Chapter 1

Recent Application of Probiotics in Food and Agricultural Science

Danfeng Song, Salam Ibrahim and Saeed Hayek

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1. Introduction

Probiotic foods are a group of functional foods with growing market shares and large commercial interest [1]. Probiotics are live microorganisms which when administered in adequate amounts confer a beneficial health benefit on the host [2]. Probiotics have been used for centuries in fermented dairy products. However, the potential applications of probiotics in nondairy food products and agriculture have not received formal recognition. In recent times, there has been an increased interest to food and agricultural applications of probiotics, the selection of new probiotic strains and the development of new application has gained much importance. The uses of probiotics have been shown to turn many health benefits to the human and to play a key role in normal digestive processes and in maintaining the animal's health. The agricultural applications of probiotics with regard to animal, fish, and plants production have increased gradually. However, a number of uncertainties concerning technological, microbiological, and regulatory aspects exist [3].

1.1. Definition of probiotics

Probiotics are live microbes that can be formulated into many different types of products, including foods, drugs, and dietary supplements. Probiotic is a relatively new word that is used to name the bacteria associated with the beneficial effects for the humans and animals. The term probiotic means ''for life'' and it was defined by an Expert Committee as ''live microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition'' [4]. FAO/WHO Expert Consultation believes that general guidelines need to provide to how these microorganisms can be tested and proven for safety and potential health benefits when administered to humans.

Lactobacillus and *Bifidobacterium* are most commonly used probiotics in food and feed (Table 1). Other microorganisms such as yeast *Saccharomyces cerevisiae* and some *Escherichia coli* and *Bacillus* species are also used as probiotics. Lactic acid bacteria (LAB) which have been used for food fermentation since the ancient time, can serve a dual function by acting as food fermenting agent and potentially health benefits provider. LAB are GRAS (general recognized as safe) with no pathogenic, or virulence properties have been reported. For the use of LAB as probiotics, some desirable characteristics such as low cost, maintaining its viability during the processing and storage, facility of the application in the products, resistance to the physicochemical processing must be considered.

Lactobacillus species	Bifidobacterium species	Others
L. acidophilus	*B. adolescentis*	*Bacillus cereus*
L. amylovorus	*B. animalis*	*Clostridium botyricum*
L. brevis	*B. breve*	*Enterococcus faecalis*[a]
L. casei	*B. bifidum*	*Enterococcus faecium*[a]
L. rhamnosus	*B. infantis*	*Escherichia coli*
L. crispatus	*B. lactis*	*Lactococcus lactis* subsp. *cremoriss*
L. delbrueckii subsp. *bulgaricus*	*B. longum*	*Lactococcus lactis* subsp. *lactis*
L. fermentum		*Leuconostoc mesenteroides* subsp. *dextranicum*
L. gasseri		*Pediococcus acidilactici*
L. helveticus		*Propionibacterium freudenreichii*[a]
L. johnsonii		*Saccharomyces boulardii*
L. lactis		*Streptococcus salivarius* subsp. *thermophilus*
L. paracasei		*Sporolactobacillus inulinus* [a]
L. plantarum		
L. reuteri		
L. salivarius		
L. gallinarum[a]		

[a] mainly applied in animals

Table 1. Probiotic microorganisms. Adapted from [5, 6]

1.2. Characteristics of probiotics

Characteristics of probiotics will determine their ability to survive the upper digestive tract and to colonize in the intestinal lumen and colon for an undefined time period. Probiotics are safe for human consumption and no reports have found on any harmfulness or production of any specific toxins by these strains [7, 8]. In addition, some probiotics could produce antimicrobial substances like bacteriocins. Therefore, the potential health benefit will depend on the characteristic profile of the probiotics. Some probiotic strains can reduce intestinal transit time, improve the quality of migrating motor complexes [9], and temporarily increase the rate of mitosis in enterocytes [10, 11].

The most common probiotics are *Lactobacillus* and *Bifidobacterium*. In general most probiotics are gram-positive, usually catalase-negative, rods with rounded ends, and occur in pairs, short, or long chains [7]. They are non-flagellated, non-motile and non-spore-forming, and are intolerant to salt. Optimum growth temperature for most probiotics is 37°C but some strains such as *L. casei* prefer 30 °C and the optimum pH for initial growth is 6.5-7.0 [7]. *L. acidophilus* is microaerophilic with anaerobic referencing and capability of aerobic growth. *Bifidobacterium* are anaerobic but some species are aero-tolerant. Most probiotics bacteria are fastidious in their nutritional requirements [12, 13]. With regard to fermentation probiotics are either obligate homofermentative (ex. *L. acidophilus*, *L. helvelicas*), obligate heterofermentative (ex. *L. brevis, L. reuteri*), or facultative heterofermentative (ex. *L. casei, L. plantarum*) [14]. Additionally, probiotics produce a variety of beneficial compounds such as antimicrobials, lactic acid, hydrogen peroxide, and a variety of bacteriocins [15, 16] . Probiotics should have the ability to interact with the host microflora and competitive with microbial pathogens, bacterial, viral, and fungal [16].

2. Probiotics health benefits

Probiotic research suggests a range of potential health benefits to the host organism. The potential effects can only be attributed to tested strains but not to the whole group of probiotics. Probiotics have shown to provide a diverse variety of health benefits to human, animal, and plans. However, viability of the microorganisms throughout the processing and storage play an important role in transferring the claimed health effects. Therefore, the health benefits must be documented with the specific strain and specific dosage [17].

2.1. Human health

Probiotics display numerous health benefits beyond providing basic nutritional value [4]. These evidences have been established by the scientific testing in the humans or animals, performed by the legitimate research groups and published in peer-reviewed journals [16, 18]. Some of these benefits have been well documented and established while the others have shown a promising potential in animal models, with human studies required to substantiate these claims [18]. Health benefits of probiotic bacteria are very strain specific; therefore, there is no universal strain that would provide all proposed benefits and not all strains of the same species are effective against defined health conditions [18].

Probiotics have been used in fermented food products for centuries. However, nowadays it has been claimed that probiotics can serve a dual function by their potentially importing health benefits. The health benefit of fermented foods may be further enhanced by supplementation of *Lactobacillus* and *Bifidobacterium* species [19]. *L. acidophilus*, *Bifidobacterium* spp. and *L. casei* species are the most used probiotic cultures with established human health in dairy products, whereas the yeast *Saccharomyces cerevisiae* and some *E. coli* and *Bacillus* species are also used as probiotics [20].

Several studies have documented probiotic effects on a variety of gastrointestinal and extraintestinal disorders, including prevention and alleviation symptoms of traveler's diarrhea and antibiotic associated diarrhea [21], inflammatory bowel disease [21], lactose intolerance [22], protection against intestinal infections [23], and irritable bowel syndrome. Some probiotics have also been investigated in relation to reducing prevalence of atopic eczema later in life [24], vaginal infections, and immune enhancement [25], contributing to the inactivation of pathogens in the gut, rheumatoid arthritis, improving the immune response of in healthy elderly people [26], and liver cirrhosis.

In addition, probiotics are intended to assist the body's naturally occurring gut microbiota. Some probiotic preparations have been used to prevent diarrhea caused by antibiotics, or as part of the treatment for antibiotic-related dysbiosis. Although there is some clinical evidence for the role of probiotics in lowering cholesterol but the results are conflicting. Probiotics have a promising inhibitory effect on oral pathogens especially in childhood but this may not necessarily lead to improved oral health [27]. Antigenotoxicity, antimutagenicity and anticarcinogenicity are important potential functional properties of probiotics, which have been reported recently. Observational data suggest that consumption of fermented dairy products is associated with a lower prevalence of colon cancer, which is suggested that probiotics are capable of decreasing the risk of cancer by inhibition of carcinogens and pro-carcinogens, inhibition of bacteria capable of converting pro-carcinogens to carcinogens [18].

2.2. Animal health

Probiotics which are traditional idea in the human food have been extended to animals by developing fortified feed with intestinal microbiota to benefit the animals. The microflora in the gastrointestinal tracts of animals plays a key role in normal digestive processes and in maintaining the animal's health. Probiotics can beneficially improve the intestinal microbial balance in host animal. Commercial probiotics for animal use are claimed to improve animal performance by increasing daily gain and feed efficiency in feedlot cattle, enhance milk production in dairy cows, and improve health and performance of young calves [28] and in improving growth performance of chickens [29]. Probiotics can attach the mucosal wall, adjust to immune responses [30], and compete the pathogenic bacteria for attachment to mucus [31, 32]. Probiotics provide the animal with additional source of nutrients and digestive enzymes [33, 34]. They can stimulate synthesis vitamins of the B-group and enhancement of growth of nonpathogenic facultative anaerobic and gram positive bacteria by producing inhibitory compounds like volatile fatty acids and hydrogen peroxide that inhibit the growth of harmful bacteria enhancing the host's resistance to enteric pathogens [32, 35]. Probiotics stimulate the direct uptake of dissolved organic material mediated by the bacteria, and enhance the immune response against pathogenic microorganisms [36, 37]. Finally, probiotics can inhibit pathogens by competition for a colonization sites or nutritional sources and production of toxic compounds, or stimulation of the immune system.

2.3. Plant health

The more beneficial the bacteria and fungi are, the more "fertile" the soil is. These microorganisms break down organic matter in the soil into small, usable parts that plants can uptake through their roots. The healthier the soil, the lower the need for synthetic herb/pesticides and fertilizers.The concept that certain microorganisms 'probiotics' may confer direct benefits to the plant acting as biocontrol agents for plants. The plant probiotic bacteria have been isolated and commercially developed for use in the biological control of plant diseases or biofertilization [38]. These microorganisms have fulfilled important functions for plant as they antagonize various plant pathogens, induce immunity, or promote growth [38-40]. The interaction between bacteria and fungi with their host plants has shown their ability to promote plant growth and to suppress plant pathogens in several studies [41-44].

3. Food applications of probiotics

Today an increase in knowledge of functional foods has led to develop foods with health benefits beyond adequate nutrition. The last 20 years have shown an increased interest among consumers in functional food including those containing probiotics. The presence of probiotics in commercial food products has been claimed for certain health benefits. This has led to industries focusing on different applications of probiotics in food products and creating a new generation of 'probiotic health' foods. This section will summarize the common applications of probiotics in food products.

3.1. Dairy-based probiotic foods

Milk and its products is good vehicle of probiotic strains due to its inherent properties and due to the fact that most milk and milk products are stored at refrigerated temperatures. Probiotics can be found in a wide variety of commercial dairy products including sour and fresh milk, yogurt, cheese, etc. Dairy products play important role in delivering probiotic bacteria to human, as these products provide a suitable environment for probiotic bacteria that support their growth and viability [45-48]. Several factors need to be addressed for applying probiotics in dairy products such as viability of probiotics in dairy [19, 48], the physical, chemical and organoleptic properties of final products [49-51], the probiotic health effect [52, 53], and the regulations and labeling issues [4, 54].

3.1.1. Drinkable fresh milk and fermented milks

Among probiotics carrier food products, dairy drinks were the first commercialized products that are still consumed in larger quantities than other probiotic beverages. Functional dairy beverages can be grouped into two categories: fortified dairy beverages (including probiotics, prebiotics, fibers, polyphenols, peptides, sterol, stanols, minerals, vitamins and fish oil), and whey-based beverages [55]. Among the probiotic bacteria used in the manufacture of dairy beverages, *L. rhamnosus* GG is the most widely used. Owing to *L. rhamnosus* GG acid and bile resistance [56], this probiotic is very suitable for industrial applications. Özer and Avnikirmaci have reported several examples of commercial probiotic dairy beverages showing that *L. acidophilus*, *L. casei*, *L. rhamnosus*, and *L. plantarum* as most applied probiotics [55].

Several factors have been reported to affect the viability of probiotic cultures in fermented milks. Acidity, pH, dissolved oxygen content, redox potential, hydrogen peroxide, starter microbes, potential presence of flavoring compounds and various additives (including preservatives) affect the viability of probiotic bacteria and have been identified as having an effect during the manufacture and storage of fermented milks [19, 48, 57]. Today, a wide range of dairy beverages that contain probiotic bacteria is available for consumers in the market including: Acidophilus milk, Sweet acidophilus milk, Nu-Trish A/B, Bifidus milk, Acidophilus buttermilk, Yakult, Procult drink, Actimel, Gaio, ProViva, and others [55].

Probioticts such as *Lactobacillus* and *Bifidobacterium* strains grow weakly in milk due to their low proteolytic activity and inability to utilize lactose [47, 57]. These bacteria also need certain compounds for their growth which is missing in milk [19, 58, 59]. To improve growth and viability of probiotics in dairy beverages various substances have been tested in milk. Citrus fiber presence in fermented milks was found to enhance bacterial growth and survival of probiotic bacteria in fermented milks [60]. Addition of soygerm powder has shown certain positive effects on producing fermented milk with *L. reuteri*. Soygerm powder may release important bioactive isoflavones during fermentation that could protect *L. reuteri* from bile salt toxicity in the small intestine [61]. Other substances include fructooligosaccahrides (FOS), aseinomacropeptides (CMP), whey protein concentrate (WPC), tryptone, yeast extracts, certain amino acids, nucleotide precursors and an iron source were also documented [59, 63, 64]. Additionally, the selection of probiotic strains and optimization of the manufacturing conditions (both formulation properties and storage conditions) are of utmost importance in the viability of probiotic bacteria in fermented milk [47, 65].

3.1.2. Yogurt

Yogurt is one of the original sources of probiotics and continues to remain a popular probiotic product today. Yogurt is known for its nutritional value and health benefits. Yogurt is produced using a culture of *L. delbrueckii* subsp. *bulgaricus* and

Streptococcus salivarius subsp. *thermophilus* bacteria. In addition, other lactobacilli and bifidobacteria are also sometimes added during or after culturing yogurt. The probiotic characteristics of these bacterial strains that form the yogurt culture are still debatable. The viability of probiotics and their proteolytic activities in yoghurt must be considered. Numerous factors may affect the survival of *Lactobacillus* and *Bifidobacterium* spp. in yogurt. These include strains of probiotic bacteria, pH, presence of hydrogen peroxide and dissolved oxygen, concentration of metabolites such as lactic acid and acetic acids, buffering capacity of the media as well as the storage temperature [19, 66, 67].

Although yogurt has been widely used as probiotics vehicle, most commercial yogurt products have low viable cells at the consumption time [19, 68]. Viability of probiotics in yogurt depends on the availability of nutrients, growth promoters and inhibitors, concentration of solutes, inoculation level, incubation temperature, fermentation time and storage temperature. Survival and viability of probiotic in yogurt was found to be strain dependant. The main factors for loss of viability of probiotic organisms have been attributed to the decrease in the pH of the medium and accumulation of organic acids as a result of growth and fermentation. Among the factors, ultimate pH reached at the end of yogurt fermentation appears to be the most important factor affecting the growth and viability of probiotics. Metabolic products of organic acids during storage may further affect cell viability of probiotics [66]. The addition of fruit in yogurt may have negative effect on the viability of probiotics, since fruit and berries might have antimicrobial activities. Inoculation with very high level of probiotics with attempts to compensate the potential viability loss, might result in an inferior quality of the product. The present of probiotic was found to affect some characteristics of yogurt including: acidity, texture, flavor, and appearance [69]. However, encapsulation in plain alginate beads, in chitosancoated alginate, alginate-starch, alginate-prebiotic, alginate-pectin, in whey protein-based matrix, or by adding prebiotics or cysteine into yogurt, could improve the viability and stability of probiotics in yogurt [70-79].

3.1.3. Cheese

Yogurt and milk are the most common vehicles of probiotics among dairy products. However, alternative carriers such as cheese seem to be well suited. Cheeses have a number of advantages over yogurt and fermented milks because they have higher pH and buffering capacity, highly nutritious, high energy, more solid consistency, relatively higher fat content, and longer shelf life [80, 81]. Several studies have demonstrated a high survival rate of probiotics in cheese at the end of shelf life and high viable cells [45, 48, 82, 83]. Probiotics in cheese were found to survive the passage through the simulated human gastrointestinal tract and significantly increase the numbers of probiotic cells in the gut [82]. However, comparing the serving size of yogurt to that of cheese, cheese needs to have higher density of probiotic cells and higher viability to provide the same health benefits. Cheese was introduced to probiotic industry in 2006 when Danisco decided to test the growth and survival of probiotic strains in cheese [84]. At that time, only few probiotic cheese products were found on the market. The test showed that less than 10% of the bacteria were lost in the cheese whey. Based on the process, a commercial probiotic cheese was first developed by the Mills DA, Oslo, Norway. Nowadays, there are over 200 commercial probiotic cheeses in various forms, such as fresh, semi-hard, hard cheese in the marketplaces. Semi-hard and hard cheese, compared to yogurt as a carrier for probiotics, has relatively low recommended daily intake and need relatively high inoculation level of probiotics (about 4 to 5 times). Fresh cheese like cottage cheese has high recommended daily intake, limited shelf life with refrigerated storage temperature. It may, thus, serve as a food with a high potential to be applied as a carrier for probiotics.

3.1.4. Other dairy based products

Other dairy products including quark, chocolate mousse, frozen fermented dairy desserts, sour cream, and ice cream can be good vehicles of probiotics. Quark was tested with two probiotic cultures to improve its nutrition characteristics and the results showed that probiotics can ensure the highest level of utilization of fat, protein, lactose, and phosphorus partially in skimmed milk [85]. Chocolate mousse with probiotic and prebiotic ingredients were developed [86]. Probiotic chocolate mousse was supplemented with *L. paracasei* subsp. *paracasei* LBC 82, solely or together with inulin and the results showed that chocolate mousse is good vehicle for *L. paracasei* [86]. Sour cream was investigated as probiotic vehicle and the results showed that using sour cream as a probiotic carrier is proved feasible [87]. Ice creams are among the food products with high potential for use as probiotic vehicles. Cruz and others have reviewed the technological parameters involved in the production of probiotic ice creams [88]. They have pointed several factors that need to be controlled, including the appropriate selection of cultures, inoculums concentration, the appropriate processing stage for the cultures to be added, and the processing procedures and transport and storage temperatures. They concluded that probiotic cultures do not modify the sensory characteristics of the ice-creams and frozen desserts also these products hold good viability for probiotics during the product storage period.

3.2. Non dairy based probiotic products

Dairy products are the main carriers of probiotic bacteria to human, as these products provide a suitable environment for probiotic bacteria that support their growth and viability. However, with an increase in the consumer vegetarianism throughout the developed countries, there is also a demand for the vegetarian probiotic products. Nondairy probiotic products have shown a big interest among vegetarians and lactose intolerance customers. According to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the U.S. National Institutes of Health, about 75% of the world population is lactose intolerant. The development of new nondairy probiotic food products is very much challenging, as it has to meet the consumer's expectancy for healthy benefits [89, 90]. Granato and others have overview of functional food development, emphasizing nondairy foods that contain probiotic bacteria strains [91]. From their review, some nondairy probiotic products recently developed are shown in Table 2.

3.2.1. Vegetable-based probiotic products

Fermentation of vegetables has been known since ancient time. Fermented vegetables can offer a suitable media to deliver probiotics. However, it shows that the low incubation temperature of vegetable fermentation is a problem for the introduction of the traditional *L. acidophilus* and *Bifidobacterium* probiotic bacteria. Probiotic of *L. rhamnosus*, *L. casei* and *L. plantarum* are better adapted to the vegetable during fermentation [94]. Nevertheless, when the temperature is adjusted at 37ºC, probiotic bacteria grow quite rapidly in plant-based substrates [95].

Category	Product
Fruit and vegetable based	Vegetable-based drinks
	Fermented banana pulp
	Fermented banana
	Beets-based drink
	Tomato-based drink
	Many dried fruits
	Green coconut water
	Peanut milk
	Cranberry, pineapple, and orange juices
	Ginger juice
	Grape and passion fruit juices
	Cabbage juice
	Carrot juice
	Noni juice
	Onion
	Probiotic banana puree
	Nonfermented fruit juice beverages
	Blackcurrant juice
Soy based	Nonfermented soy-based frozen desserts
	Fermented soymilk drink
	Soy-based stirred yogurt-like drinks
Cereal based	Cereal-based puddings
	Rice-based yogurt
	Oat-based drink
	Oat-based products
	Yosa (oat-bran pudding)
	Mahewu (fermented maize beverage)
	Maize-based beverage
	Wheat, rye, millet, maize, and other cereals fermented probiotic beverages
	Malt-based drink
	Boza (fermented cereals)
	Millet or sorghum flour fermented probiotic beverage
Other nondairy foods	Starch-saccharified probiotic drink
	Probiotic cassava-flour product
	Meat products
	Dosa (rice and Bengal gram)

Table 2. Some nondairy probiotic products recently developed. Adapted from [91]

To develop new probiotic vegetable products, many studies have been carried out. The suitability of carrot juice as a raw material for the production of probiotic food with Bifidobacterium strains was investigated [96]. Kun and others have found that Bifidobacteria were capable of having biochemical activities in carrot juice without any nutrient supplementation [96]. Yoon and others studied the suitability of tomato juice for the production of a probiotic product by *L. acidophilus, L. plantarum, L. casei* and *L. delbrueckii.* They reported that the four LAB were capable of rapidly utilizing tomato juice for cell synthesis and lactic acid production without nutrient supplementation and pH adjustment [109]. Yoon and others also tested the suitability of cabbage to produce probiotic cabbage juice and suggested that fermented cabbage juice support the viability of probiotics and serve as a healthy beverage [97]. The viability of various bifidobacteria in kimchi was investigated under various conditions and the results show the acceptable levels of probiotics in kimchi [98]. In addition, sauerkraut-type products such as fermented cabbage, carrots, onions, and cucumbers based on a lactic fermentation by *L. plantarum* could be good probiotic carrier. Yoon and others have evaluated the potential of red beets as substrate for the production of probiotic beet juice by four strains of lactic acid bacteria and all strains were capable of rapidly utilizing the beet juice for the cell synthesis and lactic acid production [99]. However, traditional methods of production might result in inactivation of the probiotic cultures and the use of probiotics in fermented vegetables would require low temperature storage of the products [94].

Moreover, soybean has received attention from the researchers due to its high protein and quality. Soymilk is suitable for the growth of LAB and bifidobacteria [100, 101]. Several studies have focused on developing fermented soymilk with different strains of LAB and Bifidobacteria to produce a soymilk product with improved health benefits [62, 101-103]. Soymilk is now known for their health benefits such as prevention of chronic diseases such as menopausal disorder, cancer, atherosclerosis, and osteoporosis, therefore, soymilk fermented with bifidobacteria may be a unique functional food [62, 104]. In probiotic soy products, fermentation by probiotics has the potential to (1) reduce the levels of some carbohydrates possibly responsible for gas production in the intestinal system, (2) increase the levels of free isoflavones, which has many beneficial effects on human health, and (3) favor desirable changes in bacterial populations in the gastrointestinal tract. Supplementing soymilk with prebiotics such as, fructooligosaccharides (FOS), mannitol, maltodextrin and pectin, was found to be a suitable medium for the viability of probiotic bacteria [105].

3.2.2. Fruit-based probiotic products

Nowadays, there is increasing interest in the development of fruit-juice based probiotic products. The fruit juices contain beneficial nutrients that can be an ideal medium for probiotics [106, 107]. Fruit juices have pleasing taste profiles to all age groups and they are perceived as being healthy and refreshing. The fruits are rich in several nutrients such as minerals, vitamins, dietary fibers, antioxidants, and do not contain any dairy allergens that might prevent usage by certain segments of the population [107, 108]. Those characteristics allow the selection of appropriate strains of probiotics to manufacture enjoyable healthy fruit juice. However, the sensory impact of probiotic cultures would have different taste profiles compared to the conventional, nonfunctional products. The different aroma and flavors have been reported when *L. plantarum* was added to orange juices which consumers do not prefer. But if their health benefits information is provided the preference increases over the conventional orange juices. Different attempts have been made to reduce the sensations of unpleasant aromas and flavors in probiotic fruit juice. Luckow and others reported that the perceptible off-flavors caused by probiotics that often contribute to consumer dissatisfaction may be masked by adding 10% (v/v) of tropical fruit juices, mainly pineapple, but also mango or passion fruit [108].

To develop probiotic fruits, many studies have been carried out. The suitability of noni juice as a raw material for the production of probiotics was studied by Wang and others and found that *B. longum* and *L. plantarum* can be optimal probiotics for fermented noni juice [109]. Suitability of fermented pomegranate juice was tested using *L. plantarum, L. delbruekii, L. paracasei, L. acidophilus.* Pomegranate juice was proved to be a suitable probiotic drink as results have shown desirable microbial growth and viability for *L. plantarum* and *L. delbruekii* [110]. Optimized growth conditions of *L. casei* in cashew apple juice were studied. *L. casei* has shown suitable survival ability in cashew apple juice during 42 days of refrigerated storage. It was observed that *L. casei* grew during the refrigerated storage and cashew apple juice showed to be suitable probiotic product [111]. Tsen and others reported that *L. acidophilus* immobilized in Ca-alginate can carry out a fermentation of banana puree, resulting in a novel probiotic banana product with higher number of viable cells [112]. Kourkoutas and others reported that *L. casei* immobilized on apple and quince pieces survived for extended storage time periods and adapted to the acidic environment, which usually has an inhibitory effect on survival during lactic acid production [113].

3.2.3. Cereal-based probiotic products

Cereal-based probiotic products have health-benefiting microbes and potentially prebiotic fibers. The development of new functional foods which combine the beneficial effects of cereals and health promoting bacteria is a challenging issue. Nevertheless, cereal-based products offer many possibilities. Indeed, numerous cereal-based products in the world require a lactic fermentation, often in association with yeast or molds. Cereals are good substrates for the growth of probiotic strains and due to the presence of non-digestible components of the cereal matrix may also serve as prebiotics [114, 115]. Due to the complexity of cereals, a systematic approach is required to identify the factors that enhance the growth of probiotic in cereals [116]. Champagne has listed number of cereal-based products that require a lactic fermentation, often in association with yeast or molds. We have found it useful to include part of these products in Table 3.

Food	Country	Ingredients	Microorganisms
Adai	India	Cereal, legume	*Pediococcus* spp., *Streptococcus* spp., *Leuconostoc* spp.
Anarshe	India	Rice	Lactic acid bacteria
Aya-bisbaya	Mexico	Rice	Lactic acid bacteria
Bhatura	India	Wheat	Lactic acid bacteria, yeasts
Burukutu	Nigeria	Sorghum, cassava	Lactic acid bacteria, *Candida* spp., *S. cerevisiae*
Fermented oatmeal (ProViva)	Sweden	Oatmeal	*L. plantarum*
Llambazi, lakubilisa	Zimbabwe	Maize	Lactic acid bacteria, yeasts, molds
Injera	Ethiopia	Sorghum, tef, corn, millet, barley, wheat	*L. plantarum*, *Aspergillus* spp., *Penicillium* spp., *Rhodotorula* spp., *Candida* spp.
Kishk, kushuk, trahanas	Egypt, Syria, Lebanon	Milk (yoghurt), wheat	*L. casei, L. plantarum, L. brevis, B. subtilis, B. licheniformis, B. megaterium*, yeasts
Kisra	Sudan, Irak, Arabian Gulf	Sorghum, millet	*Lactobacillu.* spp., *L. brevis, L. fermentum, E. faecium, Acetobacter* spp., *S. cerevisiae*
Togwa	Tanzania	Maize, sorghum	*L. plantarum, L. brevis, L. fermentum, L. cellobiosus P. pentosaceus, W. confusa, S. cerevisiae, C. tropicalis*

Table 3. Fermented cereal products that carry a lactic fermentation [94]

A multitude of fermented cereal products have been created, but only recently probiotic microorganisms involved in traditional fermented cereal foods have been reported. Strains of *L. plantarum,Candida rugosa* and *Candida lambica* isolated from a traditional Bulgarian cereal-based fermented beverage exhibited probiotic properties, being resistant up to 2% bile concentration, which enables them to survive bile toxicity during their passage through the gastrointestinal system [117]. More studies are being done to demonstrate that cereals are suitable substrates for the growth of some probiotic bacteria. Rozada-Sa´nchez and others have studied the growth and metabolic activity of four different *Bifidobacterium* spp. in a malt hydrolisate using four *Bifidobacterium* strains with the aim of producing a potentially probiotic beverage [92]. The study has reported potential use for malt hydrolysate as probiotic beverage with the addition of a growth and yeast extract. Angelov and others have used a whole-grain oat substrate to obtain a drink with probiotics and oat prebiotic beta-glucan. They have found that viable cell counts reached at the end of the process were about 7.5×10^{10} cfu/ ml. Also the addition of sweeteners aspartame, sodium cyclamate, saccharine and Huxol (12% cyclamate and 1.2% saccharine) had no effect on the dynamics of the fermentation process and on the viability of the starter culture during product storage [93]. Charalapompoulos and others have done experiments with different cereals to determine the main parameters that need to be considered in the growth of probiotic microorganisms, defining them as follows: the composition and processing of cereal grains, the substrate formulation, the growth capability and productivity of the starter culture, the stability of the probiotic strain during storage, the organoleptic properties and the nutritional value of the final product [114]. They reported that many cereals supported the growth of probiotics with some differences. Malt medium supported the growth of all examined strains (*L. plantarum, L. fermentum, L. acidophilus* and *L. reuteri*) better than barley and wheat media due to its chemical composition. Also, wheat and barley extracts were found to exhibit a significant protective effect on the viability of *L. plantarum, L. acidophilis* and *L. reuteri* under acidic conditions (pH 2.5).

Oat is often used in studies of cereal fermented by probiotic bacteria. Several studies have evaluated the potential of oat as substrates for the development of a probiotic product. Kedia and others have explored the potential of using mixed culture fermentation to produce cereal-based foods with high numbers of probiotic bacteria. In this study, LAB growth was enhanced by the introduction of yeast and the production of lactic acid and ethanol were increased in comparison against pure LAB culture. They have fermented whole oat flour with *L. plantarum* along with white flour and bran in order to compare the suitability of these substrates for the production of a probiotic beverage. Those substrates were found to enhance probiotic viability at the end of fermentation above the minimum required in a probiotic product [118]. Martensson and others have studied the development of nondairy fermented product based on oat [119]. Yosa is a snack food made from oat bran pudding cooked in water and fermented with LAB and Bifidobacteria. It is mainly consumed in Finland and other Scandinavian countries. It has a texture and a flavor similar to yogurt but it is totally free from milk or other animal products. It is lactose-free, low in fat, contains beta-glucan and it is suitable for vegetarians [120]. Yosa is therefore considered a healthy food due to its content of oat fiber and probiotic LAB, which combine the effect of beta-glucan for cholesterol reduction and the effect of LAB benefits to maintain and improve the intestinal microbiota balance of the consumer.

Other cereals and cereal components that can be used as fermentation substrates for probiotics have been studied. Survival of probiotics in a corn-based fermented substrate was reported [121]. Autoclaved maize porridge was fermented with probiotic strains (grown separately): *L. reuteri*, *L. acidophilus* and *L. rhamnosus* for 24h at 37 ◦C. All strains examined showed good growth in maize porridge with added barley malt. Probiotic fermented maize products could have a good world-wide acceptance, since maize fermentation induces fruity flavors in traditional Mexican foods. Prado and others have summarized some of the international cereal based probiotic beverages including: *Boza* made from wheat, rye, millet and other cereals in Bulgaria, Albania, Turkey and Romania, *Bushera* made from sorghum, or millet flour in Western highlands of Uganda, *Mahewu* (amahewu) made from corn meal in Africa and some Arabian Gulf countries, *Pozol* made from maize in the Southeastern Mexico, and *Togwa* made from maize flour and millet malt in Africa [5].

Normally sourdoughs are the cereal products fermented by LAB cultures. However, baking will kills most probiotic bacteria and only probiotics which synthesize a thermostable bioactive compound during leavening can be of use in bread making. Different studies have shown the ability of human derived strains of *L. reuteri* to resist simulated gastric acidity and bile acid, and also to grow well in a number of cereal substrates [89, 116]. In this perspective, *L. reuteri* has potential use in bread making due to reuterin synthesis [122]. The *L. reureri* cells might be inactivated by heating, but the bioactive compound might remain active. Probiotic *Bacillus* strains could better adapt to bread making due to their spore-forming characteristics.

3.2.4. Meat-based probiotic foods

Probiotic applications are restricted to fermented meats, such as dry sausages. The idea of using probiotic bacteria in fermenting meat products has introduced the idea of using antimicrobial peptides, i.e. bacteriocins, or other antimicrobial compounds as an extra hurdle for meat products. Meat starter culture was defined as preparations which contain living or resting microorganisms that develop the desired metabolic activity in the meat [123]. LAB are the most common used starter culture in meat which produce lactic acid from glucose or lactose. As meat content of these sugars are low, sugar is added at 0.4–0.7% (w/w) for glucose and 0.5–1.0% (w/w) for lactose to the sausage matrix [124]. Some LAB strains such as *L. rhamnosus* GG are not able to utilize lactose, therefore, the starter culture properties have to be taken into account for successful applications. From pentoses, such as arabinose and xylose, meat starter LAB produce both lactic acid and acetic acid [125]. As indicated in commercial catalogues LAB strains currently most employed in meat starter cultures are *L. casei, L. curvatus, L. pentosus, L. plantarum, L. sakei, Pediococcus acidilactici* and *Pediococcus pentosaceus* [124].

LAB have been used for dry sausage manufacturing process since 1950s in order to ensure the safety and quality of the end product. Dry sausages are non heated meat products, which may be suitable carriers for probiotics into the human gastrointestinal tract [124]. Dry sausage is made from a mixture of frozen pork, beef and pork fat with the addition of sugars, salt, nitrite, and nitrate, ascorbates and spices. The raw sausage material is stuffed into casing material of variable diameters and hung vertically in fermentation and ripening chambers for several weeks. Salt, nitrite, and added spices are the main contributors in the inhibition of different bacteria on the surface of the sausages. Lactic acid bacteria and staphylococci used as starter cultures to ferment the sausage. Salt decreases the initial water activity inhibiting or at least delaying the growth of many bacteria while favoring the growth of starter LAB and starter staphylococci. During the first day of fermentation the growth of microbes in sausage material uses up all the oxygen mixed in the sausage matrix during the chopping. After few days of fermentation, LAB decrease the pH to about 5.0 which acts as a hurdle for several Gram-negative bacterial species [126, 127]. The presence LAB in the food suggests that bacteriocins may be active in the human small intestine against food pathogens as long as they are able to survive the environment of gastrointestinal tract [27]. Likewise, probiotic strains with antimicrobial effects on food act similarly and therefore might be more successful than commonly used food fermenting bacteria. It could be concluded that dry sausage is suitable carrier for probiotics. However, human clinical studies are needed before the final answer concerning the health promoting effects of probiotic dry sausage.

Some traditional Indian fermented fish products such as Ngari, Hentak and Tungtap have been analyzed for microbial load [128]. LAB were identified as *Lactococcus lactis* subsp. *cremoris, Lactococcus plantarum, Enterococcus faecium, L. fructosus, L. amylophilus, L. coryniformis* subsp. *torquens*, and *L. plantarum*. Most strains of LAB had a high degree of hydrophobicity, indicating that these microorganisms have a probiotic potential.

4. Agricultural applications of probiotics

Probiotics applications have been extended from human applications to diversity of agricultural application. Agricultural applications include animal and plants.

4.1. Animal

Probiotics, with regard to animal applications, were defined as live microbial feed supplements beneficially improve the intestinal microbial balance in host animal [26]. They have been approved to provide many benefits to the host animal and animal products production. They are used as animal feed to improve the animal health and to improve food safety with examples of the application in poultry, ruminant, pig and aquaculture.

The microflora in the gastrointestinal tracts of poultry plays a key role in normal digestive processes and in maintaining the animal's health. Some feed additives can substantially affect this microbial population and their health promoting effects. Recently, concerns about some unwanted harmful side effects caused by antibiotics [129] has grown in many countries, so that there is an increasing interest in finding alternatives to antibiotics in poultry production. Probiotic has provided a possible natural alternative to antibiotics in poultry production to produce foods of reliable quality and safety [130]. In addition, the application of probiotic to chicken feed was shown to increase the internal and external quality of eggs. Addition of probiotic to chicken feed increased egg weight shell thickness, shell weight, albumen weight, and specific gravity and decreased shape index [131]. Farm animals are often subjected to environmental stresses which can cause imbalance in the intestinal ecosystem and could be a risk factor for pathogen infections. Applications of probiotics in feed have decreased the pathogen load in the farm animals. Feeding probiotic LAB and yeast to calve was found to promote the growth and suppress diarrhea in Holstein calve [132]. Gaggia and others have reviewed the applications of probiotics and prebiotics in animal feeding that can introduce to safe food production [133]. Probiotics has been used to intervene in decreasing pathogen load and in ameliorating gastrointestinal disease symptoms in pigs. Beside the in vitro test to identify the best potential probiotics, several studies are conducted in vivo utilizing different probiotic microorganisms. Most of the studies showed a beneficial role of improving the number of beneficial bacteria, decreasing the load of pathogens, stimulating the immune cell response towards pathogens in comparison to control, and increasing defensive tools against pathogenic invasion. In contrast, some authors reported an enhancement of the course of infection or a partial alleviation of diarrhea.

Applications of probiotics in aquaculture generally depend on producing antimicrobial metabolites and their ability to attach to intestinal mucus. *Aeromonas hydrophila* and *Vibrio alginolyticus* are common pathogens in fish, however, addition of probiotics strains (isolated from the clownfish, *Amphiprion percula*) were found capable to prevent the adhesion of these microbes to fish intestinal mucus and to compete with the pathogens [31]. Feeding probiotics to shrimp was found to reduce disease caused by *Vibrio parahaemolyticus* in shrimp [36]. Balcazar and others have reviewed the use of probiotics for prevention of bacterial diseases in aquaculture [134].

4.2. Plant

A strong growing market for plant probiotics for the use in agricultural biotechnology has been shown worldwide with an annual growth rate of approximately 10%. Based on the mode of action and effects, the plant probiotics products can be used as biofertilizers, plant strengtheners, phytostimulators, and biopesticides [38]. Berg has reported several advantages of using plant probiotics over chemical pesticides and fertilizers including: more safe, reduced environmental damage, less risk to human health, much more targeted activity, effective in small quantities, multiply themselves but are controlled by the plant as well as by the indigenous microbial populations, decompose more quickly than conventional chemical pesticides, reduced resistance development due to several mechanisms, and can be also used in conventional or integrated pest management systems [38]. Plant growth promotion can be achieved by the direct interaction between beneficial microbes and their host plant and also indirectly due to their antagonistic activity against plant pathogens. Several model organisms for plant growth promotion and plant disease inhibition are well-studied including: the bacterial genera *Azospirillum* [44, 135], *Rhizobium* [136], *Serratia* [137], *Bacillus* [138, 139], *Pseudomonas* [140, 141], *Stenotrophomonas* [142], and *Streptomyces* [143] and the fungal genera *Ampelomyces*, *Coniothyrium*, and *Trichoderma* [144]. Some examples of commercial products that have plant probiotics are listed in Table 4.

Several mechanisms are involved in the probiotics-plant interaction. It is important to specify the mechanism and to colonize plant habitats for successful application. Steps of colonization include recognition, adherence, invasion, colonization and growth, and several strategies to establish interactions. Plant roots initiate crosstalk with soil microbes by producing signals that are recognized by the microbes, which in turn produce signals that initiate colonization [43, 51]. Colonizing bacteria can penetrate the plant roots or move to aerial plant parts causing a decreasing in bacterial density in comparison to rhizosphere or root colonizing populations [43]. Furthermore, in the processes of plant growth, probiotic bacteria can influence the hormonal balance of the plant whereas phytohormones can be synthesized by the plant themselves and also by their associated microorganisms [38].

Microorganism	Name of the product	Plant pathogens, or pathosystem	Company
Ampelomyces quisqualis M-10	AQ10 Biofungicide	Powdery mildew on apples, cucurbits, grapes, omamentals, strawberries, and tomatoes.	Ecogen
Azospirillum spp.	Biopromoter	Paddy, millets, oilseeds, fruits, vegetables, sugarcane, banana	Manidharma Biotech
Bacillus subtilis GB03	Kodiak	Growth promotion; *Rhizoctonia* and *Fusarium* spp.	(Gustafson); Bayer CropScience
Bradyrhizobium japonicum	Soil implant	Soy bean	Nitragin
Bacillus pumilus GB34	YiedShield	Soil-born fungal pathogens	(Gustafson); Bayer CropScience
Coniothyrium minitans	Contans WG, Intercept WG	*Sclerotinia sclerotiorum, S. minor*	Prophyta Biologischer Pflanzenschutz
Delftia acidovorans	BioBoost	Canola	Brett-Young Seeds Limited
Phlebiopsis gigantea	Rotex	*Heterobasidium annosum*	E~nema Biologischer Pflanzenschutz
Pseudomonas chlororaphis	Cedomon	Leaf stripe, net blotch, *Fusarium* sp., sot blotch, leaf spot, etc. on barley and oats	BioAgri AB
Streptomyces griseoviridis K61	Mycostop	*Phomopsis* spp., *Botrytis* spp., *Pythium* spp.,*Phythophora* spp.	Kemira Agro Oy
Trichoderma harzianum T22	RootShield, PlantShield T22, Planter box	*Pythium spp., Rhizoctonia solani, Fusarium* spp	Bioworks
Pseudomonas spp.	Proradix	*Rhizoctonia solani*	Sourcon Padena

Table 4. Examples of commercial products that have plant probiotics. Adapted from [38]

Besides these mechanisms, probiotic bacteria can supply macronutrients and micronutrients. They metabolize root exudates and release various carbohydrates, amino acids, organic acids, and other compounds in the rhizosphere [43]. Bacteria may contribute to plant nutrition by liberating phosphorous from organic compounds such as phytates and thus indirectly promote plant growth [145]. Furthermore, probiotic can reduce the activity of pathogenic microorganisms through microbial antagonisms and by activating the plant to better defend itself, a phenomenon termed “induced systemic resistance” [146, 147]. Microbial antagonism includes the inhibition of microbial growth, competition for colonization sites and nutrients, competition for minerals, and degradation of pathogenicity factors [38, 43]. In Japanese composting, at least three groups of compositing bacteria were used individually, or in combination. The following species were used: *Bacillus* bacteria groups, Lactic acid bacteria groups and *Actinomycetous* groups. These bacteria species can protect plant products from cropping hazards. They do this by expelling against various bad worms and insects, such as nematodes with potatoes and some types of insects with soybeans and maize. They are also effective in controlling fungi such as powdery mildew, downy mildew, *phythium* (damping off with many plants), *plasmodipophora brosscae* (club-root with the cabbage Jamily); *Crucijert1e* (plants. and fusarium of wilt with tomato and banana) [148].

5. Probiotics application challenges

From a technological standpoint, Champagne has listed many challenges in the development of a probiotic food product including: strain selection, inoculation, growth and survival during processing, viability and functionality during storage, assessment the viable counts of the probiotic strains particularly when multiple probiotic strains are added and when there are also starter cultures added, and the effects on sensory properties [94]. Champagne has focused in his chapter on three of these challenges: inoculation, processing and storage issues. Other challenges such as: maintaining of probiotics, diversity and origin of probiotics, probiotic survival and being active, dealing with endogenous microbiota, and proving health benefits have also been discussed [149]. This section will focus on the viability and sensory acceptance as we have found these are the most important challenges to ensure transferring the health benefits and the commercial success.

5.1. Viability and survival

Probiotics have been proved to provide many health benefits. However, the claimed health benefits can’t be achieved without high number of viable cells. Many probiotic bacteria have shown to die in the food products after exposure to low pH after fermentation, oxygen during refrigeration distribution and storage of products, and/or acid in the human stomach [150, 151]. Probiotic products need to be supplemented with additional ingredients to support the viability throughout processing, storage, distribution, and gastrointestinal tract to reach the colon. Several reports have shown that survival and viability of probiotic bacteria is often low in yogurt. The efficiency of added probiotic bacteria depends on dose level and their viability must be maintained throughout storage, products shelf-life and they must survive the gut environment [151]. Several studies have focused on the effect of adding certain compounds to enhance the probiotic viability. Many evidences have shown that inulin, oligosaccharides, and fructooligosaccharides (FOS) have good impacts on the probiotics viability. However, the effect of these compounds are strain specific. Martinez-Villaluenga and others have examined the influence of raffinose on the survival of *Bifidobacteria* and *L. acidophilus* in fermented milk. The results showed that retention of viability of *Bifidobacteria* and *L. acidophilus* greater in fermented milk with raffinose [65]. Supplementing probiotic products with FOS, mannitol, maltodextrin and pectin were found to provide a suitable viability for probiotic bacteria [105]. Inulin and FOS were found to support the growth and viability of *L. acidophilus* but did not significantly affect growth and viability of *Bifidobacterium* and *L. casei* [152]. During food formulation step several things need to be considered such as the composition (nutrients, antimicrobials), structure (oxygen permeability, water activity) and pH of the food matrix, and possible interactions with starter microbes in fermented food matrices. Growth of probiotics in non-fermented foods is not desirable (due to possible off flavor formation), but their growth during the production of fermented foods can lower process costs and increase the adaptation of probiotics leading to enhanced viability. The starter microbes in fermented foods can sometimes inhibit probiotics but they can also enhance their survival by producing beneficial substances or by lowering the oxygen pressure. In beverages the most important factor affecting probiotic viability is probably the pH. Shelf-stable beverages typically have pH values below 4.4 to ensure their microbial stability and this low pH value combined with long storage periods is very demanding for most probiotic strains, especially those representing bifidobacteria. The packaging material should be a good oxygen barrier to promote the survival of especially anaerobic probiotic bacteria (bifidobacteria) [153]. Transportation and storage temperature is an important determinant of the shelf-life; with increasing temperatures viability losses can occur rapidly [154].

The viability and survival of probiotics are strain specific. To maintain the viability of very sensitive strains, encapsulation is often the only option, especially microcapsulation that do not affect the sensory properties of the food produced. Microencapsulation technologies have been developed and successfully applied using various matrices to protect the bacterial cells from the damage caused by the external environment [155]. Overall microencapsulation improved the survival of probiotic bacteria when exposed to acidic conditions, bile salts, and mild heat treatment [156]. The immobilization of probiotics using microencapsulation may improve the survival of these microorganisms in products, both during processing and storage, and during digestion [157, 158].

Some probiotic bacteria, such as the spore-forming bacteria, GanedenBC[30] provides better viability and stability, making it an ideal choice for product development, compared to other probiotic bacteria strains, such as *L. acidophilus* and bifidobacteria. This spore safeguards the cell's genetic material from the heat and pressure of manufacturing processes, challenges of shelf life and the acid and bile it is exposed to during transit to the digestive system. GanedenBC[30] can withstand manufacturing processes. and survive through high temperature processes such as baking and boiling, low temperature processes such as freezing and refrigeration and high pressure applications like extrusion and roll forming. GanedenBC[30] requires no refrigeration and can be formulated into products to have up to a two-year. Once it is safely inside the small intestine, the viable spore is then able to germinate and produce new vegetative cells or good bacteria [159].

5.2. Sensory acceptance

Probiotic foods must show, at least, the same performance in any sensory test as conventional foods. In most probiotic foods sensory tests are aiming to determine acceptance of the products, without, obtaining details concerning the addition of the probiotics to the food and their interaction with the consumer. Therefore, it is important to development sensory tests for probiotic foods that can be accompanied by specific sensory analyses. Sensory testing must cover all characteristics with regard to change over time during storage. Some studies have reported the possibility of obtaining similar, or even better, performance with probiotic products as compared to conventional products such as: functional yogurt supplemented with *L. reuteri* RC-14 and *L. rhamnosus* GR-1 [160], chocolate mousse with added inulin and *L. paracasei* [86] , curdled milk with inulin, and *L. acidophilus* [152], and milk fermented with *B. animalis* and *L. acidophilus* La-5, and supplemented with inulin [161].

Sensory methodology will allow obtaining important data for developing the probiotic foods. In most cases the developed products need to match similar commercial products in parallel. In general, metabolism of the probiotic culture can result in the production of components that may contribute negatively to the aroma and taste of the food product, probiotic off-flavor. For example, acetic acid produced by *Bifidobacterium* spp. can result in a vinegary flavor in the product, prejudicing the performance in sensory assessments.

Masking is one technique that has been used to reduce the off flavors in foods and it has been performed successfully through the addition of new substances or flavors to reduce the negative sensory attributes contributed by probiotic cultures. The addition of tropical fruit juices, mainly pineapple, but also mango or passion fruit, might positively contribute to the aroma and flavor of the final product and might avoid the identification of probiotic off-flavors by consumers [162]. The influence of exposure has been identified in many consumer studies [91, 163] that the frequency of exposure to a food stimulus is increased, food stimuli have been shown to be better liked. Therefore, repeated exposure and increased familiarity to sensory off-flavors, may influence consumer attitudes in a positive way, therefore increasing willingness to consume probiotic juices. Nonsensory techniques have proven useful in enhancing the sensory quality of products, such as providing consumers with health benefit information associated with probiotic cultures. Health information has been shown to be a vital tool in the consumer acceptance of a variety of probiotic food products [164-166]. Finally, microcapsules of probiotics may help prevent the off flavor of cultures [167].

6. The future of probiotics

Dairy based products containing live bacteria are the main vehicles of probiotics to human. Non-dairy beverages would be the next food category where the healthy bacteria will make their mark. Microencapsulation technologies have provided the necessary protection for probiotics and moved them outside the pharmaceutical and supplemental use to become food ingredients.

6.1. Nanotechnology, encapsulation, and probiotics

The word "nano" comes from the Greek for "dwarf ". A nanometer is a thousandth of a thousandth of a thousandth of a meter (10^{-9} m). Nanoparticles are usually sized below 100 nanometers which will enable novel applications and benefits. Nanotechnology of probiotics is an area of emerging interest and opens up whole new possibilities for the probiotics applications. Their applications to the agriculture and food sector are relatively recent compared with their use in drug delivery and pharmaceuticals. The basic of probiotic nanotechnology applications is currently in the development of nano-encapsulated probiotics. The nanostructured food ingredients are being developed with the claims that they offer improved taste, texture and consistency. Applications of nanotechnology in organic food production require precaution, as little is known about their impact on environment and human health. Some recent food applications of nanotechnology, safety and risk problems of nanomaterials, routes for nanoparticles entering the body, existing regulations of nanotechnology in several countries, and a certification system of nanoproducts were reported [168, 169]. Currently, no regulations exist that specifically control or limit the production of nanosized particles and this is mainly owing to a lack of knowledge about the risks [169]. Nanoencapsulation is defined as a technology to pack substances in miniature using techniques such as nanocomposite, nanoemulsification, and nanoestructuration and provides final product functionality and control the release of the core [170]. Encapsulation of food ingredients may extend the shelf life of the product. Nanoencapsulation of probiotic is desirable technique that could deliver the probiotic bacteria to certain parts of the gastrointestinal tract where they interact with specific receptors [170]. These nanoencapsulated probiotic bacterial may also act as *de novo* vaccines, with the capability of modulating immune responses [171].

Microencapsulation with alginate can be applied to many different probiotic strains and results show better survival than free cells at low pH of 2.0, high bile salt concentrations, and moderate heat treatment of up to 65 ◦C [156]. Microencapsulation may prove to be an important method of improving the viability of probiotic bacteria in acidic food products and help deliver viable bacteria to the host's gastrointestinal tract. Furthermore, microencapsulation appeared to be effective in protecting cells from mild heat treatment and thus could stimulate research in functional food products that receive a mild heat treatment [156]. The microencapsulation allows the probiotic bacteria to be separated from its environment by a protective coating. Several studies have reported the technique of the microencapsulation by using gelatin, or vegetable gum to provide protection to acid-sensitive *Bifidobacterium* and *Lactobacillus* [172-176].

6.2. Biotechnology and probiotics

With the revolution in sequencing and bioinformatic technologies well under way it is timely and realistic to launch genome sequencing projects for representative probiotic microorganisms. The rapidly increasing number of published lactic acid bacterial genome sequences will enable utilizing this sequence information in the studies related to probiotic technology. If genome sequence information is available for the probiotic species of interest, this can be utilized, e.g. to study the gene expression (transcription) profile of the strain during fermenter growth. This will enable better control and optimization of the growth than is currently possible. Transcription profiling during various production steps will allow following important genes for probiotic survival during processing (e.g., stress and acid tolerance genes) and identifying novel genes important for the technological functionality of probiotics [177].

Increasing knowledge of genes important for the technological functionality and rapid development of the toolboxes for the genetic manipulation of *Lactobacillus* and *Bifidobacterium* species will in the future enable tailoring the technological properties of probiotic strains. However, before wide application of tailored strains in probiotic food products, safety issues are of utmost importance and have to be seriously considered for each modified strain [178].

7. Regulations and guidelines for probiotics

Depending on intended use of a probiotic (drug *vs*. dietary supplement), regulatory requirements differ greatly. If a probiotic is intended for use as a drug, then it must undergo the regulatory process as a drug, which is similar to that of any new therapeutic agent. An Investigational New Drug application must be submitted and authorized by the Food and Drug Administration before an investigational or biological product can be administered to humans. The probiotic drug must be proven safe and effective for its intended use before marketing [14]. In the United States, probiotic products are marketed to a generally healthy population as foods or dietary supplements. For dietary supplements, premarketing demonstration of safety and efficacy and approval by the Food and Drug Administration are not required; only premarket notification is required. The law allows that in addition to nutrient content claims, manufacturers of dietary supplements may make structure/function or health claims for their products. The ''health claims'' must be defensible when placed under the scrutiny by the controlling authorities. Efforts are being made to establish meaningful standards or guideline for probiotic products worldwide (Table 5). The Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics developed guidelines could be used as the global standards for evaluating probiotics in food that could lead to the substantiation of health claims.

Organization	Region of impact	Action
Food Agriculture Organization (FAO)/ World Health Organization (WHO)	Worldwide	Developed guidelines for the evaluation of probiotics in foods.
International Dairy Federation	Worldwide	Has begun working on methods to determine certain functional and safety properties outlined in the FAO guidelines for the evaluation of probiotics in food.
European Food and Feed Culture Association	Europe	Developed guidelines for use of probiotics in foods.
Codex Standard for Fermented Milks (Codex Stan 243-2003)	Worldwide	Among other composition stipulations, this standard specifies minimum numbers of characterizing and additional labeled microbes in yoghurt, acidophilus milk, kefir, kumys and other fermented milks.
National Yogurt Association	USA	Petition under consideration by the FDA which would change the standard of identity of yoghurt, including the requirement of minimum levels of live cultures in yoghurt, but not specifically levels for any additional probiotic cultures.
International Scientific Association for Probiotics and Prebiotics	Worldwide	Industry Advisory Committee and Board of Directors to consider method validation and establishment of laboratory sites to assess microbiological content of probiotic products.

Table 5. Organizations involved in attempting to establish standards for probiotics in commercial products. Adapted from [179]

8. Conclusion

The uses of probiotics and their applications have shown tremendous increase in the last two decades. Probiotics can turn many health benefits to the human, animals, and plants. Applications of probiotics hold many challenges. In addition to the viability and sensory acceptance, it must be kept in mind that strain selection, processing, and inoculation of starter cultures must be considered. Probiotics industry also faces challenges when claiming the health benefits. It cannot be assumed that simply adding a given number of probiotic bacteria to a food product will transfer health to the subject. Indeed, it has been shown that viability of probiotics throughout the storage period in addition to the recovery levels in the gastrointestinal tract are important factors [3, 48, 83]. For this purpose, new studies must be carried out to: test ingredients, explore more options of media that have not yet been industrially utilized, reengineer products and processes, and show that lactose-intolerant and vegetarian consumers demand new nourishing and palatable probiotic products.

Author details

Danfeng Song*, Salam Ibrahim and Saeed Hayek
Department of Family and Consumer Science,
North Carolina Agricultural and Technical State University, Greensboro, NC, USA

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* Corresponding Author

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