reduced the hepatotoxic effects of the aflatoxin B_1.

Microcystins are toxins produced by freshwater cyanobacteria which can cause acute hepatoxicity and act as tumour promoters (*143*). The probiotics *Lactobacillus rhamnosus* strain GG and *Bifidobacterium lactis* strain Bb12 have demonstrated the ability to bind to microcystin-LR, the most common and most toxic variant of microcystins. A higher removal of microcystin-LR was observed when *Lactobacillus rhamnosus* strain GG was heat-treated (46 %) (*144*).

Recent studies have also suggested that probiotics could have beneficial effects beyond some metabolic disorders such as hypertension. Primary hypertension is caused by various factors and the predominant causes include hypercholesterolemia (*145*). Rising evidence has indicated that lactobacilli and bifidobacteria could cause,

when ingested, a significant reduction in serum cholesterol. This is because cholesterol synthesis mainly occurs in the intestines, hence the gut microflora promote effects on lipid metabolism. Some studies demonstrated that probiotics could promote a decrease in the blood cholesterol levels and increase the resistance of low-density lipoprotein to oxidation, therefore leading to a reduced blood pressure (*146*).

Liong and Shah (*147*), using *in vitro* experiments, reported that cholesterol could be removed from a medium by *L. acidophilus* not only through assimilation during growth, but also through binding of cholesterol to the cellular surface. This mechanism was proposed when both non-growing cells and dead cells were also found to remove cholesterol. Another hypocholesterolemic mechanism described involves the ability of certain probiotic strains to enzymatically deconjugate bile acids through bile salt hydrolase catalysis. Enzymatic activity was detected in the gut microflora such as *Lactobacillus* and *Bifidobacterium* sp. (*147,148*). Since cholesterol is the precursor for the synthesis of new bile acids, the use of cholesterol to synthesize new bile would lead to a decreased concentration of cholesterol in blood (*145*). Nguyen *et al*. (*149*) evaluated *L. plantarum* PH04 as a potential probiotic with cholesterol-lowering effect in mice. Kaushik *et al*. (*150*) demonstrated that the indigenous *L. plantarum* Lp9 exhibited cholesterol-lowering properties. *B. longum* SPM1207 reduced serum total cholesterol and LDL levels significantly, and slightly increased serum HDL (*151*).

Cavallini *et al*. (*152*) observed the effects of *Enterococcus faecium* CRL183, a mixture of isoflavones and simvastatin (drug used to treat hypercholesterolemia), on lipid parameters and atherosclerosis development in rabbits with induced hypercholesterolemia. *E. faecium* strain could be used to improve the lipid profile as an alternative or an adjuvant to drug therapy. Placebo-controlled studies (*57,153–156*) evaluate the effects of probiotic strains on cholesterol metabolism in hypercholesterolemia-induced mice and rats. It was found in all these works that the serum cholesterol levels decreased in the rats fed with a diet supplemented with probiotics.

Environment and lifestyle such as high-fat diet are some of the factors that play a key role in the development of obesity. Recent advances have identified the gut microbiota as one such environmental factor that modulates host energy and lipid metabolism. However, the molecular mechanisms of these complex host-microbe interactions have not been well identified (*157*). Most of the data obtained have been done in experimental animal studies, but promising effects are also shown in humans, thereby supporting the interest in the nutritional modulation of the gut microbiota in the management of metabolic diseases in obese patients. The observations of Cani *et al.* (*158*) suggest that increased levels of bifidobacteria may decrease intestinal permeability and lower the circulating levels of endotoxin. High-fat feeding reduces the numbers of bifidobacteria, which have many physiologically positive effects, including improved mucosal barrier function. Supplementing the diet of high-fat fed mice with prebiotics restores the levels of bifidobacteria and decreases endotoxaemia. Tanida *et al.* (*159*) found that long-term ingestion of *Lactobacillus paracasei* ST11 (NCC2461) reduced body and abdominal fat mass. Their results suggest that *L. paracasei* NCC2461 has an anti-obese action, and in this mechanism, autonomic nerves may function to facilitate the lipolytic and thermogenic responses *via* the sympathetic excitation and to suppress the parasympathetic nerve activity in rats.

Probiotics also convert milk protein into bioactive peptides, which have anthihypertensive effect. Milk peptides may exert antihypertensive effects also through other mechanisms, such as inhibition of the release of endothelin-1 by endothelial cells, stimulation of the bradykinin activity, enhancement of the endothelium-derived nitric oxide production and enhancement of the vasodilatory action of binding to opiate receptors. Angiotensin I-converting enzyme (ACE), a dipeptidyl carboxypeptidase, catalyzes the conversion of angiotensin I to the potent vasoconstrictor angiotensin II and plays an important physiological role in regulating blood pressure and fluid and salt balance in mammals. ACE inhibitory peptides from caseins and whey proteins are termed casokinins and lactokinins, respectively. *In vivo* studies have demonstrated that several ACE inhibitory peptides significantly reduce blood pressure, either after intravenous or oral administration (*160–162*). The hypotensive and immunomodulatory peptides Val-Pro-Pro and Ile-Pro-Pro, for example, can be released from precursor proteins by enzymes from *Lactobacillus helveticus*. These peptides could be applied as initial treatment in mildly hypertensive individuals or as supplemental treatment. They would also represent a low-cost alternative treatment for hypertension.

Probiotics in Food and Beverages

As it was reported by Chow (*26*), the notion that food could serve as medicine was first conceived thousands of years ago by the Greek philosopher and father of medicine, Hippocrates, who once wrote: 'Let food be thy medicine, and let medicine be thy food'. However, during recent times, the concept of food having medicinal value has been reborn as 'functional foods'. A probiotic may also be a functional food (*156*).

Functional foods are defined as: 'foods that contain some health-promoting component(s) beyond traditional nutrients'. Functional foods are also known as designer foods, medicinal foods, nutraceuticals, therapeutic foods, superfoods, foodiceuticals, and medifoods. In general, the term refers to a food that has been modified in some way to become 'functional'. One way in which foods can be modified to become functional is by the addition of probiotics (*24*).

New food products have been formulated with the addition of probiotic cultures. Different types of food matrices have been used such as various types of cheese, ice creams, milk-based desserts, powdered milk for newborn infants, butter, mayonnaise, powder products or capsules and fermented food of vegetable origin (*163*).

Dairy products

In the production of probiotics an important factor is the food substrate. Besides buffering the bacteria through the stomach, it may contain functional ingre-

dients that interact with the probiotics, altering their activities. Fat content, type of protein, carbohydrates and pH can affect probiotic growth and survival.

Dairy products are especially considered as ideal vehicle for delivering probiotic bacteria to the human gastrointestinal tract. The matrices used most frequently are cheese, yoghurt, ice cream and other dairy products, as shown in Table 2.

The most common means to incorporate probiotics to fermented milk include: (*i*) addition of probiotics together with the starter cultures (DVI culture); (*ii*) the production of two batches separately, one containing the probiotic microorganism in milk to achieve a high concentration of viable cells and another with starter cultures. When the fermentation stages are completed, the batches are mixed; (*iii*) the use of a probiotic microorganism as a starter culture. In this situation, the time of fermentation is generally higher than traditional processes using non-probiotic starter cultures (*163*). In this respect, it is necessary to consider the supplementation of the culture medium and the production conditions (*e.g.* incubation temperatures), since metabolites produced by probiotics can lead to off-flavours (*164,165*). In addition, the probiotic strains must be compatible with starter cultures, since the latter could produce inhibitory substances that damage the probiotics (*166*).

Yoghurts with high fat content showed inhibitory effects against probiotic cultures, particularly *B. bifidum* BBI (*167*). The supplementation with vitamins (*e.g.* ascorbic acid) has been reported to improve the viability of *L. acidophilus* in yoghurts (*168*). The addition of substances such as whey protein may also enhance the viability of some probiotics, probably due to their buffering property. In addition, the employment of prebiotics in yoghurt formulations could stimulate the growth and activity of probiotics. In this regard, fructooligossacharides showed to be most effective in maintaining the probiotic viability (*169*).

The utilization of probiotics in the cheese elaboration presents some challenges: low moisture content; presence of salt; starter cultures competing for nutrients and developing acid and flavour during the maturation stage; extended storage (over 3 months), which can influence biochemical activities, redox potential, and alter the cheese structure. Moreover, probiotics should survive the entire shelf life of the cheese, not produce metabolites that affect the cheese quality and the starter culture activities, and also, they should be able to grow in starter culture media (*e.g.* whey-based and phage inhibitory media). Several studies related by Tamime *et al.* (*163*) reported that Turkish white brined, Feta-type, Cheddar, Philippine white soft, Edam, Emmental, Domiati, Ras, Herrgård cheese, Quarg, and cheese-based dips can be compared with yoghurts in delivering probiotics.

The proteolytic patterns can be influenced by the addition of probiotic strains. Ong *et al.* (*170*) reported that the addition of probiotic microorganisms (*L. acidophilus* 4962, *L. casei* 279, *B. longum* 1941, *L. acidophilus* LAFTI® L10, *L. paracasei* LAFTI® L26, *B. lactis* LAFTI® B94) as dairy starter adjuncts has lead to high concentration of free amino acids by a secondary proteolysis during ripening, and this was reduced when the cheese was stored at 4 °C. It was found that all probiotic strains survived the manufacturing process and produced a level of acetic acid higher than the control Cheddar cheese.

Other vehicles that could be used to deliver probiotics are ice cream and frozen dairy desserts. These products have the advantage to be stored at low temperatures, which makes them less exposed to abusive temperatures having higher viability at the time of consumption (*171*). Besides, they are consumed by people of all ages and are composed of milk proteins, fat and lactose as well as other compounds that are required for bacterial growth. However, some probiotic species showed a decrease in the viability during the manufacture and freezing of ice cream (*172*). Some prebiotics could be used to improve the characteristics of the probiotic ice creams. Inulin demonstrated to be beneficial to the firmness, melting properties and dripping time of the ice creams (*173*). Besides, the inulin level in ice cream enhanced the viability of *L. acidophilus* and *B. lactis* (*174*). The addition of oligofructose in low-fat ice cream also improved the survival of *L. acidophilus* La-5 and *B. animalis* ssp. *lactis* Bb-12 during storage at –18 °C for 90 days (*173*). However, to

Table 2. Commercial probiotic dairy products on the European market

Type of product	Trade name	Probiotic microorganism
Fermented milk with high viscosity	Bifisoft, Bifidus, Bioghurt, Biofit, BiofardePlus, Biola, Biologic bifidus, Cultura Dofilus, Dujat Bio Aktiv, Ekologisk Jordgubbs Yoghurt, Fit&Aktiv, Fjäll Yoghurt, Gaio Dofilus, Gefilac, Gefilus, LC 1, Probiotisches Joghurt, ProViva, RELA, Verum, Vifit Vitamel, Vitality, Weight Watchers, Yogosan Milbona	*L. acidophilus*, *L. acidophilus* LA5, *L. rhamnosus* (LGG, LB21 and 271), *L. casei*, *L. casei* L19, *L. johnsonii*, *L. plantarum* 299v, *L. reuteri*, *Lactococcus lactis* ssp. *lactis* L1A, *B. bifidum*, *B. animalis* ssp. *lactis* BB-12, *B. animalis* ssp. *animalis*
Fermented milk with low viscosity (*e.g.* cultured buttermilk, yoghurt drink, dairy drink)	A-fil, Actimel, Aktifit, AB-piimä, Bella Vita, Bifidus, Biofit, Biola, Casilus, Cultura, Emmifit, Everybody, Fit&Aktiv, Fundo, Gaio, Gefilac, Kaiku Actif, LC 1 Go!, LGG+, Onaka, Öresundsfil, Philura, Probiotic drink, ProViva, Pro X, Verum, ViktVäktarna, Vitality, Le'Vive+, Yakult, Yoco Acti-Vit	*L. acidophilus*, *L. acidophilus* LA5, *L. casei* (F19, 431, Imunitas, Shirota), *L. rhamnosus* (LGG, LB21 and 271), *L. johnsonii*, *L. plantarum* 299v, *L. reuteri*, *L. fortis*, *Lactococcus lactis* ssp. *lactis* L1A, *B. bifidum*, *B. animalis* ssp. *lactis* BB-12, *B. animalis* ssp. *animalis*, *B. longum* BB536
Non-fermented dairy products (*e.g.* milk, ice cream)	Gefilus, God Hälsa, RELA, Vivi Vivo	*L. rhamnosus* LGG, *L. plantarum* 299v, *L. reuteri*

From Tamime *et al.* (*163*)

efficiently produce probiotic ice cream, it is important to select oxygen-resistant strains since the incorporation of air (overrun) in the mixture occurs in the production process, which is harmful to microaerophilic and anaerobic strains such as *Lactobacillus* sp. and *Bifidobacterium* sp. This type of challenge can be resolved by the use of microencapsulation technique. As an alternative, aerated dairy dessert (*e.g.* chocolate mousse) has also been used as a potential agent to deliver probiotics (*175*).

Non-dairy products

Some limitations of the use of dairy products to deliver probiotics are the presence of allergens and requirement of cold environments. This fact has led to the launch of new products based on non-dairy matrices. Some claims related to probiotic products are lactose intolerance and fat content.

Some matrices have been used in the development of non-dairy probiotic products such as fruits, vegetables, legumes and cereals. Fruits and vegetables can be considered good matrices since they contain nutrients such as minerals, vitamins, dietary fibres, and antioxidants. The development of different probiotic fruit juices has been studied (*176,177*). Prado *et al*. (*178*) described a revision about a variety of non-dairy probiotic beverages.

However, the incorporation of probiotics in fruit juices requires the protection against acid conditions. This can be achieved by microencapsulation technologies, which allow the entrapment of cells into matrices with a protective coating. Gelatin and vegetable gum have been demonstrated to provide a good protection for acid-sensitive *Bifidobacterium* and *Lactobacillus* (*179–181*). Encapsulation processes in milk protein have also been studied (*182*). When *B. lactis* were microencapsulated, incorporated into African fermented beverages (amasi and mahewu) and assayed for physiological conditions of the stomach, they showed a high survival rate, *i.e.* the microencapsulation enhanced the viability in comparison with free cells (*183*).

Probiotic strains usually found in vegetable materials are species belonging to *Lactobacillus* and *Leuconostoc* genera. *L. plantarum*, *L. casei* and *L. delbrueckii*, for example, were able to grow in cabbage juice without nutrient supplementation and reached 10^8 CFU/mL after 48 h of incubation at 30 °C (*184*). In addition, it was found that these same bacteria grew in beet juice (*185*).

In the case of cereals, the fermentation with probiotic microorganisms could be beneficial due to the decrease of nondigestible carbohydrates (poly- and oligosaccharides), the improvement of the quality and level of lysine, the availability of the vitamin B group, as well as the degradation of phytates and release of minerals (*e.g.* manganese, iron, zinc, and calcium) (*186*). Oat-based substrates have proved promissory for the growth of *L. reuteri*, *L. acidophilus* and *B. bifidum* (*187*). In addition, cereals such as oats and barley contain high levels of β-glucan, which is believed to have hypocholesterolemic effect (*188*). Boza, an acid and low-alcohol beverage produced in the Balkan Peninsula, is a fermented product based on maize, wheat and other cereals. Todorov *et al*. (*189*) studied the microflora of boza and verified the presence of several lactic acid bacteria with probiotic characteristics.

Malt, wheat and barley extracts demonstrated to have a good influence in increasing bile tolerance and viability of *L. acidophilus*, *L. reuteri* and *L. plantarum* (*190,191*). Fermented foods with probiotic strains had an increment in the content of the vitamin B complex. Arora *et al*. (*192*) found an enhancement of 14 and 11 % in thiamine and niacin contents, respectively, when food mixture based on germinated barley flour with whey powder and tomato pulp were autoclaved and fermented by *L. acidophilus*. Also, non-germinated and germinated mixture showed an increase of 31 and 34 % in lysine content, respectively, after autoclaving and fermentation, highlighting the importance of the germination and fermentative process on the bioavailability and improvement of the nutritional quality of foods.

Soybean is an important cereal because it has a high nutritive value. However, the unpleasant bean flavour and the content of oligosaccharides (*e.g.* stachyose and raffinose) can cause flatulence. Besides the improvement of the flavour of soybean products, fermentation can reduce flatulence (*193*), since lactic acid bacteria are able to hydrolyze α-1,6-galactosidic linkages, releasing α-D--galactose (*194*) and making these products more digestible. The survival of probiotics has been assayed in soymilk and this substrate has shown to be efficient for the growth of species such as *L. casei* (*195*), *L. acidophilus* (*196*), *B. infantis*, and *B. longum* (*197*). In addition, the antioxidative activities of soymilk can be increased after fermentation by lactic acid bacteria and bifidobacteria (*167*). This has led to the designing of the probiotic soybean yoghurt (*198*).

Application of Probiotics in Animal Feed and Aquaculture

Animal feed companies and researchers have been looking for alternative products and strategies that can help to maintain animal gut health in order to prevent or reduce the prevalence of pathogens in the food chain. An alternative and effective approach to antibiotic administration to livestock is the use of probiotics, which can help to improve gut microbial balance and therefore the natural defence of the animal against pathogenic bacteria (*199,200*).

In recent years, there has been a considerable interest in using some probiotic microorganisms and organic acids as an alternative to the use of antibiotics in feed. Probiotics are viable microorganisms and supportive substances that, once ingested by humans and animals, produce beneficial physiological effects by assisting in the establishment of an intestinal population which is beneficial to the host's entity and antagonistic to harmful bacteria. The natural adaptation of many lactic acid bacteria to the gut environment and the antimicrobial substances produced by them (organic acids and bacteriocins) has provided these organisms with a competitive advantage over other microorganisms to be used as probiotics (*6,201*).

The use of probiotics and commercial products containing probiotics in aquaculture (*e.g.* shrimp production)

has shown similar results compared to the antimicrobials currently used (*202*). It could be an interesting alternative to overcome the problem of antibiotic resistance.

Multiple ways exist in which probiotics could be beneficial and these could act either singly or in combination forming a single probiotic. These include inhibition of a pathogen *via* production of antagonistic compounds, competition for attachment sites, competition for nutrients, alteration of enzymatic activity of pathogens, immunostimulatory functions, and nutritional benefits such as improving feed digestibility and feed utilization (*203–205*). It is often reported that a probiotic must be adherent and colonize within the gastrointestinal tract, it must replicate to high numbers, it must produce antimicrobial substances, and it must withstand the acidic environment of the gastrointestinal tract (*2,206–208*).

Verschuere *et al.* (*209*) suggested a new definition of a probiotic for aquatic environments: 'a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host's response towards disease, or by improving the quality of its ambient environment', or that 'a probiotic is an entire microorganism or its components that are beneficial to the health of the host' (*210*). Table 3 (*202,211–226*) presents the application of probiotic strains in animal nutrition, during feed processing and aquaculture.

Probiotics and Prebiotics

In a recent review, Ranadheera *et al.* (*227*) reported that food substrate/diet is considered as one of the major factors in regulating colonization of microorganisms in the gastrointestinal tract. Food helps to buffer the bacteria through the stomach and may contain other functional ingredients that could interact with probiotics to alter their functionality. Colonic foods, which encourage the growth of favourable bacteria, are referred to as prebiotics. Oligosaccharides such as lactulose, galactooligo-

Table 3. Application and effects of probiotics in animal feed and aquaculture

Probiotic strain	Application	Probiotic effects	Ref.
Bacillus subtilis, *Bacillus licheniformis*	shrimp production	reduce stress, improve health, the quality of water, clean effluent water, control pathogenic bacteria and their virulence, stimulate the immune system, improve gut flora, substitute antibiotics, improve growth	*202*, *211*
Bacillus spp. and yeasts	mollusc production	minimize diseases caused by *Vibrio* spp. and *Aeromonas* spp., which results in mollusc mortality	*212*
Clostridium spp.	freshwater fish feed	produces digestive enzymes, which facilitate feed utilization and digestion, antibacterial activity against pathogenic microorganisms	*213*
Bacillus spp., *Saccharomyces cerevisiae*	aquaculture	improve water quality and interaction with phytoplankton, possess adhesion abilities, produce bacteriocins, provide immunostimulation	*209*
Bacillus spp., *S. cerevisiae*	aquaculture	stimulate the growth of microalgae that produce organic extracts capable of inhibiting pathogens and vibrios, then some microalgae species produce the antibiotic thiotropocin against some pathogens	*214*, *215*
S. cerevisiae	aquaculture	immunostimulatory activity, produces inhibitory substances against pathogens	*209*
Bifidobacterium longum, *L. plantarum*	chicken feed	produce antimicrobial substances against pathogens such as *Campylobacter*	*216*
Pediococcus acidilactici, *Lactococcus lactis*, *L. casei*, *Enterococcus faecium*	weaned piglet	stimulate animal growth, reduce coliform counts by the production of antimicrobial metabolites	*217*
S. cerevisiae	lactating ruminants	facilitates increased mobilization of body reserves, increases milk fatty acid production	*218*
S. cerevisiae	camel feed	increases total mass gain and improves feed utilization	*219*
S. cerevisiae	buffalo feed	increases digestion of cellulose	*220*
Pediococcus acidilactici	broiler chickens	improves performance, reduces serum cholesterol	*221*
Lactobacillus, *Bifidobacterium*, *Streptococcus*, *Enterococcus* ssp.	layer hens	reduces mortality	*222*
L. sporogenes	broiler chickens	reduces serum total cholesterol and triglycerides	*223*
Lactobacillus ssp.	chicken feed	immunomodulating properties	*224*
Lactobacillus spp., *Bacillus* spp.	poultry feed	reduces zoonosis in poultry meat	*216*
L. reuteri LPB P01-001	swine feed	mass gain, antimicrobial activity against *E. coli* and *S. aureus*	*225*
Enterococcus faecalis, *E. faecium*	canine feed	bacteriocin-like inhibitory substances, antimicrobial activity against Gram(+) bacteria, colonize transiently	*226*

saccharides, inulin, fructooligosaccharides, and other food carbohydrates are some of the well known examples of prebiotics. There is an obvious potential for a synergetic effect when combining probiotics and prebiotics appropriately, because prebiotics promote the growth and activities of probiotics. By increasing the amount of prebiotics in the diet, it is possible to increase and maintain healthy bacterial gut flora in the host (*228*,*229*). Ingredients in certain food products may naturally contain prebiotics, which help to improve the functional efficacy of probiotics. Many other foods such as dairy and meat products, cereals, beverages and infant formulas can be fortified with prebiotics during manufacturing process to increase probiotic efficacy (*230*). In addition, a number of other suitable food components including non-specific substrates, plants and their extracts, metabolites of microorganisms and polyunsaturated fatty acids may also be important in probiotic efficacy (*231*).

The Technology of Probiotics

Probiotics are certainly very sensitive to many environmental stresses, such as acidity, oxygen and heat. Before a probiotic can benefit human health, it must fulfill several criteria related to the safety and stability (activity and viability in products; adherence; invasive potential; resistance to low pH, gastric juice, bile acid and pancreatic juice; colonisation/survival *in vivo*) and functional and physiological aspects (adherence to intestinal ephitelium/tissue/virulence, antagonism to pathogenes, antimicrobial activity, stimulation/supression of immune response, selective stimulation of beneficial bacteria and clinical side effects in voluteers/patients). The viability of probiotics is a key parameter for developing probiotic foods. Several factors shown in Fig. 1 affect the viability of probiotic bacteria until they reach the target site of the host (*232*).

A strain is commercially demanded for its tecnological and health properties. Consequently, the search for new technologies that enable high cell yield at large scale and ensure probiotic stability in food remains strong, because many strains of intestinal origin are difficult to propagate and they must survive for economic and health reasons. In addition, more efficient technologies could lead to greater product efficacy and strain diversification.

Some authors have presented developments in fermentation technologies for producing probiotic bacteria as well potential new approaches for enhancing the performance of these organisms during fermentation, downstream processing, and utilization in commercial products, and for improving functionality in the gut (*232*–*234*).

Until now, very few data have been reported on continuous fermentations with probiotics, although this approach could provide benefits, as recently reviewed by Doleyres and Lacroix (*233*) for bifidobacteria. However, continuous fermentations can be more difficult to operate under industrial conditions, because they are highly susceptible to contamination and cell characteristics can be lost over time. This technology is worth investigating and could be used to produce cells with different physiologies and to apply various stresses under well-controlled conditions (*232*).

Membrane systems with continuous feeding of fresh medium where cells are retained in the bioreactor by an ultrafiltration or microfiltration membrane are also an interesting technological possibility. In this case, small molecules diffuse through the pores of the membrane according to their size. Therefore, inhibitory metabolic products are eliminated from the permeate and cells are concentrated on the retentate side. The concentrated cell fraction can be harvested batch-wise or continuously with no, or minimal additional downstream treatment for cell concentration before freezing or freeze drying.

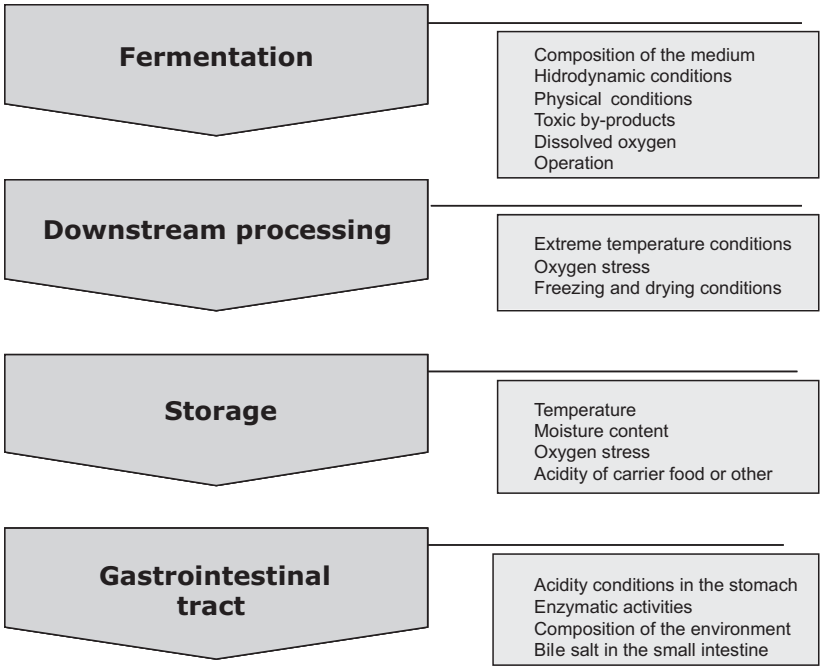

Fig. 1. Factors influencing the stability of probiotics during processing steps (adapted from Lacroix and Yildirim (*232*))

Different approaches that increase the resistance of these sensitive microorganisms against adverse conditions have been proposed, including appropriate selection of acid- and bile-resistant strains, use of oxygen-impermeable containers, two-step fermentation, stress adaptation, incorporation of micronutrients such as peptides and amino acids, and microencapsulation (*2*). Lacroix and Yildirim (*232*) reported that cell immobilization can be used to perform high cell density fermentations for both cell and metabolite production.

Microencapsulation is defined as a technology of packaging solids, liquids or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under the influences of specific conditions (*235–237*). A microcapsule consists of a semipermeable, spherical, thin, and strong membrane surrounding a solid/liquid core, with a diameter varying from a few microns to 1 mm (*238*). Encapsulation in hydrocolloid beads entraps or immobilizes the cells within the bead matrix, which in turn provides protection in such an environment (*239*). There are several techniques such as spray drying, freeze drying, fluidized bed drying for encapsulating the cultures and converting them into a concentrated powdered form. However, the bacteria encapsulated by these techniques are completely released in the product. In this case, the cultures are not protected from the product environment or during the passage through the stomach or intestinal tract (*240*).

Food-grade polymers such as alginate, chitosan, carboxymethyl cellulose (CMC), carrageenan, gelatin and pectin are mainly applied, using various microencapsulation technologies (*238*). The most widely used encapsulating material is alginate, a linear heteropolysaccharide of D-mannuronic and L-guluronic acids extracted from various species of algae (*241*). Alginate beads can be formed by both extrusion and emulsion methods (*238*). The use of alginate is favoured because of its low cost, simplicity, and biocompatibility (*242–244*). Other materials used with the emulsion technique which avoid the release of the cultures in the food product are a mixture of κ-carrageenan and locust bean gum (*245–247*), cellulose acetate phthalate (*248*), chitosan (*249*), and gelatine (*239–250*).

Several factors, such as the capsule size (*181*), the method of microencapsulation (*251*), the coating of the capsules (*252*), the technological properties of probiotic strains with regard to processing and heat stability, the resistance of probiotic strains to the acidic conditions present in the gut, and the presumed synergistic effects of pro- and prebiotics by combining them in a single product, have been observed to strongly influence the viability of the probiotic cultures and, as a result, further research is still needed in this area.

Microcapsules and microspheres can be engineered to gradually release active ingredients (*239*). A microcapsule may be opened by many different means, including fracture by heat, solvation, diffusion, and pressure (*253*). A coating may also be designed to open in the specific areas of the body. A microcapsule containing acid-labile core materials that will be consumed by gastrointestinal fluids must not be fractured until after it passes through the stomach. A coating must therefore be used that is able to withstand acidic conditions in the stomach and allows active ingredients to pass through the stomach (*237,254*).

Several advantages of entrapped-cell over free-cell fermentations have been demonstrated: high cell densities, reuse of biocatalysts, improved resistance to contamination and bacteriophage attack, enhancement of plasmid stability, prevention from washing-out during continuous cultures, and the physical and chemical protection of cells (*234*). Table 4 (*179–181,238,248,250–252, 255–281*) presents some applications and properties of immobilized probiotic cells.

Reliable and convenient biomarkers need to be developed for process monitoring and product assessment. In this regard, the 'omics' technnologies could be particularly useful for identifying such functionality-relevant biomarkers. These approaches could also help to identify the mechanisms for cell fitness and stress adaptation, which will be needed to develop more generic and science-based technologies for the production of sensitive probiotics. This fact would surely enlarge the range of commercial probiotics and product applications. Moreover, these tools might facilitate screening approaches to identify new probiotic strains that combine suitable technological and functional qualities (*232*).

New Trends in Probiotic Products and Processing

In general, consumer's understanding of the potential benefits of foods containing viable bacteria/probiotics is poor, particularly in the countries without a tradition of cultured/sour dairy products. There are many barriers to communicating messages about probiotics and the role of diet in the gut flora modulation. However, in the countries where there have been well planned educational programmes among consumers and health professionals, the degree of awareness has increased (*282,283*). In the future, health claims may help inform consumers of the potential benefits, but it is crucial that appropriate communication guidelines are adhered to and that all claims are scientifically substantiated (*14*).

As it was presented by Reid (*11*), the number of scientific publications on probiotics has doubled in the past three years and this recent interest (*284*) has been further stimulated by several factors: (*i*) exciting scientific and clinical findings using well documented probiotic organisms; (*ii*) concerns over limitations and side effects of pharmaceutical agents; and (*iii*) consumer's demand for natural products. The key to the future of probiotics will be the establishment of a consensus on product regulation, including enforcement of guidelines and standards, appropriate clinical studies that define strengths and limitations of products, and basic science studies that uncover the mechanisms of action of strains. Besides, the molecular elucidation of the probiotic actions *in vivo* will help to identify true probiotics and select the most suitable ones for the prevention and/or treatment of a certain illness (*82*). In fact, not only new probiotic food must be developed, but the study and development of new medications to combat diseases should be continously performed.

Table 4. Microencapsulation of probiotic bacteria using different technologies

Bacteria	Polymer	Microencapsulation technology	Functionality	Ref.
Bifidobacterium	alginate/glycerol	gel beads	biomass production	*255*
Bifidobacterium	carrageenan/locust bean gum	gel beads		*256,257*
Bifidobacterium	alginate/chitosan	gel beads	acid/storage stable	*258*
Bifidobacterium	alginate/pectin/whey protein	gel beads	acid/storage stable	*259*
Bifidobacterium	resistant starch	gel beads	acid/storage stable	*260*
Bifidobacterium	waxy maize starch	gel beads/emulsification	acid/storage stable	*180*
Bifidobacterium	alginate/starch	gel beads	acid/storage stable	*179,261*
Bifidobacterium	modified waxy maize starch	spray-dried powder		*180*
B. bifidum	alginate	gel beads	acid/thermo/storage stable	*252*
B. bifidum	κ-carrageenan		freeze-dried powder	*262*
B. breve	alginate microspheres	emulsification	acid stable	*263*
B. breve	powder of freeze-dried culture	micronization	thermo/storage stable	*264*
B. longum				
B. infantis	gellan gum and xanthan gum	gel beads	acid/storage stable	*265*
B. lactis	alginate	gel beads/extrusion	acid/bile salt stable	*263*
B. lactis (Bb-12)	cellulose acetate phthalate	gel beads		*266*
B. longum	whey protein	micronization	acid stable	*251,264,267*
B. longum	κ-carrageenan	gel beads/emulsion		*268,269*
B. longum	κ-carrageenan/locust bean gum	gel beads/emulsion		*270*
B. longum	alginate	gel beads/extrusion		*276*
B. pseudolongum	cellulose acetate phtalate	gel beads	acid and bile salt stable	*271*
Lactobacillus	carrageenan	gel beads	biomass production	*248*
	alginate	gel beads	acid stable	*181*
	alginate/starch	gel beads	acid/storage stable	*179,261*
	carrageenan/locust bean gum	gel beads		*238*
L. acidophilus	alginate	direct compression	acid stable	*272*
L. acidophilus LA14 and *B. lactis* BI07	alginate/xanthan gum	gel beads	acid/storage stable	*273*
L. acidophilus (La-05)	cellulose acetate phthalate	gel beads	acid/bile salt stable	*266*
L. acidophilus	alginate	gel beads	acid/thermo/storage stable	*274*
L. acidophilus	powder of freeze-dried culture	micronization	thermo/storage stable	*264*
L. rhamnosus	alginate	gel beads	acid/storage stable	*275*
L. bulgaricus	carrageenan/locust bean gum	gel beads	biomass production	*276*
L. casei	carrageenan/locust bean gum	emulsification	acid stable	*269*
L. casei	alginate	gel beads	acid stable	*277*
L. delbrueckii	alginate/sodium lauryl sulphate	gel beads	biomass production	*278*
L. lactis	gelatin/toluene-2,4-diisocyanate	gel beads	biomass production	*250*
L. reuteri	Ca-alginate and κ-carrageenan	gel beads	storage	*279*
P. acidilactici	corn and olive oil microcapsules emulsified by peptides	emulsification	acid/storage stable	*280*

Conclusions

Probiotics have been extensively studied and explored commercially in many different products in the world. Recent studies have suggested that probiotics have demonstrated beneficial effects to human and animal health. Much of the clinical probiotic research has been aimed at infantile, antibiotic-related and traveller's diarrhoea. The non-pathogenic organisms used as probiotics consist of a wide variety of species and subspecies, and the ability to adhere, colonise and modulate the human gastrointestinal system is not a universal property. *Lactobacillus* and *Bifidobacterium* are the main probiotic groups; however, there are reports on the probiotic potential of yeasts. Some of the identified probiotic strains exhibit anti-inflammatory, anti-allergic and other important properties. Besides, the consumption of dairy and non-dairy products stimulates the immunity in different ways. Future research must investigate the mechanisms by which gut microflora interacts with the intestinal epithelium in health and disease. With this knowledge, optimal probiotic strains can be developed. The viability of probiotics is a key parameter for developing probiotic food products. New technologies have been developed to enable high cell yield at large scale and ensure probiotic stability for a long period in food. Various food matrices, dairy and non-dairy, have been used with probiotics and were briefly documented. With different technologies, such as microencapsulation, cell immobilization and continuous fermentation, the probiotics will become an important and viable ingredient in the functional foods, expanding the probiotic application outside the pharmaceutical and supplement industries.

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