Chapter 3
Industrial Production of Active Probiotics for Food Enrichment

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Abstract Functional foods are defined as foods that have health benefits beyond their inherent nutritional value. The incorporation of probiotics in food products is one of the most popular forms of such products and acceptable to most consumers. In this chapter, various industrial probiotic products are discussed, including the type of microorganisms used and the production process. Details of processing conditions and choice of probiotics for retaining the viability of the microorganism through production are included. Advanced non-food application of probiotics and the potential for such products are also presented.

Keywords Probiotics · Fortified products · Stability and safety · Non-food applications

3.1 Introduction to Probiotics

Functional foods are foods that have a potentially positive effect on health beyond basic nutrition. Probiotics are living bacteria and yeasts that are incorporated into food products because they help to maintain the microbial flora in the gut, which in turn results in good overall health. An estimated 500 to 1000 species of microorganisms inhabit the digestive system. Maintaining a good distribution of beneficial microorganisms prevents the inhabitation of pathogenic microbes and is also known to improve the immune response.

An ideal probiotic microbe must be of human origin, with beneficial physiological effects, and should be generally regarded as safe (GRAS) (Singh et al. 2011). An efficient probiotic should have important properties including good stability under storage and distribution conditions, and should be non-pathogenic, non-toxic, sustainable in the host body, with effective adhesion, resistance to low

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pH and bile salts in the gut, and should have good activity, with beneficial effects on the host (e.g. enhancing immunity) (Fuller 1989).

The common criteria for probiotics used in industry are as follows:

- **Biosafety**: Microbes should be generally regarded as safe (GRAS) (e.g. *Lactobacillus, Bifidobacterium, Enterococcus*).
- **Origin**: Microbes used in probiotics should preferably be autochthonous in origin.
- **Resistance in in vitro and in vivo conditions**: Microbes should be tolerant to the defense mechanisms, pH, bile and pancreatic conditions in the host body.
- **Adherence**: Microbes should adhere to the intestinal epithelium and colonize to survive for longer periods.
- **Antimicrobial activity**: An important criteria for probiotic organisms is that they are effective in protecting the host against pathogens. Therefore, they should exhibit certain antagonistic properties. For example, *Lactobacillus* sp., a widely used microbe in probiotics, produces inhibitors (e.g. bacteriocins), reduces redox potential, produces hydrogen peroxide, decreases pH through the production of lactic acid and protects the human host against harmful organisms (Kosin and Rakshit 2006).

Over the past two decades, a considerable amount of research and development has been focused on probiotics and their inclusion in many traditional foods. Consumer acceptance has resulted in the availability of large numbers of probiotic food products and their industrial production. The present chapter reviews the major industrial probiotic products, including the type of probiotics involved, the production processes, and the related stability and food safety issues. The application of probiotics in non-food applications in feed, medicine, aquaculture and veterinary products is also covered. Table 3.1 provides a list of common microbes, their application and probiotic properties.

3.2 Industrial Probiotic Products

There is an increased awareness of the health benefits of dietary food and functional foods with probiotics. According to market reports, up to 93 % of consumers in North America believe that the risk of disease can be reduced by the consumption of functional foods (Champagne and Møllgaard 2008). Industries are competing for the production of efficient probiotic functional foods, as high growth rates and low processing costs make them good candidates for industrial production. In 2011, the market for probiotics products was valued at $27.9 billion, and this is expected to increase substantially, by 6.8 % annually, reaching $44.9 billion by 2018 (Chr. Hansen 2013). The Asia-Pacific region is currently experiencing the highest rate of growth in probiotics production, followed by Europe (Chr. Hansen 2013). Some of the dairy-based industrial probiotic strains in use in a variety of market products are listed in Table 3.2.

Functional dairy-based foods can be divided into fortified dairy and whey (protein)-based products (Özer and Kirmaci 2010).

Table 3.1 Common probiotic bacteria, properties and applications (BC Diary 2015)

Bacteria	Properties	Use	Potential probiotic effects
Lactobacillus acidophilus	Acid-resistant Grows slowly and is less viable in fermented products	Used in acidophilus milk and in kefir; may be used in yogurt	Fights intestinal infection and may prevent colon cancer. Reduces intestinal transit time
Lactobacillus GG	Good adherence. Colonies do not last long; therefore, recommended to consume a few times a week Minimum levels for colonization are • 10^8 cells in milk • 10^9 cells in fermented milk or enteric tablets • 10^{10} cells in gelatin capsules	Some new fermented dairy products using LGG are available in Europe	Inhibits pathogens. Reduces tumor development Prevents traveler's diarrhea, antibiotic-associated diarrhea and infant diarrhea
Lactobacillus casei	Some strains are acid-tolerant Does not colonize	Used in kefir and many cheeses including cheddar, also used in some new yogurt-like products	Induces the activity of intestinal microflora. Decreases incidence and duration of diarrhea Induces levels of immunoglobulins, γ-interferon and phagocytic activity. Reduces the risk of colon cancer
Bifidobacterium	Some strains are acid-tolerant, but unclear on adherence and colonization Produces both lactic acid and acetic acid	Can be used in yogurt	Restricts growth of pathogens Prevents and cures diarrhea Reduces intestinal transit time and may reduce colon cancer Induces production of secretory immunoglobulin

3.2.1 Fortified Dairy Products

Dairy products are considered to be good vectors for the delivery of probiotics to humans, because of their inherent characteristics that favor probiotic growth and make

Table 3.2 Some of the prevailing probiotic products in the market

Brand name	Mode of delivery	Strains	Scope	Producer	Effect	Recommended dose	Reference
Bifidus Regularis® Activia	Yogurt	*Bifidobacterium lactis* CNCM I-2494	Human	Dannon (USA)	Reduces irritable bowel syndrome (IBS) symptoms, reduces GI discomfort	10^{10} CFU × 2/day	(Activia; Guyonnet et al. 2007; Aureli et al. 2011; Agostini et al. 2012)
Actimel	Fermented milk	*L. casei* DN-114 001	Human	Danone (UK)	Prevention of *C. difficile* infection (CDAD) in adults	10^{10} CFU × 2/day	(Danon Actimel; Hickson et al. 2007; Aureli et al. 2011)
Yakult Light	Fermented dairy drink	*L. casei* Shirota	Human	Yakult (USA)	Reduces the incidence of antibiotic-associated diarrhea (AAD), positive effect on natural killer (NK) cell activity	6.5×10^9 CFU × 1/day	(Yakult; Activity et al. 2007; Wong et al. 2014)
Florastor®	Capsule (250 mg)	*Saccharomyces cerevisiae boulardii*	Human	Biocodex (Canada)	Prevention of AAD	250 mg ($\sim 5 \times 10^9$ CFU) × 2/day	(Sazawal et al. 2006; Florastor 2009; Aureli et al. 2011)
BioGaia Gastrus	Chewable tablets	*L. reuteri* 17,938, *L. reuteri* ATCC PTA 6475	Human	Biogaia (Sweden)	Treatment of *H. pylori* infection, acute infectious diarrhea in children	Each tablet (2×10^8 CFU)/day	(BioGaia; Francavilla et al. 2014; Szajewska et al. 2014)

(continued)

Table 3.2 (continued)

Brand name	Mode of delivery	Strains	Scope	Producer	Effect	Recommended dose	Reference
Culturelle	Capsule	*L. rhamnosus* GG (LGG)	Human	Valio Dairy (Finland)	Treatment of acute infectious diarrhea in children, AAD, irritable bowel syndrome, adjuvant in *H. pylori* eradication	Each capsule (1×10^{10} CFU)/day	(Aureli et al. 2011; Culturelle® 2013)
LIFE START®2	Goat milk (2.50 oz powder)	*Bifidobacterium infantis*	Human	Natren (USA)	Alleviation of irritable bowel syndrome, reduces risk of necrotizing enterocolitis	1 gm (2×10^{9} CFU)/day	(KeLATOX; Aureli et al. 2011; UC Davis 2014)
inner-ēco Coconut Water Probiotic Kefir	Fermented coconut water	*Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *diacetylactis, Leuconostoc mesenteroides* subsp. *cremoris, Lactobacillus kefiri, Kluyveromyces marxianus* var. *marxianus, Saccharomyces unisporus*	Human	inner-ēco (USA)	Promotes healthy microflora ecology in the GI tract, reduces sugar cravings	1 tbsp* (1×10^{11} CFU)/day	(Wegmans; Aureli et al. 2011; TrendMonitor 2013)
Enviva® PRO	Dry granules	*Bacillus subtilis* (3 strains)	Poultry	Danisco Animal Nutrition (UK)	Reduces coliform colonization in the gut, enhances immunity reduces nutritional stress in the gut	1.5×10^{5} CFU/g of feed	(Danisco; Lee et al. 2011)
Enviva® MPI	Fermented milk product	*L. rhamnosus*, *L. farciminis*	Piglets	Danisco Animal Nutrition (UK)	Supports piglet gut health, protects the growing pig against zoonotic bacteria such as *E. Coli*, *Clostridium*, *Brachyspira, Campylobacter jejuni*	5 g (5×10^{8} VFU)/kg of feed	(Bernardeau et al. 2009; Enviva 2013; Tareb et al. 2013)

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Table 3.2 (continued)

Brand name	Mode of delivery	Strains	Scope	Producer	Effect	Recommended dose	Reference
Life Products 10-G	Dry granules or oil	*Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus plantarum, Pediococcus acidilactici*	Dairy cattle	Life Products (USA)	Reduces the growth of *E. coli* and other pathogenic bacteria, improves immune system, improves milk productivity and feed consumption	1 g (2×10^9 CFU)/head/day	(Lifeproducts)
BACTOCELL® PA10	Lyophilized live bacteria	*Pediococcus acidilactici* MA 18/5 M	Shrimp & fish	LALLEMAND Inc. (Canada)	Supports piglet gut health, protects the growing pig against zoonotic bacteria such as *E. Coli*, *Clostridium*, *Brachyspira, Campylobacter jejuni*	**Salmonids and shrimp:** 100–1000 g/ton of complete feed **Marine fish:** 100–200 g/ton of feed (1 g = $1*10^{10}$ CFU)	(Lallemand; Castex et al. 2009)
Probiotics Spilac	Powder	*L. sporogenes*	Shrimp & fish	Guybro Chemical (India)	Improves growth, enhances biomass and immunity	**Fish:** 1 kg/ton of feed **Shrimp/prawn:** 2 kg/ton of mash feed, 5 kg/ton of pellet feed (1 kg = $33.2*10^9$ CFU)	(Guybro; Seenivasan et al. 2014)
SUKAFEED-B. Sub	Powder	*Bacillus subtilis*	Pigs, poultry, ruminants and aquatic animals	Sukahan (Weifang) Bio-Technology Co. LTD (China)	Improves feed efficiency and promotes digestion and absorption of nutrients in feed, enhances immune function, reduces stress, prevents diarrhea and dysentery	500–1000 g per ton of feed (1 g = 2×10^9 CFU)	(Sukahan; Soccol et al. 2013)

(continued)

Table 3.2 (continued)

Brand name	Mode of delivery	Strains	Scope	Producer	Effect	Recommended dose	Reference
P-Lact Plus	Powder	*Lactobacillus casei, Streptococcus faecium, Lactobacillus acidophilus, Lactobacillus lactis, Bacillus coagulans, Bacillus licheniformis, Saccharomyces cerevisiae*	Shrimp/prawn	Marine Aqua Technologies (India)	Enhances growth and body weight, maintains optimal health and immunity	**Regular use**: 10 g/kg of pellets **Stress/poor growth/disease condition:** 20 g/kg of pellets 10 days (kg = 4.5×10^5 CFU)	(PVS Laboratories Limited)

*tbsp: tablespoon; *L.*: *Lactobacillus*

them sustainable through the storage period. For the application of probiotics in dairy products, industries must focus on specific parameters (e.g. acidity, pH, dissolved oxygen content, redox potential and hydrogen peroxide effect) to meet essential requirements of probiotic properties (Akin 2005; Bais et al. 2006; Akin et al. 2007), health effects and regulations (Sanders and Klaenhammer 2001; Parvez et al. 2006). For good therapeutic effects, it is normally accepted that probiotic bacterial concentration in milk products should range from 10^6 to 10^7 colony-forming units per milliliter (CFU/mL). This can be maintained by reducing the dissolved oxygen level using microencapsulated cells (Ozer et al. 2005; Akin et al. 2007).

Probiotic dairy drinks were the first commercialized functional beverages, and are consumed worldwide in huge amounts in the form of a wide variety of products (Halliwell 2002; Hilliam 2004). Some of the commercial dairy-based probiotics and their manufacturers are listed in Table 3.3. *Lactobacillus rhamnosus* GG (LGG) is the most widely used probiotic bacterium in dairy industries because of its viability in the human gut and good acid tolerance. Some of the dairy product brands containing this bacteria are Gefilus, developed by Valio Ltd. (Finland), Aktifit, Biola, BioAktiv, YOMO, LGG+, Yoplait 360° and Kaiku Actif, which include milk, yogurt, buttermilk and drink products (Özer and Kirmaci 2010).

3.2.1.1 Probiotic Milk

Milk is the primary food through which probiotics can be delivered to the human body. The bacterium most widely used as a probiotic strain in milk is *Lactobacillus acidophilus*, because of its low growth rate and stability in milk. Another commonly used probiotic bacterium in such products is *Bifidobacterium bifidum*. In order to allow the growth of *L. acidophilus*, an acidic pH (5.5–6.0) must be maintained in the medium. However, the fermentation of milk often results in a drop in pH to below these levels, leading to a reduction in bacterial count.

Production Process

The production of *L. acidophilus* milk includes heat treatment, inoculation and fermentation. Initially, milk is heated to 95 °C for 1 h or for 15 min at 125 °C (Vedamuthu 2006) for the production of denatured proteins and release of peptides, which stimulates the growth of *L. acidophilus.* The milk is then cooled to 37 °C and kept for 3–4 h to allow spores present to germinate, followed by sterilization to destroy any vegetative cells and cooling to 37 °C. An active bulk culture of *L. acidophilus* is inoculated into the heat-treated milk at a rate of about 2–5 %, and is allowed to ferment until the pH drops to 5.5–6.0 or approximately 1 % of lactic acid is obtained (Surono and Hosono 2011). The number of viable *L. acidophilus* colonies increases to 2–3 × 10^9 CFU/mL during fermentation for 18–24 h., but this decreases with time, and the count may be reduced with extended incubation time. Co-culturing of *L. acidophilus* with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* (subs. *bulgaricus*) is often preferred. Finally, the product is rapidly cooled to less than 7 °C and then pumped into the packaging containers (Kosikowski and Mistry 1997; Vedamuthu 2006).

Table 3.3 Production and characteristics of acidophilus milk

Product	Starter organism	Fermentation	Additives	Specifications
Acidophilus milk	*Lactobacillus acidophilus*	At 37 °C until pH 5.5–6.0 (usually takes 18–20 h). Inoculation level 2–5 %	Enrichment with minerals and vitamins is possible	Distinctive tangy flavor and slightly thickened texture
Sweet acidophilus milk	*Lactobacillus acidophilus*	No fermentation is allowed. *L. acidophilus* is added to pasteurized milk at >5 °C	Enrichment with minerals and vitamins is possible	Sweet flavor, extended shelf life to 14 days (if freeze-dried, shelf life can be extended up to 28 days)
Acidophilin	*Lactobacillus acidophilus*, kefir yeasts	Fermentation is achieved at 35 °C	Whole or skimmed milk is fortified with skim milk powder, sucrose or cream	Acidophilin is used to treat colitis, enterocolitis, dysentery and other intestinal diseases. The product is sweeter than acidophilus-yeast milk
Diphilus milk	*Lactobacillus acidophilus*, *B. bifidum*	Fermentation is achieved at 37°C until pH 4.5–4.6.	N/A	Produced from cow's milk and has a specific taste and aroma. Used in therapy for intestinal disorders.
Acidophilus Bifidus milk	*Lactobacillus acidophilus*, *B. bifidum*	Fermentation is achieved at 37 °C until pH 4.5–4.6	Protein enrichment and fat standardization are common practices	Shelf life of the product is around 20 days, with an average number of probiotic bacteria of 10^8–10^9 CFU /mL
Bifighurt	*Bifidobacterium longum* (CKL 1969) or *Bifidobacterium longum* (DSM 2054)	Fermentation is achieved at 42 °C until pH 4.7 For human strains of probiotic bacteria Fermentation is set at 37 °C	N/A	Slimy texture with a characteristic slightly acidic flavor

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Table 3.3 (continued)

Product	Starter organism	Fermentation	Additives	Specifications
		Inoculation level of probiotic strains is around 6 %		
Bifidus milk	*Bifidobacterium bifidum* or *B. longum*	Inoculation level is 10 %. Fermentation is achieved at 37 °C until pH 4.5–4.6	Protein enrichment and fat standardization are common practices	Slightly acidic flavor and characteristic aroma with a lactic acid-to-acetic acid ratio of 2:3
Yakult	*Lactobacillus casei* Shirota	Fermentation is achieved at 37 °C until pH 4.5–4.6 (usually takes 16–18 h)	Total solids and sugar levels are adjusted to 3.7 and 14 % prior to heat treatment. It is common practice to add nature-identical flavors (e.g. tomato, celery, carrot)	The shelf life of the product is about 30 days. Regular consumption of this product has a positive effect on natural killer (NK) cell activity in middle-aged people
Yakult Miru-Miru	*Lactobacillus casei, Bifidobacterium bifidum* or *Bifidobacterium breve*, *Lactobacillus acidophilus*	Fermentation is achieved at 37 °C until pH 4.5–4.6	Addition of saccharides is a common practice	This product is broadly similar in composition to cow's milk
Acidophilus-yeast milk	*Lactobacillus acidophilus, Saccharomyces lactis*	Fermentation is achieved at 35 °C until 0.8 % lactic acid and 0.5 % ethanol levels are reached	N/A	Viscous and slightly acidic product with a yeasty taste

Modified from Özer and Kirmaci (2010)
N/A Not available

The production of bifidus milk, which contains *Bifidobacterium bifidum*, is similar to that of acidophilus milk. Bifidus milk often contains lactic and acetic acids in a ratio of 2:3, making it acidic in nature. The production of probiotic milk with both acidophilus and bifidum bacteria is also possible, and such milk has a characteristic aroma and is slightly acidic (Homayouni et al. 2012).

The protein content of fermented and non-fermented milk is similar, but higher amounts of free amino acids are present in acidophilus milk. It can also be enriched by the addition of calcium, iron and vitamins. In acidophilus milk products, lactose is hydrolyzed by β-galactosidase enzymes present in *Lactobacillus acidophilus*, which makes them consumable by lactose-intolerant individuals. However, due to the high acid content, their sour taste can reduce the market for these products. This has led to the development of sweet acidophilus milk. The bacteria used in probiotic milk products are viable for 14 days when stored at <10 °C after packaging. Freeze-dried cultures, however, have shown better results, with viability for 23 days at 4 °C reported for sweet acidophilus milk. Therefore, frozen products have gained much attention in the production of probiotic foods (Vedamuthu 2006).

3.2.1.2 Probiotic Cheese

Cheese is another dairy product which supports the survival of probiotic microbes, and is thus considered a good vehicle for transporting probiotics into the human body. This can be achieved either by producing cheese that favors the requirements of probiotic strains, or by developing appropriate probiotic strains to adapt to the cheese (Homayouni et al. 2012).

Both *L. acidophilus* and *B. bifidum* are common probiotics used in cheese products. Various cheese varieties such as cheddar (Dinakar and Mistry 1994; Gardiner et al. 1998), gouda (Gomes et al. 1995), cottage (Blanchette et al. 1996; O'Riordan and Fitzgerald 1998) and white-brined (Ghoddusi and Robinson 1996) cheeses have been assessed with these probiotic strains.

Production Process

Cheddar cheese has been found to be an efficient carrier of probiotics into the gastrointestinal tract. Certain microbial strains such as *Enterococcus faecium* (Gardiner et al. 1999) have shown better viability, stability and activity in cheddar cheeses than in other varieties of cheese. Gardiner et al. (1999) reported that when the probiotic culture was exposed to porcine gastric juice with a pH of 2, cheddar cheese showed a greater protective effect than yogurt. Cheddar cheese is manufactured under controlled bacteriological conditions to reduce contamination (McSweeney et al. 1994). The standard method of preparing cheddar cheese is initiated by curdling with the addition of starter and probiotic microbes followed by the rennet enzyme. Curds are cooked and milled at 39 °C and the pH is maintained at 6.1. Salt is added to a level of 2.8 % (wt/wt). The curds are then placed in the template molds, and pressure (200–400 kPa) is applied. The produced curd is then vacuum-packed. It is recommended that the packed cheese is stored at or below 8 °C in order to maintain the viability of the probiotic culture (Gardiner et al. 1998).

Research on the production of probiotic cheese has given rise to precautions and insights, which in turn have resulted in better products. It has been suggested that oxygen content and water activity of probiotic cheese must be evaluated before packaging (Dave and Shah 1997). Roy and Mainville (1997) reported that a high

survival rate and stability of probiotic microorganisms could be achieved by cooling the product. In addition, low temperature inhibits the interaction between the probiotic microorganism and cheese components. The subtle interaction of probiotic microbes in the cheese depends on the type and quantity of sugars available, degree of hydrolysis of milk proteins and lipids, availability of free amino acids and availability of short-chain fatty acids (Fox et al. 1996).

Antagonism between probiotic microbes and the starter organisms in cheese has also been reported. This is mainly caused by bacteriocins, peptides or proteins with antibiotic properties, which are considered limiting factors in production (Joseph et al. 1998). Such antagonism may also be caused by other constituents such as hydrogen peroxide, benzoic acid, biogenic amines and lactic acid. These effects also depend on whether the probiotics are added before or after fermentation. Interactions and metabolic activity may be reduced by lowering the temperature (<8 °C).

The final taste and flavor of the probiotic cheese is mainly affected by the proteolytic and lipolytic properties of the probiotic microbes (Kunji et al. 1996). Cheddar cheese is considered a potent vehicle for carrying these probiotic microbes to the human gastrointestinal tract, due to its dense solid structure and fat content, which helps to protect the probiotic bacteria. In addition, low pH creates a buffer environment around it and thus provides favorable conditions for the survival of the probiotic strains (Ross et al. 2002; Bergamini et al. 2005).

3.2.1.3 Probiotic Yogurt

Yogurt is the most popular probiotic consumer product, and is preferred for its nutritional value, health benefits and various therapeutic effects. Increased awareness of the importance of probiotics has made probiotic yogurt a common product in most grocery stores. Traditionally, yogurt has been made with *Lactobacillus bulgaricus* and *Streptococcus salivarius* as starter cultures. However, they are not able to survive the gastrointestinal tract environment and are thus not considered for use as probiotics in fermented products. Therefore, the addition of probiotic microbes (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) is preferred due to their value and viability.

Production Process

Probiotic yogurt production is similar to traditional yogurt manufacturing except for the inoculation of additional probiotic microbial cultures. Initially, starter microbial cultures (*L. bulgaricus* and *S. salivarius*) are added to the heat-treated homogenized milk with increased protein content (3.6–3.8 %) and incubated for 3.5 h at 45 °C or 9 h at 37 °C. The probiotic cultures are either added with the starter culture or after the first fermentation. The product is cooled to 4 °C before packaging. The viability of the probiotic microorganisms depends on several factors, including carbohydrate composition, fermentation medium, antagonism, culture conditions, dissolved oxygen (especially for *Bifidobacterium* spp.), incubation, and storage temperature and duration.

It has been reported that the viability of probiotic bacteria, especially *Bifidobacterium*, is reduced at refrigerated storage temperatures due to antagonistic effects (Dave and Shah 1997). One way to overcome this is to grow the *Bifidobacterium* spp. separately, wash out the free metabolites, and then transfer the cells to the probiotic yogurt (Homayouni et al. 2012). *Bifidobacterium* species are highly anaerobic in nature, and therefore, high dissolved oxygen content in milk is a critical constraint on their growth. To address this problem, a high-oxygen-utilizing bacteria, *S. thermophilus*, has been used along with *Bifidobacterium* species to maintain low levels of dissolved oxygen in yogurt. Yogurt must be stored at low temperatures for better viability of probiotic microbes.

3.2.2 Whey (Protein)-Based Probiotic Products

Whey, also referred to as a milk serum-based product, has gained much attention in recent years because of its high nutritional and low calorific value, thirst-quenching character and lower acidity than fruit juices. The nutritional value of whey basically depends on the milk. Some of the constituents in whey include lactose (70 %), proteins (beta-lactoglobulin, alpha-lactalbumin, serum albumin) and some minerals, along with certain vitamins (most of the B complex vitamins and some others) (Goyal and Gandhi 2008). Extensive research has focused on the health benefits of whey products. Weight gain and reduced incidence of diarrhea in pigs (Shilovskya 1983) and calves (Navetal et al. 1987) have been reported.

High lactose (sugar) content in whey supports the growth of probiotic microbes. Whey protects the microbes against the highly acidic environment of the gastrointestinal tract by increasing the level of pH in its immediate environment, and promotes the survival and viability of probiotic microorganisms. Therefore, whey is considered an efficient carrier for the transport of probiotics into the gut. In addition, whey provides a favorable medium for the survival of probiotic microbes during storage at low temperatures. Hernandez-Mendoza et al. (2007) studied changes in microbial count, pH values and titratable acidity during the storage of whey-based probiotic beverages. The authors reported no change in product parameters during storage for 30 days at 4 °C, except for slight acidification, but the beverage retained its acceptable flavor.

Lactobacillus and *Bifidobacterium* species are widely used probiotic microbes in whey-based products as well. Whey probiotic drinks containing *L. acidophilus* have shown preventive effects against diarrhea in children. The health-promoting probiotic microbes not only increase the flavor and texture of the whey-based products, but they also provide nutrition and various strain-specific health benefits (Katz 2001). One of the limitations of probiotic whey products is high transportation costs due to the volume occupied by liquid whey. This can be overcome, however, by concentrating the product through evaporation, reverse osmosis or ultrafiltration to occupy less volume but with the same relative composition.

In addition to probiotic microbes, a number of bioactive components with similar activity are being used in various functional food products. Some of the manufacturers of non-probiotic functional milk products are listed in Table 3.4 (**Advanced Non-food Applications of Probiotics**).

Probiotics have been shown to confer various health benefits to humans (Aureli et al. 2011), animals (Iannitti and Palmieri 2010) and plants (Song et al. 2012). However, it is necessary to ensure that processing conditions applied during probiotics production enable them to retain their activity and viability. Probiotics can be applied across a wide range of areas beyond food and beverages.

3.3 Applications of Probiotics

3.3.1 Medical Applications

Probiotics exert certain effects on various pathological diseases and gastrointestinal and extra-intestinal disorders, including the prevention or alleviation of symptoms of traveler's diarrhea and antibiotic-associated diarrhea, inflammatory bowel disease (Marteau et al. 2002) and lactose intolerance (de Vrese et al. 2001), and protection against intestinal infections (Reid et al. 2001) and irritable bowel syndrome. Though all probiotic microorganisms show various health benefits, there is no single organism capable of conferring all proposed benefits (Vasiljevic and Shah 2008). In addition to improved immune response in elderly people, medicinal properties observed in probiotics include improvements in patients suffering from rheumatoid arthritis and liver cirrhosis (Ibrahim et al. 2010), reduced prevalence of atopic eczema (Gueimonde et al. 2006), and reduced risk of colon cancer through inhibition of carcinogens and of bacteria capable of converting pro-carcinogens into carcinogens (Vasiljevic and Shah 2008).

Some researchers (Burns and Rowland 2000) have reported important medicinal characteristics of probiotics including anti-genotoxicity, anti-mutagenicity and anti-carcinogenicity. However, clinical trials show mixed results. Some studies suggest that certain probiotics may help in maintaining remission of ulcerative colitis and preventing relapse of Crohn's disease and recurrence of pouchitis (a complication of surgery to treat ulcerative colitis). These studies are still in the research phase, and further work is needed to prove strain-specific effects.

Probiotics have been suggested to be helpful in maintaining the microflora in the urogenital ecosystem. Lactobacilli play an important role in inhibiting the growth of pathogenic microbes by creating highly acidic environments, but this can be disturbed by various factors such as antibiotics, spermicides and birth control pills. Probiotic treatment may restore the microflora balance and help in curing some common female urogenital problems such as bacterial vaginosis, yeast infection and urinary tract infection (Harvard 2005).

Current research on probiotic applications in disease control has proven that some probiotic microbes (e.g. lactic acid bacteria) are helpful for delivering cytokines directly to the targeted sites in the host body (Behnsen et al. 2013).

3.3.2 Soil Fertility in Agriculture

Soil fertility plays a major role in agriculture, and is dependent on the bacteria and fungi in the soil. These microorganisms grow on organic matter and degrade into small molecules which can be taken up by plants through their roots. Good soil fertility can be achieved by using very low levels of synthetic herbs or pesticides and fertilizers, and therefore by using probiotic microorganisms. Nowadays, specific microorganisms are isolated from plant sources and cultured on a commercial scale, and are then used as bio fertilizers or biological control agents for plant diseases (Berg 2009). These microorganisms help to suppress plant pathogens and promote plant growth (Perrig et al. 2007; Saleem et al. 2007; Sheng et al. 2008; Compant et al. 2010).

3.3.3 Veterinary Applications

Recent studies have shown enormous health benefits of probiotics in animals. Animal feed with efficient probiotic microorganisms benefits animal health, maintains the microbial balance in the gut and aids in the digestion of food in the gastrointestinal tract. The use of probiotics in animal feed can enhance immunity, increase daily weight gain and feed efficiency in feedlot cattle, enhance milk production in cows, and improve health performance in calves (Seo et al. 2010) and chickens (Kalavathy et al. 2003). Probiotic microorganisms compete with the pathogenic bacteria in the gut for attachment to the mucosal wall and adjust to the immune response (Vine et al. 2004). They can aid the growth of non-pathogenic microbes and gram-positive bacteria in the gut by inhibiting pathogenic bacterial growth. Probiotic strain do this by producing hydrogen peroxide and volatile fatty acids (Jin et al. 2000). They are also efficient in stimulating the synthesis of B complex vitamins and improving the immune response in animals (Excelife 2006).

3.3.4 Aquaculture Enhancement

Probiotics are well known for their beneficial properties such as pathogen inhibition in aquatic organisms. In China alone, the market demand for commercial probiotics was reported at over 50,000 tons, with an approximate value of €50 million (Qi et al. 2009). Probiotics are used in the form of feed as growth promoters for

Table 3.4 Some commercial non-probiotic functional drinks

Country	Producer	Brand	Type of product	Bioactive components
Australia	PB food Australia	Heart plus	Low-fat milk	Fish oil
	Dairy farmers	Farmers best	Low-fat milk	Vegetable oil
	Parmalat	Physical	Milk	Calcium
		Calcium Plus	Milk	Calcium
Belgium	Danone	Zen	Fermented milk drink	Magnesium
Canada	Danone	Danacol	Low-fat dairy drink	Phytosterol
	Parmalat	Beatrice	Chocolate milk	
	Neilson	Dairy Oh	Fresh and chocolate milk	
	Lactantia Parmalat	Lactantia Nature Addition	Low-fat milk	Flax seed oil
	Natrel	Natrel Omega-3	Low-fat milk	Organic flax seed oil
		Natrel Calcium	Milk	Calcium
France	Candia	Candia with omega-3	Low-fat milk	
		Viva	Milk	Magnesium
	Lactalis	Magnesio	Milk	Magnesium
Finland	Valio	Evolus®	Fermented dairy fruit beverage	Bioactive peptides obtained by milk fermentation Val-Pro-Pro/Ile-Pro-Pro
	Ingman Dairy	Night-Time Milk	Low-fat milk	Melatonin
Ireland	Dawn Dairy	Dawn omega Milk	Low-fat milk (fresh)	Fish oil
Japan	Calpis	Ameal S	Cultured milk	Bioactive peptides
	Meiji-Milk	Meiji Love	Milk	Calcium and iron
	Meiji-Love	Fe-Milk	Milk	Iron
	Stolle Milk	Alpha	CPP- and IgG-rich milk	CPP and IgG
	Kyodo Milk Japan	*Lactobacillus casei* with lactoferrin	Probiotic fermented milk	*Lactobacillus casei*, lactoferrin

(continued)

Table 3.4 (continued)

Country	Producer	Brand	Type of product	Bioactive components
Malaysia	Nestle	Omega Plus	Low-fat milk (UHT)	Vegetable oil
New Zealand	Anchor	Vital	Low-fat milk	
Singapore	Nestle	Omega Plus	Low-fat milk (UHT)	Corn oil
Spain	Corporación Alimentaria Peñasanta S. A.	Natura Linea	Milk-fruit juice drink	Conjugated linoleic acid (Cognis Tonalin brand)
UK	Unilever	Flora pro•activ	Yogurt drink	Phytosterol
			Low-fat milk	
	Waitrose and Red Kite Farms	Slumber Bedtime Milk	Low-fat milk	Melatonin
	Dairy Crest	St. Ivel Advance	Fresh milk	
	Mc Neil Nutritionals	Benecol	Yogurt drink	Phytosterol

Compiled from Özer and Kirmaci (2010)

cultivated species in aquaculture. Colony formation of probiotic microbes in the gut of the aquatic species depends on factors such as body temperature, enzyme level, genetic resistance and water quality (Martínez Cruz et al. 2012). Microalgae such as *Chaetoceros* spp., which are considered central diatoms, have been used as carriers for probiotic microorganisms including *Vibrio alginolyticus* C7b and grown together to feed shrimp (Gomez-Gil et al. 2002). In 2003, Lara-Flores et al. (2003) reported that providing a probiotic *Streptococcus* strain in the diet of Nile tilapia (*Oreochromis niloticus*) led to a significant increase in crude protein, lipid content and weight (from 0.154 to 6.164 g) observed at 9 weeks of culture feeding. In addition to the growth-promoting factor, probiotics act as inhibitors of pathogens and control certain diseases in aquatic organisms. The pathogens *Aeromonas hydrophila* and *Vibrio alginolyticus* were inhibited using probiotic strains isolated from the gastrointestinal tract of clownfish (*Amphiprion percula*) (Vine et al. 2004). Because of their anti-pathogenic activity, probiotics are increasingly being used in place of antibiotics. Many importing countries have banned animal and aquaculture products produced with antibiotic supplementation in feed, as the use of antibiotics in feed has already been shown to lead to the development of antibiotic resistance in many food pathogens (EU Ban 2005; Gilchrist et al. 2007; Nunes et al. 2012; Burt 2014).

3.4 Probiotic Stability

The production of stable probiotics supplements possessing desirable organoleptic characteristics and capable of imparting prolonged health benefits to a targeted individual, while simultaneously retaining the viability of the incorporated strains, is one of the greatest technological challenges at present. Probiotic strains included in food supplements must tolerate various unfavorable conditions during industrial processes (manufacturing and storage) and must survive the harsh and competitive environment of the gastrointestinal tract and other environmental conditions. Many of the prevalent probiotics in the market are fastidious microorganisms which are nutritionally demanding and extremely sensitive to parameters such as ambient temperature, pH, oxygen content, water activity and the presence of other chemicals and microorganisms (Chávarri et al. 2012; Gueimonde and Sánchez 2012). A major hindrance in the production of probiotic supplements is the loss of viability of the probiotic strains during the industrial processing of the product. In order to circumvent this problem, a number of technological and microbiological approaches have been adopted by various manufacturing units (Anal and Singh 2007; Gueimonde and Sánchez 2012).

3.4.1 Technological Approach for Stability of Industrial Probiotics

For a probiotic product to be effective, it must maintain the viability of the strain during its manufacture, distribution and storage. An accepted benchmark is that a person should consume at least 100 g (containing at least 10^8 to 10^9 viable cells) of probiotic cultures in order to meet the minimum required effective concentration (at least 10^6 CFU/g) to show beneficial health effects (Kechagia et al. 2013; Mitropoulou et al. 2013). In addition, a probiotic culture should not reduce the desirable organoleptic quality of the product. The viability of the probiotic strains and the organoleptic properties of products containing such strains appear to be negatively affected during manufacturing and storage. For instance, the viability of *Bifidobacterium,* a probiotic bacterium, in food products such as yogurt preparations is significantly affected by various physiochemical factors including pH, concentration of lactic and acetic acids, hydrogen peroxide, dissolved oxygen content and low storage temperatures (Shah et al. 1995; Mitropoulou et al. 2013). Thus it is of the utmost importance to develop technology capable of preserving the viability of *Bifidobacterium* and other probiotics in the product in order to impart the desired health benefits to the consumer. Furthermore, the health benefits must be achieved in a cost-effective manner.

Immobilization of viable cells is an important technique for preserving the viability of probiotic bacteria, as it helps to protect the probiotic microbes from adverse environmental conditions including changes in pH, temperature and various harmful microbial attacks (Gbassi and Vandamme 2012; Heidebach et al. 2012; Mitropoulou et al. 2013). One may find the terms "immobilization", "entrapment" and "encapsulation" used interchangeably in most of the literature on the microencapsulation of probiotics (Gbassi and Vandamme 2012; Mitropoulou et al. 2013). Immobilization is defined as the process of attaching a cell or entrapping it within a suitable inert material (called a matrix), while encapsulation is the process of forming a continuous coating around an inner matrix that is wholly contained within the capsule wall as a core of encapsulated material (Gbassi and Vandamme 2012). Among immobilization techniques (covalent bonding, adsorption, entrapment and encapsulation), probiotic encapsulation technology (PET) has emerged within the past decade as an exciting and rapidly developing technology. Another efficient method used in the probiotics industry is microencapsulation, which is the process of coating or entrapping a useful core material. This method results in tiny capsules ranging in size from a few micrometers to a few millimeters (Heidebach et al. 2012). This increases the mouth feel of the product.

Selection of a matrix material is a crucial step in carrying out any sort of immobilization technique, and it becomes more sensitive when the product is made in the food industry for human consumption. The carrier used in immobilization of probiotics should be chemically, physically and biologically stable, mechanically robust, easily available and non-toxic, and should have easy handling requirements. Other factors to consider, depending upon its application, include physical characteristics (porosity, swelling, compression and mean particle behavior) and the possibility for microbial growth, biodegradability and solubility (Mitropoulou et al. 2013). An immobilization carrier (support) to be used in the food industry must meet stringent rules and regulations; few are considered to be industrially applicable. Generally, biopolymers and natural supports of food-grade purity are preferred, and materials containing non-digestible carbohydrates are being explored as potential carriers for use in the future (Mitropoulou et al. 2013). In addition to alginate, which is most commonly used as support for the encapsulation of probiotics, potential probiotic carriers include chitosan-coated alginate beads, apple pieces, pear pieces, wheat grains, oat pieces, whey proteins, carrageenan, gelatin, cellulose acetate phthalate and locust bean gum (Gbassi and Vandamme 2012; Cláudia et al. 2013; Mitropoulou et al. 2013). Encapsulation techniques used in the probiotics food industry include spray drying, spray cooling, fluidized-bed agglomeration and cooling, freeze and vacuum drying, emulsion-based techniques, and coacervation and extrusion. (Chávarri et al. 2012). However, the majority of microcapsules of probiotic strains for use in the food industry are generated by either extrusion or emulsion. The use of spray drying as an alternative encapsulation technique has also recently emerged (Heidebach et al. 2012).

3.4.1.1 Extruded Beads

In this technique, probiotics strains are mixed with an aqueous hydrocolloid solution and then extruded. Typically, a syringe is used that extrudes the gelling liquid in the form of droplets. One of the most common extrusion techniques using sodium alginate involves dropping the mixture of probiotic strains and sodium alginate solution into calcium chloride solution from an appropriate height using a syringe. The size of the encapsulating capsules depends upon the diameter of the orifice of the extruder, dropping height and the viscosity of the hydrocolloid–cell mixture. The size of beads produced with this method generally ranges from 0.5 to 3 mm (Gbassi and Vandamme 2012; Heidebach et al. 2012).

3.4.1.2 Emulsion Precipitation

In this technique for encapsulation of probiotic cells, a water-in-oil (W/O) emulsion of aqueous hydrocolloid–cell mixture (discontinuous phase) and vegetable oil (continuous phase) is formed using a small volume of discontinuous phase and larger volume of continuous phase. Once this emulsion is formed, the dispersed hydrocolloid–cell mixture is insolubilized to form small beads within the oil phase. In the case of the formation of alginate capsules, the microcapsules are hardened by slow addition of calcium chloride solution to the emulsion while stirring the mixture. The hardened droplets settle to the bottom of the reservoir. With this technique, capsules less than 100 μm in size can be generated. Emulsification produces oily or aqueous droplets known as capsules, while extrusion produces gelled droplets, called beads. In addition, capsules differ in size and shape, compared to uniformly shaped beads (Gbassi and Vandamme 2012; Heidebach et al. 2012).

3.4.1.3 Spray Drying

In order to enhance the longevity of probiotic microcapsules, they are usually dried after production. Freeze drying is a common method for drying the capsules produced by extrusion or emulsification. Spray drying has emerged as an alternative method for achieving capsule formation and drying in a single step. The efficacy of this method in terms of protecting probiotic cell concentrates from various adverse conditions was investigated by spray drying the probiotic cell mixtures using aqueous solution of different polymers including modified starch, gum arabic, gelatin, whey protein isolate, maltodextrin mixed with gum arabic, and ß-cyclodextrin mixed with gum arabic. However, one of the biggest drawbacks of this technique for microcapsule formation is that the microcapsules in most cases are water-soluble, which makes them unsuitable for use in aqueous food products and the need for further protection during gastrointestinal transit (Heidebach et al. 2012).

3.4.2 Biological Approaches for Producing Industrially Stable Probiotics

Most probiotic strains used in industrial production either have inherent resistance to severe conditions they are expected to face or are adapted to help them acquire such characteristics. The selection approach includes (i) the selection of naturally available strains with the desired properties, (ii) stress adaptation of the naturally occurring strains, and (iii) genetic manipulation of the desired probiotic strains to produce genetically modified organisms (GMOs). (Gueimonde and Sánchez 2012; Novik et al. 2014).

3.4.2.1 Screening for Naturally Resistant Strains

Probiotic strains show varied resistance against pH, temperature and oxygen conditions, which in turn affects the stability and shelf life of probiotic foods. The selection of naturally available strains with desired traits for the production of probiotic products is desirable during manufacturing. One phenomenon that affects the stability of commonly available probiotics products such as yogurts and fermented milks is "post-acidification" or continuous acid production by the starter cultures during storage. This problem can be overcome by selecting starter cultures in which post-acidification will not occur. Secondly, the product is often exposed to oxygen-abundant conditions during its manufacture and storage. The use of aerotolerant species such as *Bifidobacterium animalis* subsp. *lactis* is often preferred. Thirdly, strains capable of producing exopolysaccharides (EPS), which are considered to have better tolerance of various stresses, have been suggested for industrial applications (Gueimonde and Sánchez 2012).

3.4.2.2 Acclimatization of Naturally Occurring Probiotic Strains

Probiotic strains with desirable characteristics and better adaptation to various stressful conditions can be produced from naturally occurring wild-type strains. Three major approaches are presently employed for producing more robust, industrially desirable strain: adaptation by employing gradually increasing stress, mutagenic treatment and treatment with selective pressure (Gueimonde and Sánchez 2012).

(a) **Adaptation by employing gradual increasing stress**
In this method strains are subjected to increasing sub-lethal stress conditions before exposing them to harsh conditions. This method has been employed for enhancing the heat and acid tolerance of the microorganisms in order to produce more stable probiotic products.

(b) **Mutagenic treatment**
Treatment of probiotic strains with various mutagens such as UV light or chemicals has improved the stability of the products in terms of acid tolerance, sensory attributes and metabolic activity (Gueimonde and Sánchez 2012). For example, UV mutagenesis and subsequent incubation in acidic medium led to improved stability of *B. animalis* subsp. *lactis* in low-pH juice (Saarela et al. 2011). Similarly, high production of acetic acid by the *Bifidobacterium* species has been a major limiting factor for its inclusion as a probiotic in fermented dairy products, as it confers undesirable organoleptic properties to the final product. Hence, new probiotic strains of *Bifidobacterium animalis* subsp. *lactis* CECT 7953 capable of producing low amounts of acetic acid have been developed from wild strains (*Bifidobacterium animalis* subsp. *lactis)* by random UV mutagenesis. These new strains, with reduced capacity for producing acetic acid, are desirable for the production of fermented dairy products (Margolles and Sánchez 2012).

(c) **Selective pressure treatment**
Stress-resistant derivatives may be obtained by exposing sensitive strains to selective pressure (stress factor). For example, sensitive strains of *Lactobacilli* and *Bifidobacterium* exposed to selective pressure were found to produce derivative strains with improved resistance to acid, bile, heat and oxygen (Collado and Sanz 2006; Berger et al. 2010). Additionally, such microbial derivatives have been shown to have stable phenotypes and cross-resistance to other stresses. This is an important advantage from a commercial point of view (Gueimonde and Sánchez 2012).

3.4.2.3 Producing Genetically Engineered Strains

The use of genetic engineering for improving the stability of probiotic strains is another exciting alternative. However, food infused with genetically modified organisms (GMOs) is still unacceptable in many countries and among large groups of consumers. Genetic modification of probiotic strains commonly involves one of the following (Gueimonde and Sánchez 2012; Novik et al. 2014):

(a) **Homologous expression**
This involves modifying the expression level of the prevailing gene of the microbial strain. For example, overexpression of a chaperone in *Lactobacillus paracasei* was found to increase strain stability (Desmond et al. 2004; Gueimonde and Sánchez 2012).

(b) **Heterologous expression**
This involves introducing the desired gene from one microbe into the probiotic strain of interest. For example, heterologous expression of the betaine uptake system (BetL) of *Listeria* into *Lactobacillus salivarius* was found to increase tolerance to acid and high osmolar conditions (Gueimonde and Sánchez 2012; Novik et al. 2014).

3.5 Safety of Probiotics

Probiotics are generally considered safe for human consumption, as no significant negative effects have been reported. Many members of probiotic strains such as *Lactobacillus* have been consumed along with various dairy products. Among the probiotics, *Bifidobacterium* and members of the lactic acid bacteria (LAB) genera *Lactococcus* and *Lactobacillus* are commonly believed to be safe and given the status "generally recognized as safe" (GRAS), whereas other genera, including *Streptococcus* and *Enterococcus*, are considered to be opportunistic pathogens (Salminen et al. 1998; Snydman 2008). Despite the many controlled clinical trials on the use of probiotics that have demonstrated safe use, in some instances consumption of probiotics has been linked with three health issues: (i) occurrence of diseases such as bacteremia or endocarditis, (ii) various adverse immunogenic responses, both localized and generalized, and (iii) transfer of antibiotic resistance to the other pathogens in the gastrointestinal tract. There is no evidence from population-based studies, however, of any increased risk of bacteremia or endocarditis due to probiotics (Snydman 2008). For example, *Lactobacillus* GG, a bacterial strain commonly used in probiotic therapy, has been associated with bacteremia and liver abscess in patients with short gut syndrome. However, because of the relatively small number of such cases, and the positive safety evaluation results obtained for this probiotic, it can be generally considered as safe (Salminen et al. 2002; Snydman 2008). Some concerns have been raised that probiotics might produce undesirable metabolites and cause colon cancer or degrade the intestinal mucus, but these theories are not supported by sufficient epidemiologic or clinical evidence. These hypotheses were further contradicted by experimental data obtained from research carried out in an animal model, which showed anti-tumorigenic properties of probiotics in colon cancer (Goldin et al. 1996; Snydman 2008). Other studies performed in gnotobiotic rats have shown no evidence of degradation of the intestinal mucus by probiotic bacteria (Berg 1980; Ishibashi and Yamazaki 2001; Snydman 2008). Lactic acid bacteria are intrinsically resistant to antibiotics, and the transfer of antibiotic resistance to harmful pathogens in the gastrointestinal tract has been raised as a concern for their use. In most cases, however, this resistance is not considered to be transmissible. Furthermore, these probiotics have shown sensitivity to many antibiotics in clinical use. Thus, even in cases where patients may develop lactic acid bacteria-associated opportunistic infections, they can be treated by conventional antibiotic therapy. However, transmissible enterococcal resistance against glycopeptide antibiotics (vancomycin and teicoplanin) should not be ignored, as vancomycin is one of the last remaining effective antibiotics for treating certain multidrug-resistant pathogens (Salminen et al. 1998). Additionally, many new species and more specific strains of probiotics are being isolated and characterized for probiotic use. These newly identified strains should be carefully assessed, and rigorous clinical trials and evaluation should be conducted on a case-by-case basis before their incorporation into food products. In the case of selecting novel strains, species and genera for probiotic use, current

safety assessment procedures described in the European Union (EU) novel foods directive must be strictly adhered to among EU member countries (Conzelmann 1997; Salminen et al. 1998). Although the overall safety record held by probiotics is excellent, they should be used with caution in certain patient groups, particularly in neonates born prematurely or immunocompromised individuals (Hickson 2011; Marchand 2012). It should also be noted that the efficacy of probiotics is both strain- and disease-specific, and they should be given in adequate amounts. Furthermore, the properties of probiotics vary among species and can be strain-specific. Individual strains can possess characteristics such as resistance to gastric acid and bile, the ability to colonize the mucosa, and antimicrobial activity (Jacobsen et al. 1999; Hickson 2011). Hence, it would not be wise to generalize the effects of one probiotic species or strain to others without confirmation in separate studies. For instance, *L. rhamnosus* GG is a specific bacterial probiotic strain (the nomenclature includes genus, species and strain) which is capable of preventing antibiotic-associated diarrhea (AAD), whereas other strains of *L. rhamnosus* may not have this effect. On the other hand, some species in the *Lactobacillus* genus may not act as probiotics (McFarland 2006; Hickson 2011).

3.6 Technological Hurdles

Probiotic encapsulation technology (PET) is one of the most acclaimed technologies for protecting the probiotic strains and ensuring the stability of the products against various adverse effects (Chávarri et al. 2012; Gbassi and Vandamme 2012). There has been a tremendous improvement in the use of this technology for the development of stable, high-quality food products that retain their organoleptic qualities. It has been used for the production of dairy-based probiotic products such as yogurt, milk and cheese, and has been extended to non-dairy products such as fruit juices, cookies and chocolates. Despite these improvements, however, there are still many technological hurdles that PET must overcome before it can be considered a full-fledged technology. These include the development of equipment to produce small, uniform capsules or beads, the selection of non-toxic encapsulation materials, development of capsules or beads compatible with the pH of the human digestive tract, detailed in vitro and in vivo studies of the effects of encapsulation on the safety of probiotic strains in humans, and assessing the cost of microencapsulation (Vidhyalakshmi et al. 2009; Rokka and Rantamäki 2010; Gbassi and Vandamme 2012).

Emulsification and extrusion are two of the most common PET procedures. The presence of residual oil in the encapsulated material during the production of probiotic capsules via emulsification may not be suitable for the development of low-fat dairy products, as this can cause a deterioration in texture and organoleptic characteristics. Furthermore, it is arguable that emulsifiers, surfactants and the residual oil used in the emulsification process may be toxic to probiotic cells and may interact with food components. Therefore, the development of microcapsules

using only aqueous gelling, without the use of oils, emulsifiers and surfactants, is of the utmost importance. Extrusion faces the challenges of scaling up dairy production for large quantities of beads (Gbassi and Vandamme 2012).

Another challenge is in determining the physicochemical characteristics of encapsulation materials in order to predict their disintegration or dissolution mechanisms under varying conditions of pH and salinity and their interaction with probiotic cells and other components present in the gut. In vitro studies aimed at delivering viable strains of probiotics to consumers should be undertaken through simulation using simple and reproducible gastrointestinal tract models (Vidhyalakshmi et al. 2009; Chávarri et al. 2012; Gbassi and Vandamme 2012).

PET makes use of encapsulation materials such as natural polymers and milk proteins, which are expensive, and also uses various raw materials such as oils and emulsifiers for the formation of capsules. These all add to the cost of manufacturing, making probiotics-incorporated supplements an expensive product. Thus a major challenge is in reducing the costs involved in PET (Chávarri et al. 2012; Gbassi and Vandamme 2012).

The production of hydrocolloid-based microcapsules is a common practice for the encapsulation of probiotic strains in the food industry, and it involves the use of a high-shear process (due to the highly viscous nature of the hydrocolloids) during emulsification. In some probiotic strains, this reduces the encapsulation yield (EY), a combined parameter that describes the survival of the cells and the efficacy of entrapment during encapsulation procedures (Capela et al. 2007; Ding and Shah 2009; Heidebach et al. 2012). An alternative method is protein-based microencapsulation. However, production of protein-based microcapsules sometimes requires slight modifications to existing methods or even the establishment of novel encapsulation techniques. In a number of cases, such modification have reduced the encapsulation yield (Picot and Lacroix 2004; Annan et al. 2008; Heidebach et al. 2012). Similarly, the use of lipid-based microcapsules for the protection of the probiotic strains lacks sufficient scientific evidence of its effectiveness and requires more research (Heidebach et al. 2012).

3.7 Present and Future of Probiotics

The present probiotics market is growing at a good pace. Based upon a market report from Transparency Market Research, global probiotics demand was valued at $27.9 billion in 2011, and is expected to reach $44.9 billion by 2018, representing a 6.8 % compound annual growth rate (CAGR) from 2013 to 2018 (Chr. Hansen 2013; Nutraceuticals world 2013). Demand in Asia-Pacific and Europe has dominated the global market, with Asia-Pacific expected to be the fastest-growing market in the future (Nutraceuticals world 2013).

Most probiotic supplements present in the market are dairy-based products. However, non-dairy beverages are expected to grow rapidly. Bernat et al. (2014) recently explored the production of fermented almond milk infused with probiotics

and successfully optimized the process to ensure reduced fermentation time and production of a stable product that retained the viable strains throughout manufacturing, storage and in vitro digestion (Song et al. 2012; Bernat et al. 2014).

Disruption of gut microbiota has been linked to chronic diseases such as autoimmune disorders, colon cancers, gastric ulcers, obesity, type 2 diabetes and cardiovascular disease, and the use of probiotics has been reported to mitigate or completely cure these diseases. There is also considerable evidence of the beneficial effects of probiotics for the treatment of pouchitis, inflammatory bowel disease, traveler's diarrhea, allergy, antibiotic-associated diarrhea and clostridium difficile infection (Aureli et al. 2011; Hickson 2011; Adam et al. 2012; Marchand 2012; Jz et al. 2013; Kurzweil 2014; Chen et al. 2014).

In addition to their use in maintaining digestive health, probiotics have also been explored for their use in the human immunodeficiency virus (HIV) community. Andrieu et al. (2014) reported the successful use of a vaccine consisting of inactivated simian immunodeficiency virus (SIV)mac239 particles, together with a living probiotics adjuvant (either the Calmette–Guérin bacillus, *L. plantarum* or *L. rhamnosus*), for protection against simian immunodeficiency virus (SIV) in monkeys, which is equivalent to HIV. This opens up the possibility of using probiotics for the treatment of HIV in the future (Andrieu et al. 2014). Advances in molecular biology techniques have enabled a much clearer understanding of the gut microbiota, and research is thus being focused on the production of symbiotic products (combination of prebiotics and probiotics). Since there can be variations in the types of resident bacterial groups among individuals depending upon geographical region, ethnicity, age group and dietary habits, it is crucial to undertake further studies of the gut microbiota based on these parameters. It will be necessary to develop indigenous probiotic strains based upon the target population to gain enhanced health benefits.

From an industrial perspective, probiotic products should be available in different dosage forms and customized solutions. It is also necessary to determine the mode of action of specific probiotic effects. However, due to conflicting results obtained from various clinical studies, health professionals must exercise caution in establishing the efficacy of probiotic strains or formulations in terms of specific health claims. Many of the observed effects of the use of probiotics have been strain-specific, and thus conclusions should not be extrapolated. The benefit versus potential risk of each strain must be considered, especially for their use in immunocompromised individuals or persons suffering from multiple chronic diseases. Probiotic products presently available in the market commonly contain *Lactobacillus, Bifidobacterium* and *Saccharomyces boulardii*, which represent only a fraction of microbiota. Organisms such as *Bacteroides*, which comprise the major population of gut microbiota, should be studied for their potential broader health benefits.

One of the biggest challenges faced by probiotic producers is retaining the viability of the probiotics throughout the manufacturing, storage and delivery process. To overcome this challenge, microencapsulation techniques have been employed. This concept has been extended to the production of nano-capsules

(nanotechnology) of the viable cells. Microencapsulation of different probiotic strains with alginates has been shown to improve the survival rate of those strains under highly acidic conditions (pH 2.0) and high bile salt concentrations and to improve heat tolerance compared to the free cells, thus making them more commercially attractive (Ding and Shah 2007; Song et al. 2012). In addition to alginates, microencapsulation using gelatin or vegetable gums has been carried out to protect acid-sensitive *Bifidobacterium* and *Lactobacillus* (Song et al. 2012). Similarly, desirable probiotics can be nano-encapsulated for specific delivery of those bacteria to certain parts of the gastrointestinal tract, where they will interact with specific receptors (Sekhon 2010). However, as discussed above, microencapsulation still needs to be improved, and the adoption of nanotechnology within the food industry for encapsulating live bacterial cells is still a new concept. The latter method should be used with caution, as very little is known about its impact on environmental and human health. Presently, no regulations exist that specifically control or limit the production of nano-sized particles, and this is mainly due to a lack of unbiased knowledge regarding the risks. Detailed studies of the ingredients, more options of media for industrial use, and product and process re-engineering are needed to make probiotics products more palatable and to increase market demand (Song et al. 2012; Grover et al. 2012).

3.8 Conclusions

The use of probiotics in the food industry is increasing, and is predicted to have a much larger market share in the future. Their health benefits have been studied extensively, but considerable research is still ongoing to determine their efficacy, safety and precise dosage. The use of probiotics is not limited to improvements in human health; it has also been extended to veterinary, agricultural and fishery sciences. Studies have also explored the use of probiotics for the treatment of dreadful diseases such as HIV and for their ability to treat chronic diseases such as type 2 diabetes, obesity and colon cancer. The industrial use of probiotics is limited mainly by the fact that the viability of the probiotics strains is reduced during the various manufacturing processes, storage and delivery to the consumer. Tremendous improvement has been made in encapsulation technologies, primarily extrusion and emulsion methods, which has resulted in more stable products and has helped preserve the viability of the probiotic cultures in the product. However, this technology still needs improvement, and alternative methods are being explored in order to make the manufacturing process more economical and the product more palatable.

Recent developments in molecular biology have helped to deepen our understanding of gut microbiota, and it is now known that more than 10,000 different bacteria are present in the gut. However, apart from common probiotics such as *Lactobacillus, Bifidobacterium* and *Saccharomyces boulardii,* research should be further directed towards the study of organisms such as *Bacteroides*, which constitute the predominant

flora in the gut, and which would further broaden the health application of probiotics. As there is considerable diversity of human gut microbiota by geographic region, ethnicity, age group, population and dietary habits, the choice of indigenous strains for target populations may bring greater benefit to the consumer.

The development of extensive sequencing methods has enabled metagenomic studies on the human gut microbiome to be carried out (Gueimonde and Collado 2012). With our knowledge of gut microbiota composition and activity, diseases related to microbiota aberrations in the gut will be identifiable, and it will be possible to develop probiotics that can overcome the effects of these aberrations. This will expand the scope of probiotics use for more specific health issues in the future.

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