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PROPERTIES OF PROBIOTICS AND ENCAPSULATED PROBIOTICS IN FOOD

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ABSTRACT

Probiotics are microorganisms which confer health benefits upon application in sufficiently-high viable cell amounts. Probiotics are typically members of *Lactobacillus* and *Bifidobacterium* species commonly associated with human gastrointestinal tracts. In the recent past, there has been a rising interest in producing functional foods containing encapsulated probiotic bacteria. Recent studies have been reported using dairy products like cheese, yogurt and ice cream as food carrier, and non-dairy products like meat, fruits, cereals, chocolate, etc. However, the industrial sector contains only few encapsulated probiotic products. Probiotics have been developed by several companies in a capsule or a tablet form. The review compiles probiotics, encapsulation technology and cell life in the food matrices.

Key words: encapsulation, food, *Lactobacillus*, *Bifidobacterium*, probiotics

INTRODUCTION

Probiotics, are mono- or mixed-cultures of live micro-organisms, beneficially affect the health of animals or humans when consumed in sufficient amounts, by improving the properties of the indigenous gastrointestinal flora [Guimaraes et al. 2013, Rehaiem et al. 2014]. Species belonging to *Bifidobacterium and Lactobacillus* are largely used as probiotics. The latter include *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus* and *Streptococcus genera*. These genera such as *Bifidobacterium* and *Lactobacillus* are common inhabitants of the human gut but functional properties markedly differ within species and strains [Vitali et al. 2012], the yeast *Saccharomyces cerevisiae* and some *Escherichia coli* strains are also used as probiotics [Sungsoo-Cho and Finocchiaro 2010].

Scientists first realized in the late 19th century, a wide range of traditional sour milk products had additional benefits apart from prolonged shelf-life and pleasant sensory properties. In 1900, two microbiologists, Tissier and Moro, reported their findings of isolates from the faeces of breast-fed infants. Tissier noted that morphological appearance was similar to those of lactobacilli; however, many of them appeared in bifurcated forms. Thus, he named them Bacillus bifidus. Similarly, Moro postulated that the isolate was derived from the mother's breast and normally resided in the neonate's oral cavity and intestinal content. At the same time, Nobel Laureate Ilya Metchnikoff noticed that Bulgarian peasants had an average life-span of 87 years. One of the major differences in their lifestyle in comparison with the contemporary diet was a large consumption of fermented milk. Life-span was prolonged by the consumption of sour milk and lactic acid producing bacteria. Metchnikoff's experiments led him to believe that *Lactobacillus bulgaricus* could

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successfully establish itself in the intestinal tract and prevent multiplication and even decrease the number of putrefactive bacteria. Certain strains of *Lactobacillus* were isolated and found to be capable of colonizing human digestive tract by Minoru Shirota, Japan in 1930. Scientists have continued to investigate possible benefits of bacteria to human health [Vasiljevic and Shah 2008].

SOURCES AND DESCRIPTIONS OF PROBIOTICS

Probiotics are microorganisms which confer health benefits upon application in sufficiently-high viable cell amounts. Probiotics are commonly associated with human gastrointestinal tracts [Weinbreck et al. 2010]. Some of the descriptions and definitions of probiotics commonly cited over the years in Table 1 [Vasiljevic and Shah 2008].

Species belonging to *Bifidobacterium* and *Lactobacillus* are largely used as probiotics. They are also divided two groups as intestinal and vaginal sources [Goktepe et al. 2006].

Bifidobacteria are an important group of probiotic cultures commonly used in fermented dairy products. *Bifidobacterium* are Gram positive, anaerobic, non motile and non-sporulating organisms. They may have various shapes such as short curved rods, club shaped rods, and bifurcated Y shaped rods [Prasanna et al. 2014]. The species included in the genus Bifidobacterium are 29: *B. adolescentis*, *B. angulatum*, *B. animalis*, *B. asteroides*, *B. bifidum*, *B. boum*, *B. breve*, *B. catenulatum*, *B. choerinum*, *B. coryneforme*, *B. cuniculi*, *B. dentium*, *B. gallicum*, *B. gallinarum*, *B. indicum*, *B. longum*, *B. magnum*, *B. merycicum*, *B. minimum*, *B. pseudocatenulatum*, *B. pseudolongum*, *B. psychraerophilum*, *B. pullorum*, *B. ruminantium*, *B. saeculare*,

Table 1. Probiotic descriptions according to years [Vasiljevic and Shah 2008]

Year	Description
1953	Probiotics are common in vegetable food as vitamins, aromatic substances, enzymes and possibly other substances connected with vital processes
1954	Probiotics are opposite of antibiotics
1955	Deleterious effects of antibiotics can be prevented by probiotic therapy
1965	A substance secreted by one microorganism which stimulates the growth of another
1971	Tissue extract which stimulates microbial growth
1973	Compounds that build resistance to infection in the host but do not inhibit the growth of microorganisms in vitro
1974	Organisms and substances that contribute to intestinal microbial balance
1992	Live microbial feed supplement which beneficially affects the host animal by microbial balance
1992	Viable mono- or mixed culture of live microorganism which, applied to animals or man, have a beneficial effect on the host by improving the properties of the indigenous microflora
1996	Live microbial culture or cultured dairy product which beneficially influences the health and nutrition and the host
1996	Living microorganisms which, upon ingestion in certain numbers, exert health benefits beyond inherent beyond basic nutrition
1999	Microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host
2001	A preparation of or a product containing viable, defined microorganism in sufficient numbers, which alter the microflora in a compartment of the host and by that exert beneficial health effect in this host
2002	Live microorganism which, when administered in adequate amount, confer a health benefit on the host

Table 2. Variety of probiotics [Goktepe et al. 2006]

Intestinal bacteria		Vaginal bacteria	
*Lactobacillus acidophilus** "group"	*Bifidobacterium adolescentis**	*Lactobacillus acidophilus**	*Bifidobacterium bifidum*
L. acidophilus senso strictu	*B. angulatum*	*L. fermentum*	*B. longum*
L. animalis	*B. bifidum*	*L. casei*	*B. infantis*
L. brevis	*B. breve*	*L. rhamnosus*	*B. breve*
L. bunchneri	*B. cantenulatum*	*L. cellobious*	*B. catenulatum*
L. crispatus	*B. dentium*	*L. plantarum*	*B. dentium*
L. curvatus	*B. infantis*	*L. brevis*	
L. deLrueckii	*B. longum*	*L. debrueckii*	
L. fermentum	*B. pseudocantenulatum*	*L. salivarious*	
L. gasseri	*Enterococcus faecalis*	*L. jensenii*	
L. johnsonii	*E. faecium*	*L. vaginalis*	
L. paracasei	*Leuc. mesenteroides*	*L. gassari*	
L. plantarum	*Pediococcus pentosaceus*	*L. crispatus*	
L. reuteri	*Weissella confusa*		
L. rhamnosus			
L. ruminis			
L. salivarius			
L. sakei			

B. scardovii, *B. subtile*, *B. thermacidophilum*, and *B. thermophilum*. In turn two subspecies constitute the species *B. animalis* (subsp. animalis and lactis), *B. pseudolongum* (subsp. globosum and pseudolongum), and *B. hermacidophilum* (subsp. thermoacidophilum and porcinum), and the species *B. longum* is subdivided in three different biotypes (longum, infantis, and suis) [Lee and Salminen 2009].

The effectiveness of this organism is related to its ability to colonize the intestinal tract and control undesirable intestinal bacteria. The optimum pH for the growth of Bifidobacteria is 6.0-7.0 and virtually no growth at below of 4.5 or above of 8.5. The optimum temperatures of growth are 37-41°C, the minimum are 25-28°C, and the maximum are 43-45°C. Some *Bifidobacterium* cultures used as probiotic are *B. adolescentis*, *B. longum*, *B. infantis*, *and B. breve* [Rivera-Espinoza and Gallardo-Navarro 2010].

Lactic acid bacteria – LAB are usually described as Gram-positive microorganisms, devoid of cytochromes and preferring anaerobic conditions but are aerotolerant, fastidious, acid-tolerant, and strictly fermentative [Lee and Salminen 2009]. At present, more than 125 *Lactobacillus* species have been identified [Sungsoo Cho and Finocchiaro 2010; Table 2]. The most important genera are: *Lactobacillus*, *Lactococcus*, *Enterococococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, and *Bifidobacterium*. However, *Bifidobacterium* shares certain physiological and biochemical properties with typical. Therefore, for practical and traditional reasons, bifidobacteria are still considered a part of the LAB group. Members of the LAB are usually subdivided into two distinct groups based on their carbohydrate metabolism: the homofermentative group which produce lactic acid as principal metabolite and the heterofermentative group which also

Table 3. Microbial group in the GIT [Goktepe et al. 2006]

Microbial group	Stomach 10^1-10^3 CFU·ml^{-1}	Dodenum 10^1-10^4 CFU·ml^{-1}	Jejunum+Ileum 10^5-10^8 CFU·ml^{-1}	Colon 10^9-$5 \cdot 10^{11}$ CFU·ml^{-1}
Actinomyces spp.			10^4-10^6	
Bacteroides-Prevotella-Porphyromonas group	up to 10^2	ca. 10^3	10^4-10^7	10^9-10^{11}
Bifidobacterium spp.				10^9-10^{10}
Clostridium spp.			10^4-10^5	10^8-10^9
Coprococcus cutactus				10^7-10^8
Enterobacteriaceae	up to 10^2	10^2-10^4	10^3-10^6	10^5-10^7
Enterecoccus spp.			10^2-10^4	10^3-10^6
Eubacterium spp.				10^9-10^{11}
Fusobacterium spp.			10^3-10^5	10^5-10^7
Lactobacillus spp.	10^1-10^3	10^2-10^4	10^4-10^6	10^5-10^8
Megamonas hypermegas				10^7-10^8
Megasphaera elsdenii				10^7-10^8
Methanobacteria				up to 10^9
Peptostreptococcus spp.			10^2-10^6	10^8-10^9
Proteus spp.				10^3-10^6
Pseudomonas spp.				$>10^3$
Staphylococcil				ca. 10^3
Streptococcus spp.	10^1-10^3		10^3-10^8	up to 10^7
Veillonella spp.			10^3-10^7	10^5-10^8
Yeasts				ca. 10^3

produce ethanol and carbon dioxide. Homofermentative group consists of *Lactococcus*, *Pediococcus*, *Enterococcus*, *Streptococcus* and some lactobacilli, whereas heterofermentative bacteria include *Leuconostoc*, *Weissella* and some lactobacilli [Vasiljevic and Shah 2008].

The growth of *L. acidophilus* occurs at a temperature as high as 45°C; however, the optimum is found between 35 and 40°C. The organisms grow in slightly acidic media at pH of 6.4-4.5, but growth will stop at a pH of 4.0-3.6. This bacterium tolerates from 0.3% to 1.9% titrable acidity, with and optimum pH at 5.5-6.0 [Rivera-Espinoza and Gallardo-Navarro 2010].

L. rhamnosus is a LAB with probiotic capacity. The growth activity of LAB is affected by fermentation conditions such as pH, temperature, medium composition and other factors [Pimentel-González et al. 2009].

Probiotic microorganisms are mostly of GIT origin. Microbial population are changeable throughout the GIT (Table 3), to 10^1 to $10^3 \cdot$ml^{-1} (or g^{-1}) in the stomach, 10^7 ml^{-1} in the jejunum (comprising mainly lactobacilli, *Enterobacteriaceae* and streptococci), up to 10^9 CFU·g^{-1} in the terminal ileum, and ca. $5 \cdot 10^{11}$ g^{-1} in the distal colon [Goktepe et al. 2006]. LAB, especially strains of Lactobacillus, are normally found in the human adult gastrointestinal (GI) tract, and have

Table 4. Fermented food showed probiotic characteristics [Cruz et al. 2009]

Product	Probiotic microorganisms	Substrates
Adai	LAB	cereal, legume
Agbelima	*Lb. plantarum*, *Lb. brevis*, *Lb. fermentum*, *Leuc. mesenteroides*	cassava
Atole	LAB	maise
Ben-saalga	LAB	pearl millet
Boza	*Lb. plantarum*, *Lb. brevis*, *Lb. rhamnosus*, *Lb. fermentum*, *Leuc. mesenteroides* subsp. *dextranium*	cereals
Dosa	*Leuc. mesenteroides*, *Lb. fermentum*, *Sacch. cerevisiae*	rice and bengal gram
Idli	*Leuc. mesenteroides*, LAB, yeast	cereal, legume
Ilambazi lokubilisa	LAB	maize
Kecap	LAB	wheat, soybeans
Kenkey	*Lb. casei*, *Lb. lactis*, *Lb. plantarum*, *Lb. brevis*, *Lb. acidophilus*, *Lb. fermentum*, *Lb. casei*, yeast	maize
Kishk	LAB	cereal and milk
Kisra	*Lactobacillus* sp., *Lb. brevis*	sorghum
Koko	*Lb. fermentum*, *Lb. salivarius*	millet
Mahewu	*Lb. bulgaricus*, *Lb. brevis*	maize
Mawe	*Lb. fermentum*, *Lb. brevis*, *Lb. salivarius*, *Sacch. cerevisiae*	maize
Ngari	*Lactococcus lactis* subsp. *cremoris*, *Lactococcus plantanum*, *Enterococcus faecium*, *Lb. fructosus*, *Lb. amylophilus*, *Lb. coryniformis* subsp. *torquens* and *Lb. plantarum*	fish
Ogi	*Lb. plantarum*, *Lb. fermentum*, *Leuc. mesenteroides*, and *Sacch. cerevisiae*	maize
Saurkraut	*Leuc. mesenteroides*, *Lactococcus lactis*, LAB	cabbage
Som-fug	LAB	fish
Tarhana	*Streptococcus thermophilus*, *Lb. bulgaricus*, *Lb. plantarum*	parboiled wheat meal and yogurt
Tempeh	LAB, *Lb. plantarum*	soybean
Uji	LAB	maize, sorghum cassava, finger millet

been prevalently used as probiotics, exclusively in fermented dairy products [Tulumoglu et al. 2013].

Probiotic microorganisms are mostly of human or animal origin. The dairy industry, in particular, has found probiotic cultures. Yoghurts and fermented milks are the main vehicles for probiotic cultures [Trabelsi et al. 2013]. New products such as milk-based desserts, powdered milk for newborn infants, ice-creams, butter, mayonnaise, various types of cheese are also being introduced in the international market [Cruz et al. 2009]; however, some studies show that strains recognised as probiotics are also found in non-dairy fermented substrates [Martins et al. 2013]. Fermentation has been used to preserve, improve the

quality or modify the flavor of cereals, fruits, vegetables, legumes and meat. As fermentation process involves mixed cultures such as yeast, LAB and fungi, some of traditional fermented food show probiotic characteristics (Table 4), although the research of these matrices as raw material for probiotic microorganisms is still scarce compared with their dairy counterpart [Rivera-Espinoza and Gallardo-Navarro 2010]. Products made from fruits and vegetables, such as drinks, purées, fermented vegetables, table olives and minimally processed fruit have also been used. Moreover, products containing probiotic bacteria have an ever-expanding world market [Martins et al. 2013]. These products can be in the form of capsules or powders to be dissolved in cold drinks, and fermented foods of vegetable origin [Cruz et al. 2009].

HEALTH BENEFITS

Over the last 20 years there has been an increased interest in the role of probiotic bacteria in human health [Chandramouli et al. 2004]. Probiotics are defined as live microbial feed supplements [Okuro et al. 2013] which beneficially affect the host by improving the intestinal microbial balance [Kima et al. 2008]. Several health benefits are attributed to the ingestion of foods containing probiotic cultures. Some of them proved scientifically and others still requiring further studies in humans [Cruz et al. 2009].

Some of the major benefits of probiotics are: they reduce or eliminate ailments such as colon irritation, constipation and travellers diarrhoea [Rehaiem et al. 2014]. Other health benefits include inhibition of pathogenic bacteria, synthesis of B vitamins, lowering of blood ammonia levels, cholesterol absorption, inhibition of tumour formation [Capela et al. 2006], improving the absorption of calcium [Shi et al. 2013], and improvement of lactose utilization by producing b-galactosidase [Kima et al. 2008], when there are enough probiotics in colon. In order to provide health benefits, it is essential that there is a minimum of one million viable probiotic organisms per gram of a product [Capela et al. 2006], or 10^7 CFU·g^{-1} at point of delivery [Amine et al. 2014, Li et al. 2011, Semyonov et al. 2011], or be eaten in sufficient amounts to yield a daily intake of 10^8 CFU [Chávarri et al. 2010], although the numbers vary from strain to strain [Anekella and Orsat 2013].

Many studies have reported that probiotics are very sensitive to adverse environments, and survival rates of probiotics are poor in products. Due to their vast applications in food industries, it is urgent to develop new methods to preserve the viability of probiotics [Shi et al. 2013].

Multiple reports have shown their health benefits on gastrointestinal infections, antimicrobial activity, improvement in lactose metabolism, reduction in serum cholesterol, immune system stimulation, antimutagenic properties, anti-carcinogenic properties, anti-diarrheal properties, improvement in inflammatory bowel disease and suppression of Helicobacter pylori infection by addition of selected strains to food products [Pereira et al. 2011].

The health benefits cannot be predicted for a determined species of microorganisms, and there is no single probiotic strain capable of providing all the benefits mentioned [Cruz et al. 2009].

ENCAPSULATIONS

Encapsulation may be defined as a process of entrapping one substance (active agent) within another substance (wall material). The substance that is encapsulating is often called the coating, membrane, shell, capsule, carrier material, external phase, or matrix [Nedovic et al. 2011]. The encapsulation technology has been used by the food industry for several years. The number of food compounds has been increasing, for example: flavours, dyes, stabilizers, antioxidants, enzymes, probiotics, lipids, mineral salts and vitamins, among others [Estevinho et al. 2013].

Microencapsulation in which the cells are retained within an encapsulating matrix or membrane [Pimentel-González et al. 2009], has emerged as an alternative for protection of probiotics, providing a particular and convenient micro-environment for the encapsulated microorganism, enhancing their viability, and enabling controlled release of cells in the intestinal tract. Encapsulation technology has been proved to be one of the most effective ways to protect probiotics during processing and subsequent storage. Furthermore, encapsulation systems with control-released ability can deliver probiotics to a specific target and release them at required time [Shi et al. 2013]. The benefits of encapsulation protect probiotics against stress conditions.

Non-encapsulated probiotic microorganisms may be exposed to high temperatures, low pH, high osmotic pressure and high levels of oxygen during processing and storage of foods [Kailasapathy 2006]. The survival of probiotic organisms is also affected by acid in the stomach and the bile salts in the intestine tract [Capela et al. 2006]. These microcapsules may provide a more suitable anaerobic environment for the susceptible probiotic bacteria [Antunes et al. 2013, Nag et al. 2011], as well as protecting from bacteriophage and harsh enviroment [Heidebach et al. 2009, Jiménez-Colmenero 2013, Makinen et al. 2012], such as freezing and gastric conditions [Pimentel-González et al. 2009] thus reducing cell injury [Antunes et al. 2013].

The capsules should also be able to maintain their entirety through the gastrointestinal tract until they reach colon, where they should comminute and release the probiotic bacteria. Probiotics have been shown liquid based products such as dairy products, while the effect of encapsulation on probiotic survival in dry (low water activity) food products is lower. While it is known that proteins and fats/waxes are good oxygen and moisture barriers, respectively, it is presently not understood whether these compounds are adequate to protect probiotics against these physical factors in dry food matrices. Therefore, protein- and fat-based encapsulates of a commonly used probiotic strain were prepared and the survival of these encapsulates during exposure to different water activities and oxygen levels were compared [Weinbreck et al. 2010].

The functional performance of the microcapsules depends on the morphology, the chemical nature and the surface characteristics of the polymeric shell influenced by the process parameters [Butstraen and Salaün 2014]. A microcapsule consists of a semipermeable or non-permeable, spherical, thin and strong membrane surrounding a solid/liquid core, with a diameter varying from a few microns to 1 mm [Pimentel-González et al. 2009].

Encapsulation materials, Generally Recognized as Safe (GRAS) ingredients, can be used in food applications [Matalanis et al. 2011, Shi et al. 2013]. Natural polymer based materials are preferred due to their biodegradability, compatibility food grade nature and wide availability [Antunes et al. 2013]. Xanthan gum, gellan gum, starch derivatives, cellulose acetate phthalate, casein, whey proteins [Nualkaekula et al. 2012], chitosan, carboxymethyl cellulose, carrageenan, gelatin, pectin [Pimentel-González et al. 2009] vegetable gum, fats and alginate [Chávarri et al. 2010] can be used as encapsulation material. Alginate as an encapsulating agent is non-toxicity, simplicity in entrapping living microbial cells and low cost. Alginate is also an accepted food additive and can be safely used in foods. The use of alginate is limited due to its low stability in the presence of chelating agents and in acidic conditions below pH 2.0. The coating of alginate beads and its effectiveness in protecting probiotic bacteria has been extensively studied. Alginate microcapsules has been coasted with chitosan for improving activity [Chávarri et al. 2010]. The most commonly used gum is xanthan, a heteropolysaccharide consisting of polypentasaccharide groups formed from 2 glucose, 2 mannose and 1 glucuronic unit, with its polymer backbone consisting of 1→4 linked β-(D)-glucose units. Microcapsules were produced by the emulsion method in which the discrete water phase, containing xanthan gum was cross-linked with calcium chloride while suspended in oil. This property is advantageous in bacterial applications as the conditions are gentle enough not to damage the bacteria [Cook et al. 2012]. Casein, a milk protein, has been used in a few cases as a water insoluble (below pH 6) matrix for protecting bacteria during gastric transit. Milk proteins are particularly popular as encapsulating materials, while non-milk based materials are less desirable. Skimmed-milk has been utilised in microencapsulation by using rennet to gel the proteins contained in the milk [Cook et al. 2012].

Encapsulating probiotics within a physical barrier has been investigated by many researchers. Encapsulation is considered as a technology of incorporating protective materials into small capsules that can be released at the controlled rate under specific conditions [Chávarri et al. 2010, Shi et al. 2013]. The most used techniques to encapsulate probiotics are extrusion, atomisation or spray drying, emulsion, coacervation and immobilisation in fat and starch granules [Pedroso et al. 2012]. Many encapsulation technologies have been reported extrusion, emulsion method and spray drying technology. Extrusion method probably is the mildest one among all of probiotics encapsulation technologies. These method involves

dropping hydrocolloid solution with concentrated probiotics into solidifying solution [Shi et al. 2013]. In the emulsion method, a small volume of the cell-polymer suspension is added to a large volume of a vegetable oil such as soybean oil, sunflower oil, canola oil or corn oil. The mixture is homogenized to form a water-in-oil emulsion [Krasaekoopt et al. 2003]. Spray drying is a commonly used microencapsulation technique for probiotic encapsulation [Okuro et al. 2013]. This method involves atomization of an emulsion or a suspension of probiotics and encapsulating agents in a hot air drying chamber, resulting in rapid evaporation of water [Fritzen-Freire et al. 2013].

RECENT ENCAPSULATED DEVELOPMENTS

Microencapsulation is substantial for the survival of probiotics during storage and their transit through the digestive tract. Probiotics must be encapsulated because they are very sensitive to environmental conditions such as air, moisture, temperature, stomach pH and bile salt solutions. Addition of microcapsules should not affect the sensory properties of food products [Burgain et al. 2011].

L. bulgaricus was encapsulated in alginate-milk microspheres prepared by Shi et al. [2013]. The tolerance of encapsulated *L. bulgaricus* to adverse environments such as low pH (pH 2.0 and 2.5), high concentration of bile salt (1.0% and 2.0%) and long time storage (1 month), was investigated. This study showed that encapsulation could improve the tolerance of *L. bulgaricus* to adverse environments. However, alginate can provide limited protection to probiotics because alginate microspheres are not stable in acidic environment and have porous structure which allow diffusion of acid in and out of microspheres easily.

Trabelsi et al. [2013] studied to develop the microencapsulation of a *L. plantarum* TN8 on sodium alginate. The optimal conditions identified were 2% for sodium alginate, 10^{10} CFU·ml^{-1} for biomass, and 30 min for hardening time. When the survival rates of free and microencapsulated *L. plantarum* TN8 during exposure to artificial gastrointestinal conditions were compared, the encapsulated cells exhibited significantly higher resistances to artificial intestinal juice and artificial gastric juice.

Annan et al. [2008] produced to encapsulate the probiotic *B. adolescentis* 15703T with alginate-coated gelatin microspheres. The alginate coated gelatin microspheres in simulated gastric juice (pH 2.0, 2 h) survive higher numbers due to the buffering effect of intact microspheres. Heidebach et al. [2010] produced casein-based microcapsules for coating two probiotic strains, which differ in their sensitivity against dehydration, *L.* F19 and *B.* Bb12 during freeze-drying. *L.* F19 survived in significantly higher numbers in the encapsulated state, compared to free cells (protein-cell-mixture). Encapsulation improved the survival of *B.* Bb12 during storage for up to 90 days under all tested conditions.

Kim et al. [2008] microencapsulated *L. acidophilus* ATCC 43121 with sodium alginate. They studied about the effects of microencapsulation on the changes in survival rate of the *L. acidophilus* ATCC 43121 during exposure to artificial gastrointestinal and on the change in heat susceptibility of *L. acidophilus* ATCC 43121 during the heat treatment. In addition, cholesterol assimilation and intestinal adhesion of non-encapsulated and encapsulated *L. acidophilus* ATCC 43121 were also investigated to explore the effect of microencapsulation on health beneficial effect of lactic acid bacteria. Therefore, non-encapsulated cells were completely destroyed when exposed to artificial gastric juice (AGJ) of pH 1.2 and 1.5, while the treatment diminished the viable count of encapsulated samples only by 3 log. Encapsulated cells exhibited a significantly higher resistance to artificial intestinal juice (AIJ) and heat treatment than nonencapsulated samples. However, encapsulation did not significantly affect the adherence of *L. acidophilus* ATCC 43121 onto the human intestinal epithelial cell lines HT-29. The microencapsulation effectively protected the microorganisms from heat and acid treatment in delivering the viable cells to intestine without any significant adverse effect on their functionalities.

Chávarri et al. [2010] used chitosan as a coating material to improve encapsulation of *L. gasseri* (L) and *B. bifidum* (B) in calcium alginate beads and the prebiotic quercetin (Q) with the objective of enhancing survival of the probiotic bacteria was used. These results indicated that the survival of microspheres with quercetin during storage at 4°C was possible, while probiotic bacteria microencapsulated with

quercetin did not survive. Because of this, quercetin and *L. gasseri* or *B. bifidum* were microencapsulated separately. Microencapsulated *L. gasseri* and microencapsulated *B. bifidum* were resistant to simulated gastric conditions (pH 2.0, 2 h) and bile solution (3%, 2 h). Consequently, the microencapsulation of *L. gasseri* and *B. bifidum* with alginate and a chitosan offered effective media for the microencapsulation of *L. gasseri* and *B. bifidum* to the colon and maintaining their survival during simulated gastric and intestinal juice.

L. casei ATCC 393 was encapsulated with alginate, chitosan and carboxymethyl chitosan with extrusion method by Li et al. [2011]. Results indicated that alginate-chitosan-carboxymethyl chitosan microcapsules could successfully protect *L. casei* against negative conditions.

Probiotic organisms are typically added either to fresh foods with high water activities and an expected shelf-life of weeks (e.g. yogurt) or to dry products with low water activities (a_w < 0.25) and an expected shelf-life of months (e.g. infant formula). For dry products, losses in probiotic viability cause considerable reductions in product shelf-life times [Weinbreck et al. 2010].

Microencapsulated probiotic powder formulation provides a more convenient delivery format compared to wet gelled formulations. Fritzen-Freire et al. [2013] evaluate the viability and the physical properties of BB-12 microencapsulated by spray drying, as encapsulating agent, with the prebiotics inulin, oligofructose, and oligofructose-enriched inulin (at a ratio of 1:1, 200 g/L total concentrations). These microcapsules showed a high survival rate of bifidobacteria during storage at the temperatures evaluated.

Ying et al. [2013] were encapsulated *L. rhamnosus* GG (LGG) with spray drying. Then, capsules were added into apple juice or citrate buffer (pH 3.5) and stored at 4 or 25°C over a 5-week period. The LGG was encapsulated by using whey protein isolate (WPI) alone, WPI in combination with a physically-modified resistant starch (RS) at various ratios (4:1, 1:1 and 1:4), or RS alone. Spray dried microencapsulated LGG formulated with WPI or WPI-RS mixtures protected LGG in low pH environments (pH 3.5 apple juice and citrate buffer) over 5 weeks storage at 4°C and were more effective than at high temperature storage (25°C). Microencapsulated LGG formulations containing WPI or WPI in combination with RS provided better protection to LGG in apple juice and citrate buffer solution than the formulation containing only RS under all storage conditions.

Anekella and Orsat [2013] aimed to microencapsulate a combination of *L. acidophilus* NRRL B-4495 and *L. rhamnosus* NRRL B-442 in raspberry juice through spray drying. Role of maltodextrin as a carbon source was also assessed for its prebiotic potential. High temperatures during spray drying are harmful to probiotics and can be damaged by sub-lethal thermal shock. Increasing the microencapsulating material concentration increased the survival rate of the probiotics. Non-dairy probiotic foods are becoming popular as they do not pose problems of lactose intolerance.

Most of the products containing probiotic cells have been made to identify new food carriers (Table 5) [Burgain et al. 2011].

Probiotics represent a fast developing field. In past years, probiotics have expanded from the traditional health benefit areas of digestive comfort and immune protection to diverse applications in various, sometimes unexpected, health benefit areas. The use of microencapsulation to enhance the survival rates of probiotic microorganisms in connection with an application in food can be considered to be promising. These methods were optimized towards specific requirements. Many researchers have investigated strategies to improve the storage stability of encapsulated probiotics. Different foods containing encapsulated probiotic cells are present on the market. Belgium group Barry Callebaut produces chocolate containing encapsulated probiotic cells. In some cases, inulin or other prebiotics have been added to probiotics in the manufacturing of the bar called ‘Attune’, into yoghurt-covered raisins, nutrient bars, chocolate bars, or tablets. Unilever, Hansen, and company Dos Pinos, have developed a probiotic ice cream having multiple health benefits. Many products containing encapsulated probiotic cells are available in a tablet/capsule form or in a powder form. In conclusion, probiotic market has a strong future as the consumers demand is increasing.

Table 5. Examples of encapsulated probiotics and their applications in various food systems [Burgain et al. 2011]

Food	Probiotic strains	ME technology	Materials
Cream	*L. lactis*	extrusion	Ca-alginate
Mayonnaise	*B. bifidum* *B. infantis*	emulsification	alginate
Dry beverage	*Bifidobacterium* PL1	spray drying	starch
Banana	*L. acidophilus*	extrusion	k-Carregeenan
Soft foods	*B. lactis*	extrusion	gellan/xanthan gum
Tomato juice	*L. acidophilus*		Ca-alginate
Sausages	*L. reuteri*	extrusion emulsion	alginate
Sausages	*L. reuteri* *B. longum*	extrusion	alginate
Biscuits Cranberry and vegetable juices	*L. rhamnosus*	extrusion	whey protein
Oranges and apple juices	*L. rhamnosus* *L. salivarius* *B. longum* *L. plantarum* *L. acidophilus* *L. paracasei* *B. lactis*	emulsification	
Chocolate	*L. helveticus* *B. longum*	spray-coating	fatty acids
Swine feeding	LAB	extrusion	Ca-alginate
Tomato juice	*L. acidophilus*	extrusion	
Chocolate	*L. helveticus* *B. longum*	spray-coating	
Fresh cheese	*L. bulgaricus* *S. thermophillus*	extrusion	Ca-alginate
Cheddar	*B. bifidum*	emulsification	k-Carregeenan
Fresh	*L. lactis* spp. *lactis*	emulsification	k-Carregeenan
Crescenza	*B. bifidum* *B. infantis* *B. longum*	freze drying	Ca-alginate
Cheddar	*L. paracasei*	spray drying	skim milk
Cheddar	*L. acidophilus* *B. infantis*	emulsification	alginate/starch
Feta	*L. acidophilus* *B. lactis*		alginate
Kasar	*L. acidophilus* *B. bifidum*	extrusion and emulsification	alginate
White brined	*L. acidophilus* *B. lactis*	extrusion and emulsification	alginate
Yoghurt	*L. acidophilus* *B. longum*	spray-drying	maltodextrin/gum arabic
Yoghurt	*L. acidophilus*	extrusion	alginate-chitosan
Yoghurt	*L. casei*	extrusion	alginate/pectin

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