



## **Beneficial aspects of probiotics, strain selection criteria and microencapsulation using natural biopolymers to enhance gastric survival: A review**

Ali Ahmad Leghari<sup>1,2</sup>, Sana Shahid<sup>2</sup>, Muhammad Salman Farid<sup>2</sup>, Muhammad Saeed<sup>2</sup>, Hira Hameed<sup>2</sup>, Sehar Anwar<sup>2</sup>, Zahra Nishat<sup>2</sup>, Muhammad Kamran Shahid<sup>2</sup>, Asif Meraj<sup>3</sup>

<sup>1</sup> State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, China

<sup>2</sup> National Institute of Food Science & Technology, University of Agriculture, Faisalabad, Pakistan

<sup>3</sup> Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Pakistan

Email: [drmsaeed@uaf.edu.pk](mailto:drmsaeed@uaf.edu.pk)

**Abstract:** Probiotics have attained an inevitable status in human life which upon ingestion in appropriate quantity bestow numerous curative benefits. The plethora of services rendered via probiotics include immunomodulation, alleviating the risks of benign tumors as well as malignant carcinomas, treating gastric disturbances, combating pathogenic bacterial strains and mitigating hypertension. Prebiotics being indigestible carbohydrates selectively escalate the growth patterns of good bacteria. Non digestible dietary constituents in synbiotics intensify the persistence and survivability of probiotics and ameliorate the overall health and wellness of the host. Microencapsulation offers utter protection and astounding propensity to shielded probiotic for evasion of all the obstacles of harsh gastric milieu. Well established and documented practices of microencapsulation include emulsion, spray drying and extrusion. Current review summarizes all the corresponding aspects of probiotic, their encapsulation using various biopolymers, improved survival and integration with different food stuff to impart functional properties.

[Ali Ahmad Leghari, Sana Shahid, Muhammad Salman Farid, Muhammad Saeed, Hira Hameed, Sehar Anwar, Zahra Nishat, Muhammad Kamran Shahid, Asif Meraj. **Beneficial aspects of probiotics, strain selection criteria and microencapsulation using natural biopolymers to enhance gastric survival: A review.** *Life Sci J* 2021;18(1):30-47]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). <http://www.lifesciencesite.com>. 5. doi:[10.7537/marslsj180121.05](https://doi.org/10.7537/marslsj180121.05).

**Key words:** Probiotics, Prebiotics, Synbiotics, Microencapsulation, Lactobacillus, Bifidobacteria

### **1. Introduction to probiotics**

Probiotic is a term essentially constituted by an amalgamation of two words of strikingly diverse origins. The former part “pro” is basically a preposition of Latin origin which means before nevertheless, the later part “biotic” belongs to Greek origin which is synonymous to “life”. Probiotics have attained an inevitable status in human life as being immensely beneficial and advantageous microbes that upon ingestion in appropriate quantity bestow numerous curative benefits. Their integration with food articles has been greatly established and significantly documented. A food commodity to be designated as a “probiotic food product” must possess these viable bacteria in  $10^8$  colony forming units to grant their ample benefits (Singh *et al.*, 2017). To sum up, plethora of services rendered via probiotics include immunomodulation, alleviating the risks of benign tumors as well as malignant carcinomas, treating gastric disturbances, combating pathogenic bacterial strains and mitigating hypertension. Probiotics demonstrate a competitive rivalry to the pathogenic inhabitants of the same ambiance and tend to exceed

them in colonization as well as obssessing the nutrient reservoirs. Human body constitutes a mutualistic association with these exceptional cordial colonizers and offers them nourishment accompanied by shelter and in return enjoys surplus health welfares. Although, heaps of bacterial species have been categorized as probiotics, most have their place in two important genera namely *Lactobacillus* and *Bifidobacterium* (Arbolea *et al.*, 2011). *Saccharomyces boulardii* with its conspicuous tendency to cope with harsh gastrointestinal conditions is the only yeast-based probiotic (McFarland, 2010).

### **2. Historical perspective**

The history of inadvertent manipulation of probiotics stretches its roots deep into the span well before the detection of microscopic creatures. Kollath and Stillwell in the mid of 20<sup>th</sup> century firstly introduced the familiar term “probiotic”. However, commencement of probiotic’s journey was surely well before this declaration when neither the microbial fermentation was on the scene nor the salubrious health claims conferred by these cordial entities were recognized (Iannitti and Palmeiri, 2010). Left over

imprints of Roman and Egyptian empires 2000 years before Christ, indicate the commercialization of numerous fermented products of dairy origin especially butter and cheese. Back in ancient times, Hindus were also known to be the keen consumers of fermented beverages on account of their healthful and wholesome aspects (Gogineni *et al.*, 2013). Evidence illustrate that, fermentation had been a common practice among the dwellers of Indus valley. Nonetheless, proper manifestation of fermentation expertise was recorded in the residents of Tigris-Euphrates river banks. Moreover, Tibet nomads also carried out preservation of yak milk employing the essentially identical fermentative adroitness (McFarland, 2015).

Chinese masons and subordinates used to consume vegetables in fermented form during the buildup of prestigious Wall of China. Romans and Egyptians documented the remedial properties of fermented sauerkraut in their ancient texts. Romans were the trend setters to manipulate yeasts isolated from liquors to ameliorate and valorize the textural, nutritional and organoleptic characteristics of fermented dough. Pictorial descriptions procured from archeological zones of antique European civilizations also accentuate the meaningful utilization of fermented cereal products. Their firm religious belief was “a supernatural drive is the responsible factor to lighten their breads with divine pores” Egyptians adroitly used yeast cultures for leavening. Greek food handlers were proficient in purifying and inoculating starter cultures for the fermentation of grapey beverages. Though the miscellaneous health benefits of these fermented edibles were perceived to a great extent and vastly documented, the genuine motive of these sundry advantages was still dormant and remained the same till the establishment of microbiology discipline. With this advent a meaningful association among human health, these salubrious benefits and cordial bacterial species was acquainted (Mackowiak, 2013).

A famous microbiologist Leeuwenhoek identified unicellular yeast in a liquor with the help of his hand-made combination of magnifying lenses (earliest simple microscope). Louis Pasteur also identified a handful of these fermentative agents however he could not unfold the nexus between healthy aspects and these valorized foods. *Lactobacillus acidophilus* was the very first exceptional probiotic species to be discovered by researchers back in late 19<sup>th</sup> century. In 1899, Henry Tissler witnessed a conspicuous discrepancy between persistence of some y-shaped bacterial rods in the stools of exclusively breastfed infants and those with severe diarrhea. Henry entitled these isolates as “*Bifidobacteria*” (Gogineni *et al.*, 2013). Elie

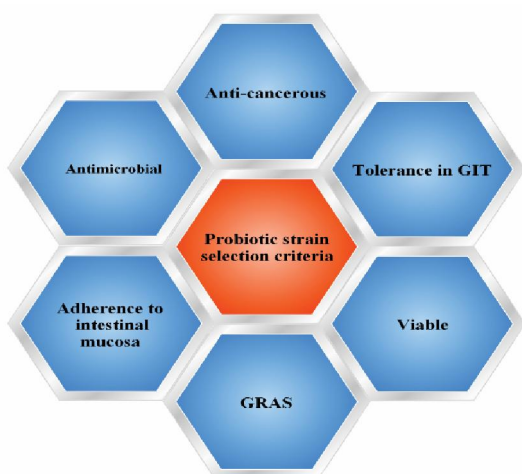
Metchnikoff verified that inhibition of pathogenic colonic clostridia was possible with the competitive potential of lactate producing bacteria detained in sour milk (McFarland, 2015). Furthermore, Metchnikoff experienced that cultures for curdling of milk into yoghurt have remedial properties for curing the patients of dementia in Bulgaria. He concluded that these cultures are the responsive creatures for bestowing these anti-aging effects. Owing to his own abode and culture identification in Bulgaria, the name *Lactobacillus bulgaricus* was dedicated to this cordial strain (Siezen and Wilson, 2010).

Seven years later after the commencement of 20<sup>th</sup> century, a German scientist was bestowed with highest scientific award Noble Prize in honor of his services for probing the mechanism of enzymatic fermentation produced by the metabolic activities in yeast cells. Henri Boulard was the first microbiologist to isolate and purify yeast cells with comprehensive probiotic potential. Similar yeast *S. boulardi* (named after his isolator) is commercially being exploited on large scale. In 1920, the consumption of *L. acidophilus* was found effective in treating constipation. Euler and Harden were also esteemed with Noble Prize for proving the mechanism of fermentation carried out by the cellular activities of yeast (Gogineni *et al.*, 2013). With the passage of time, multitudes of probiotic strains were delved and identified and indispensable reforms were made to redefine the term “probiotic” (Ezema, 2013). In 1935 a microbiologist from Japan formulated the first ever and popular dairy based probiotic drink “Yakult” utilizing *L. caesi*. In 1964, some scientists suggested that all the organic and inorganic supplements including bacteria that serve to rebalance the intestinal micro-biota should be labelled under the title of probiotics (Amara and Shibl, 2015). Current well-known definition of probiotics has been jointly put forward by World health organization (WHO) and Food and Agriculture organization (FAO).

### 3. Specifications for selection

Probiotics are continually being adjoined with various edibles to impart functional characteristics. These food items include a variety of cereal based products, neonate formula milks, some sort of cheeses, ice-creams, fruit beverages (juices, nectars, drinks) and various categories of yoghurts (Filannino *et al.*, 2013; Prasanna *et al.*, 2014). Fortification of aforementioned common foods with these gastro-friendly bacteria amends them into nutraceuticals. Imparted probiotics should be carefully picked up keeping in account the bactericidal, anti-carcinogenic and safety prospects. The probiotics strain must not be posing any toxic and allergenic effect as well as pathogenicity. On top of that, the designated strain ought to manifest clinging behavior towards gastric mucosa and easiness towards in-vivo gastrointestinal

milieu. Another important aspect to prioritize is the sensorial attributes of host foods should not be lugubriously transformed upon storage. Upon melange, the product should retain identical overall acceptability as fresh produce and probiotics must retain the tendency to cope with unfavorable ambience encountered during storage and processing (Parker *et al.*, 2018). Few significant specifications are thoroughly elaborated below.



**Figure 1:** Specifications for selection of probiotic strains for integration with food products

### 3.1. GRAS status

Probiotic strains which are intended to be integrated with ordained food article should essentially hold the GRAS (generally recognized as safe) status. It means that employed probiotics strain must not be posing any toxic and allergenic effect as well as pathogenicity. GRAS ingredients are extensively utilized as food additives and blended in conventional edibles at large scale throughout the globe. FDA emphasizes on consideration and fulfilment of various safety speculations to ensure the safety of consumers (Parker *et al.*, 2018).

### 3.2. Viability of incorporated strain

Viability and survivability together are allocated a supreme significance in rallying the specification for the selection of probiotic strain. Consequently, the species administered in food commodities ought to be robust to an extent at which it can withstand the harsh ambience of GIT until delivery to the targeted site i.e. colon where it functions to its full capacity and put forwards therapeutic and remedial benefits (Oliveira and Molero, 2016).

### 3.3. Tolerance in harsh GIT milieu

The ultimate objective of blending traditional foods with these marvelous bacteria is to convey them safely to the lateral part of digestive tract where the dominant percentage of digestion is being executed.

During their voyage in posterior part of GIT probiotics are greatly exposed to severe acidity and extremely low pH ranging from 1.5-2.0. These undesirable circumstances eventually influence the vigor, vitality and remedial potential of these salubrious entities. pH tends to fluctuates as the GIT progresses towards anus. Esophagus retains 7.0 pH however there is considerable variation of pH in proximal (6.2-7.4 approximately) and distal (6.7-7.9 approximately) parts of small intestine. Considering all these aforementioned pH levels, probiotic should possess enough tolerance to manifest stability in all these ambiances (Gandhi and Shah, 2015). Additional to these disturbances, bile owing the annihilating propensity and deteriorative enzymes also pose substantial hindrance. Apart from these aspects that majorly influence the probiotic survivability, some other factor having the tendency to effect vitality and vigor are the nature of additives blended in food products, the behavior of encapsulation polysaccharides and more importantly storage temperatures (Ying *et al.*, 2016).

### 3.4. Anti-carcinogenicity

Two substantively domineering qualifications for determining a probiotic strain are anti-cancerous and anti-mutagenic aspects which are cautiously considered while administrating them in commercial food commodities. Studies unveil the anti-proliferative trend of probiotics against malefic bacteria producing an enzyme azo-reductase (Orgil *et al.*, 2016). Another study reported the destruction of a proximal carcinogen due to Beta-glucosidase enzyme which is produced by the anti-proliferative activities of probiotics (Mahony *et al.*, 2001). Probiotics significantly balance the natural phenomenon of apoptosis in normal cells and aggravate it in carcinomas prompting nitrosamine and nitroreductase. Proto-corporation of two probiotic species *B. longum* and *L. acidophilus* in commercial plain yoghurts has been found to mitigate the probabilities of carcinogenic proliferation and significant reduction in genotoxicity (Prasanna *et al.*, 2014).

### 3.5. Antimicrobial potential

Microbicidal properties are of supreme significance to consider while deciding the appropriate probiotic to be integrated with food products. Almost all fellows of the genus *Bifidobacteria* synthesize and release bacteriocins that overwhelm the pathogenic rivals. Organic acids produced by the members of *Lactobacillus* genus percolate the cellular walls of pathogenic microorganisms and eventually abolish them. These acids upon infiltration split and decline the internal pH ultimately lysing the pathogenic cells. Additionally, some probiotics have been reported to produce microbicidal enzymes for instance, lysozyme along with anti-pathogenic peptides known as

defensins. Defensins are familiar for their great catastrophic features on growth of ominous pathogens (Eshaghi *et al.*, 2017). Gram +ve bacterial pathogens are explicitly targeted by bifidocin-B which is synthesized and released by a strain of *B. bifidum* in particular. Nisin, natamycin and lactacin are some other distinguished bacteriocins. Lactobacilli are also involved in production of some anti-mycotics for instance, methylhydantoin which suppress the growth and survival of *F. graminearum* (Hassan *et al.*, 2012).

### 3.6. Adherence to intestinal mucosa

Habituation of probiotic to the mucosa of intestine is requisite for bequeathing the anticipated health welfares that are responsible for overall wellness of health in human beings. Therefore, this colonization is deliberated as an imperative specification for qualification of incorporation within food supplies. Here an obvious antagonism is exhibited by probiotics and rivalry pathogens both contesting for dwelling and nourishment resources. As probiotics overwhelm and subjugate the rivals consuming the recourses earlier, supplies are limited and resultant obliteration of pathogens is commenced. Bifidobacteria and Lactobacilli possess particular surface proteins known as mucins that assist in adherence and occupation of enterocytes. Mucins are the pertinacious secretions submerged in cellular walls of these cordial bacteria (Sanchez *et al.*, 2010; Ossowski *et al.*, 2011).

### 4. Mode of action

Straightforward categorization split the mode of action of probiotics into three basic types. In first category both the indigenous and acquired immunity of host is modulated by the immune-modulating propensity of probiotics. This mechanism of actions plays a pivot role in establishing the functional capacities of probiotics in frightening the maladies of human digestive tract. Second mechanism of action involves the direct influence i.e. combat of probiotics with pathogenic rivals. Aforesaid mode represents a discernible role of probiotics in equilibration of gut micro-biota accompanying the exceptional remedial advantages. The last mode of this basic categorization deals with inactivation and detoxification of harmful components in food, precarious bacterial metabolites and products of digestion synthesized by host (Oelshlaeger, 2010). A comprehensive elucidation all three approaches is stated below.

#### 4.1. Immune-modulation

Products synthesized by the metabolic activities of probiotics have strong nexus in modulation of immune responses of the host. Additional to these metabolites, some cellular components of their exterior walls and nucleic particles are also involved in ameliorating these defensive mechanisms. Gut epithelia accommodate to immune cells and prime

clinging sites for these beneficial bacteria are the epithelial layering of gut cells where they adhere. Various in-vitro experimentations have reported absolute adherence of probiotics to simulated mucosa. Famous anti-inflammatory prospects of *L. plantarum* are directly attributed to teichoic acid present in its cellular wall. Probiotics uphold and rejuvenate intestinal mucosa when *E. coli* incursion in host cells is originated. A probiotic strain *L. rhamnosus* GG, invigorate the growth of intestinal cells by endowment of a specific healing protein. Studies report the production of butyric acid by some bacterial species of probiotic potential i.e. *Clostridium* and *Eubacterium* genus, which consolidates the intestinal epithelial layers (Oelshlaeger, 2010).

#### 4.2. Direct influence on competing microbes

##### 4.2.1. Production of antimicrobial substances

Bacteriocins produced by the cellular activities of probiotics counter the influence of pathogenic rivals in various ways. The genus *Lactobacillus* is engaged in the production of less molecular weight microbicidal peptides e.g. SCFAs which through various in-vitro trials have strongly claimed their inhibitory aspects on replication cycles of pathogenic opponents. Some probiotics have been reported to evident inclusive involvement in synthesis of antibiotics beside the production of low molecular weight SCFAs. *L. reuteri* was named after an antibiotic produced by it entitled "reuterin" which is chemically a hydroxy aldehyde. It has annihilating effects on broad spectrum of bacteria, algae and fungi (Cleusix *et al.*, 2007).

##### 4.2.2. Contest for scarce nutrients

Probiotics exist in antagonism with menacing pathogens to procure the scarce nourishment and dwelling supplies. Plenty of minerals and fibers are classified under these limiting supplies. One such example is the trace mineral iron which is indispensable for all bacterial entities. Probiotics belonging to the genus *Lactobacillus* have clear cut edge on other bacterial species as iron do not fall in their natural requirements. However, when iron which is critical to evolve the replication cycles of pathogenic bacteria is consumed by probiotics, pathogenic growth is severely influenced (Elli *et al.*, 2000).

##### 4.2.3. Blockage of adhesion sites for pathogens

Besides contesting for nourishment, a rivalry also exists to take the possession of common occupation sites of the epithelial mucosa. Both are in the quest of similar receptors for attachment so that they can execute their corresponding activities to their extreme capacity. Probiotics excel this occupation and make these receptors unavailable to the competitive pathogens. However, this ability of adherence is largely dependent on the severity of infectious competitor and the tendency of probiotic itself.



Lactobacilli usually synthesize adhesion proteins which assist them to accomplish the aforementioned competitive exclusion (Roos and Jonsson, 2002). Probiotics also possess some modes of anti-adhesion to exclude the pathogens for instance, via disintegration proteins acting on the competitor's receptor, by developing some sorts of biofilms or by inducing specific bio-surfactants.

#### 4.2.4. Anti-invasive impacts

Bacteria induce pathogenicity by invading the cells of mucosal epithelium. Scientists are continuously striving to probe the bacterial species of probiotic potential exclusively focusing on anti-invasion aspects. Hess *et al.* (2004) determined a novel method of quantification for invasiveness by these ominous pathogens. They demonstrated that Gentamycin particularly decimates extra-cellular bacteria thus effortlessly differentiates extra and intra cellular bacterial forms. Probiotics exclusively annihilate intra-cellular pathogens for instance, *Bifidobacterium bifidum* tends to impede the intrusion of *S. typhimurium* in host epithelial mucosa via secretion of particular anti-invasive factors (Botes *et al.*, 2008).

#### 4.3. Suppression of pathogenic toxins

Synthesis and release of precarious toxin is one of the vital intimidating factors of pathogenic strains of bacteria. Diarrhea is listed as one of the various potential implications due to these perilous toxins. Efficacy of probiotics to inhibit the expression of these notorious toxins is basically the remedial factor responsible for protecting the host against chronic diarrhea. *B. breve* has been reported to suppress the release of shiga toxins produced by *E. coli* (STEC) strain. A murine and in-vitro experimentation was conducted to assess the efficacy of *B. breve*, *B. pseudocatenulactum* and *B. longum*. Outcomes of the trial indicated that about 90% of the samples expired when exposed to STEC toxin however results were strikingly unique with *B. breve* treated samples which showed a great degree of persistence. The suppression of shiga toxin was attributed to the production and release of anti-toxin acetic acid by Yakut strain of *B. breve* (Asahara *et al.*, 2004). A similar suppression of a toxin produced by pathogenic bacterium *C. difficile* has been reported by a yeast (*S. boulardi*) with established probiotic potential (Oelshlaeger, 2010).

#### 5. Distinguished genera

Myriads of microbes from bacterial and viral origin inhabit and replicate inside human bodies. Contrary to other occupants of bacterial origin, *Bifidobacterium*, *Streptococcus* and *Lactobacillus* genera claim a sheer majority and mentioned in Table 1. Human body constitutes a mutualistic association with these exceptional colonizers and offers them nourishment accompanied by shelter and in return

enjoys surplus health welfares (Iannitti and Palmeiri, 2010; Chua *et al.*, 2017). Although heaps of bacterial species have been categorized as probiotics, most have their place in two important genera namely *Lactobacillus* and *Bifidobacterium*. Utmost significant bacterial species with established probiotic propensity belonging to *Lactobacillus* genus include *L. rhamnosus*, *L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *L. gasseri*, *L. caesi* and *L. salivarius*. While those belonging to *Bifidobacterium* genus include *B. breve*, *B. infantis*, *B. longum*, *B. bifidum*, *B. animalis*, *B. lactis* and *B. adolescentis*. *Saccharomyces boulardii* with its conspicuous tendency to cope with harsh gastrointestinal conditions is the only yeast-based probiotic (Arboleya *et al.*, 2011; Illanes *et al.*, 2016). Renowned multinational food industries such as Heinz and Nestle often manipulate *L. reuteri* DSM 17938 and *L. acidophilus* NCFM strains to develop a range of probiotic food products (Nieuwboer *et al.*, 2014).

#### 5.1. Lactobacilli

Genus *Lactobacillus* is comprised of a diverse group of 180 gram positive bacterial species ranging from shorter to lengthier rods however some may also possess round morphologies. All lactobacilli exhibit no effervescence or bubble formation with hydrogen peroxide which categorize them as catalase negative. They are microaerophilic (requiring little oxygen for their survival & multiplication) and non-sporulating. They profoundly inhabit mucosal sites of human body i.e. interior of mouth, inside lining of female reproductive tract and GIT (Arboleya *et al.*, 2011). Their deployment is indispensable in dairy sector where they are the indigenous ingredients of various fermented products e.g. all kinds of yoghurts and some sorts of cheeses. Pickling of fruits and vegetables is unable to proceed without their administration at some stage of processing. The key produce of their fermentation activity is lactic acid hence they are also entitled as lactic acid bacteria. Utmost significant bacterial species with established probiotic propensity belonging to *Lactobacillus* genus include *L. rhamnosus*, *L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *L. gasseri*, *L. caesi* and *L. salivarius* (Illanes *et al.*, 2016).

#### 5.2. Propionibacteria

Genus propionibacterium is comprised of non-motile, non-sporulating, gram positive aerotolerants. Their morphological characteristics specify them as pleomorphic or cocci clustered to form straighter chains. They are specialized for the production of Vitamin B-12 and propionic acid. They precisely stretch to the selection scale designed for bacteria to qualify as probiotics owing to their conspicuous tendency to cope with harsh gastrointestinal surroundings and the severe ambiance encountered

during storage (Rabah *et al.*, 2017). Various species of this genus hold an extensive and established historical perspective of safe integration with food commodities and also GRAS status. Their deployment for the development of Swiss cheese as starter culture is being practiced for decades (Cousin *et al.*, 2010). *P. freudenreichii* is manipulated to generate eyes on the exterior surface of Swiss cheese which are basically created due to the bubbling of carbon dioxide. This eyes formation is considered as an important sensorial parameter to enchant the consumers. Aforesaid strain as being anti-inflammatory also confers remedial benefits during the intrusion of notorious pathogen *H. pylori* (Myllyluoma *et al.*, 2008). Application of *P. thoenii* and *P. jensenii* is essentially popular in dairy sector to ameliorate the physicochemical and sensorial attributes of dairy products apart from bestowing a handful of remedial benefits especially anti-bacterial aspects.

### 5.3. Bifidobacteria

Bifidobacteria naturally inhabit colon and their multitude is a significant indicator of health and well-being. They exist as bifurcated rods resembling the English alphabet Y in exterior look. Pleomorphic, cudgel shaped or curled forms are also perceived during microscopic inspection of different species. A creamy whitish shade is reflected on observation of their colonial exteriors (Bertelsen *et al.*, 2016). They revitalize immune system, combat bacterial pathogens, avert constipation and cure irritable bowel syndrome. Additionally, evidences specify that they are highly effective in mitigating colorectal cancer biomarkers (Saulnier *et al.*, 2009). Significance of *Bifidobacteria* has been accredited in the recent past as prototypically vital probiotic genus and since then it is efficaciously being integrated to valorize several foodstuffs exclusively beverages and neonate formulations (Cronin *et al.*, 2011). The existence of this vital probiotic genus has recently been confirmed in human breast milk which blessed it with supreme prominence. Their characteristic contribution in establishing the indigenous immunity of infants owing to their inevitable role in genesis and gut micro-biome maturation further dedicates them an extreme distinction (Solis *et al.*, 2010). *Bifidobacteria* also aid in rebalancing the gut micro-biota which gets distressed in gut dysbiosis.

They may also counter the influence of pathogenic metabolites by releasing particular bacteriocins that have catastrophic consequences on pathogenic invaders of human being (Eshaghi *et al.*, 2017). *Bifidobacterium infantis* is extremely helpful in assuaging the pains of colon in neonates as well as moderating and alleviating inflammatory bowel disease (Simone *et al.*, 2014; Taipale *et al.*, 2011). Administration of Bifidobacteria significantly declines

the morbidity rate of enterocolitis up to 49% in premature infants. Likewise, the mortality is largely lessened up to 27% (Lau and Chamberlain, 2015). *B. lactis* owing to its stress resilience and immune-modulation aspects is utilized by probiotic food developers on a large scale (Braegger *et al.*, 2011). Synbiotic unifications of *Bifidobacterium breve* and *Bifidobacterium longum* with FOS offer defensive effects against inflammation (Sagar *et al.*, 2014). Shorter chained fatty acids are yielded from indigestible saccharides in correspondence to the fermentation activities of aforementioned probiotics which consequently revitalize and rejuvenate colonocytes (Cani *et al.*, 2009; Prasanna *et al.*, 2014). Various microbiological assays of conventional and contemporary cheeses e.g. Gouda and Cheddar have described the usage of bifidobacterial isolates in amalgamation with *Lactobacillus* strain. Bifidus milk and Bifidus yoghurt are prominent probiotic products developed initially in Japan.

### 6. Health claims

The discovery of probiotics fetched an infinite revolution in numerous arenas of biological science including the food science and technology (Yoon *et al.*, 2005). Their application is enormously prevalent in several overlapping sectors and unquestionably they have managed to achieve an irrevocable distinction in the lives of *Homo sapiens* via recurring conferment of numerous pragmatic advantages (Aron *et al.*, 2015). Most terrifically, they perform a dazzling part in rejuvenation of immune system and restoration of intestinal mucosa (Yoon *et al.*, 2006). The release of bactericidal factors for instance, defensins and bacteriocins have solid ruinous impacts on survivability of notorious micro invaders residing inside human body. Occupation of mutual bondage spots with pathogens by these cordial entities further remunerate the host contemporaneously imperiling the opponents. Additional Provision of excessive energy vittles extracted from catabolism of indigestible fibers also recompense the consumers (Chua *et al.*, 2017). They also synthesize some vital mineral metabolites e.g. cobalamin and alleviate the probabilities of various allergies (Aimmo *et al.*, 2012; Prasanna *et al.*, 2014). These indigenous colonizers are the occupants of substantial share of Gastro-intestinal tract where they function to their utmost extent (Zoumpopoulou *et al.*, 2017).

Their incorporation modifies conventional recipes into functional food formulations (Cruz *et al.*, 2013). Lactose intolerants are principally aided in lactose degradation via endowment of Beta galactosidase which is typically absent in them (Randheera *et al.*, 2013; Esmerino *et al.*, 2013). An upsurge in vegetarianism across the developed countries and religious obstacles to opt foods of

animal origin have drastically affected the market of dairy based probiotic products too. These actualities have compelled the development of cereal and fruit based probiotic products. In recent times, the application of probiotics is attaining reputation in cereal, and vegetable products (Nualkaekul *et al.*, 2013; Pakbin *et al.*, 2014). Researchers report that Bowl maladies are effectively eased by the therapeutic contributions of *B. bifidum* and *B. breve* in combination (Perricone *et al.*, 2014). *B. adolscensis* in

collaboration with *B. infantis* stimulates the manufacture of anti-bodies against particular viral invasion. Preventive effects of *B. animalis* against chronic diarrhea are well documented and physician around the world prescribe its routine utilization for neonates (Chua *et al.*, 2017; Freitas and Hill, 2017). Supplementation of probiotics in laxative medications can add additional curative features to overcome the complications of hardened stool (Tellez *et al.*, 2013; Perricone *et al.*, 2015).

**Table 1: Distinguished probiotic genera and their safe applications in different foodstuff**

Genus	Morphological characterization	Biochemical characterization	Important species	Applications	Reference
Lactobacillus	Gram +ve, rod shaped	Catalase -ve	<i>L. bulgaricus</i> <i>L. plantarum</i> <i>L. acidophilus</i> <i>L. rhamnosus</i>	Dairy products i.e. yoghurt and cheese. Pickles (fruits & vegetables)	Illanes <i>et al.</i> (2016), Arboleya <i>et al.</i> (2011)
Propionibacteria	Gram +ve, Pleomorphic or coccoid form	Catalase +ve	<i>P. freudenreichii</i> <i>P. thoenii</i> <i>P. jensenii</i>	Production of vitamin B-12 and propionic acid. <i>P. freudenreichii</i> is used as starter culture in Swiss cheese for eyes formation.	Rabah <i>et al.</i> (2017) Cousin <i>et al.</i> (2010)
Bifidobacteria	Gram +ve, Curled or cudgel shaped, look like Y alphabet	Catalase -ve	<i>B. infantis</i> <i>B. breve</i> <i>B. longum</i> <i>B. lactis</i>	Gouda and cheddar cheese in combination with lactobacillus species. Also, bifidus milk and bifidus yoghurt are produced.	Bertelsen <i>et al.</i> (2016) Prasanna <i>et al.</i> (2014)

Offspring of closely related parents tend to carry inherited allergies which perplexed the health care professionals around the globe. Regular consumption of LGG a strain of *Lactobacillus rhamnosus* has conferred anti-allergic impacts against infantile eczema (Savilahti, 2009). Antibiotic therapy in infants usually leaves them with Diarrhea which is a common babyhood sickness. Many probiotics have been administered to these baby patients on experimental basis out of which *L. rhamnosus* have been found most pertinent juxtaposed to others (Mantegazza *et al.*, 2018). Consumption of *L. reuteri* has been reported to lessen the crying intervals pertaining to colic aches in newborns (Savino *et al.*, 2010). Intestinal infections are impressively prevented by the incorporation of *L. fermentum* in baby food formulas. Likewise, its incorporation in pharmaceuticals also aids in the treatment of mastitis (Bertelsen *et al.*, 2016).

### 7. Safety concerns

Although lots of probiotic species have been bestowed with GRAS status, exact mechanism of immune system modulation by most of them is yet very much ambiguous. Deleterious aspects were not

traced out in controlled in-vivo trials involving many important probiotic strains however a complete acquaintance with the behavioral perspective of intended strain is indispensable to tackle the safety concerns (Prasanna *et al.*, 2014). Assessment of safety parameters for a typical probiotic strain is never a tranquil task. A probiotic strain ought to be affiliated with the genus customarily colonizing the human intestine. It is mandatory for a probiotic species to be free from risk factors pertaining to human health i.e. carcinogenicity, hemolysis and factors inducing tolerance to commercially available antibiotics (Bertelsen *et al.*, 2016). Authentication of health claims of probiotic is frequently assessed as per the directions and SOPs published by FAO and WHO concomitantly.

In the year 2002 FAO and WHO jointly directed specification to award probiotic status to a microbe (FAO/WHO, 2002). Crucial elements to be addressed while estimating the safety of probiotics are nonexistence of pathogenicity, probability of toxin discharge, probable platelet aggregation propensity, antibiotic tolerance induction factors and possible

mucus deteriorating factors (Iannitti and Palmeiri, 2010). Stability and survival in harsh gastric milieu are the vital attribute to be included in aforesaid selection criteria as well (Anadon *et al.*, 2016). A microbe with significant viability within the ambience offered by host's body is quite capable of inducing infection in individuals with malfunctioned immune defense. Often complaints of sepsis and mucous membrane degradation arise in immuno-compromised individuals (Floch, 2018). Apart from above mentioned concerns, FAO and WHO proclaim the administration of probiotics exceptionally secure (Boyle *et al.*, 2006).

#### 8. Commercially available probiotic foods

The quest for appetizing and functional foods is amassing with the technological advancement in nutritional studies. It is worth-noticing that a paramount portion of these essential products is overshadowed by the products having integrated probiotics (Tripathi and Giri, 2014). There are three specific ways of integration of probiotics with food commodities i.e. culture concentrates, fermentative agents and nutritional supplements. Enteric conveyance of these salubrious and cordial entities utilizing food articles (mostly beverages) as vehicles is

a widespread method. A gigantic percentage is detained by products of dairy origin in the market of functional foods (Cruz *et al.*, 2013). However, galactosemia, lactose intolerance, allergenic milk proteins and high cholesterol content are the notorious complications of dairy based probiotic products (Rouhi *et al.*, 2013). An upsurge in vegetarianism across the developed countries and religious obstacles to opt foods of animal origin have drastically affected the market of dairy based probiotic products too (Yoon *et al.*, 2005). These actualities have compelled the development of cereal and fruit based probiotic products (Prado *et al.*, 2015) e.g. cabbage drink, juice of beetroot, fermented coconut water, puddings developed from the mish mash of oat-bran, fermented oat and rice based beverages (Ghosh *et al.*, 2015; Salmeron *et al.*, 2015), soy based tofu (Santos *et al.*, 2014), cran berry, orange, pineapple, guava and pomegranate juices (Perricone *et al.*, 2014; Freire *et al.*, 2015), ginger drink (Bianchi *et al.*, 2014), and some sorts of chocolates with incorporation of encapsulated probiotics (Possemiers *et al.*, 2010).

#### 9. Prebiotics and their beneficial aspects

**Table 2. Established health benefits of different probiotic species and prebiotic components**

Category	Example	Health benefits	Reference
Probiotics	<i>Bifidobacterium lactis</i>	Absorption of vitamins and minerals in gut. Decomposition of body waste and tumor growth retardation.	Braegger <i>et al.</i> (2011)
	<i>Saccharomyces boulardii</i>	Treatment of digestive problems e.g. irritable bowel syndrome (IBS) & diarrhea. Relieves lactose intolerance and beneficial for high cholesterol patients.	Nieuwboer <i>et al.</i> (2014)
	<i>Lactobacillus acidophilus</i>	Kills germs as it creates an acidic environment. Boosts immune system.	Illanes <i>et al.</i> (2016)
	<i>Propionibacterium freudenreichii</i>	<i>P. freudenreichii</i> has an anti-inflammatory role during the invasion of <i>Helicobacter pylori</i> .	Myllyluoma <i>et al.</i> (2008)
	<i>Bifidobacterium infantis</i>	Relieves the pain of colon in neonates	Simone <i>et al.</i> (2014)
Prebiotics	Lactulose	Lacitol in combination with lactulose is used for treatment of liver malfunctioning.	Vandenplas <i>et al.</i> (2015).
	Inulin	Boosts calcium metabolism. Gall bladder stones are broken down by the presence of inulin and GOS.	Anadon <i>et al.</i> (2016)
	Galactooligosaccharide	Effective in reduction of bloating.	Olveira and Molero (2016)
	Pyrodextrin	Indigestible dextrin improves intestinal flora Increases stool and bowel movements.	Cronin <i>et al.</i> (2011)
	Xylooligosaccharides	Prevents constipation in pregnant ladies. Improves blood sugar level and laxation.	Florowska <i>et al.</i> (2016)

The term prebiotic was first conceptualized in 1995 by Roberfroid. Prebiotics being indigestible carbohydrates selectively escalate the growth patterns

of good bacteria. Though human gastrointestinal enzymes are incapable to digest these special dietary fibers, colon microbiota can effortlessly ferment them.



So, they simulate the function of fertilizers for probiotics. Energy extracted by the fermentation of these prebiotics is expended by probiotics to regularize their metabolic processing (Rasmussen and Hamaker, 2017). Probiotics are conspicuously selective and specific in their prebiotic fermentation profiles. One prebiotic escalates the growth patterns of only a group of corresponding probiotic species or a single probiotic species catabolizes only a relevant group of these special indigestible fibers. Consequently, the aforesaid advantageous properties are aggravated to an appreciable extent (Cronin *et al.*, 2011). Lactulose, inulin, fructooligosaccharides, galactooligosaccharides, xylooligosaccharides, pyrodextrins and carrageenan are some familiar prebiotics with established and well-documented potential of probiotic growth proliferation (Anadon *et al.*, 2016; Olveira and Molero, 2016). Evidences imply that prebiotics also assist to ameliorate and valorize the textural and organoleptic attributes of foods in which they are incorporated (Florowska *et al.*, 2016).

These indigestible saccharides are selectively catabolized to generate SCFAs which might include acetic acid, butyric acid and propionic acid in general. Additionally, some gases and corresponding metabolites are also generated owing to this catabolic mechanism. Aforesaid fatty acids basically serve to lessen pH levels which eventually ease the motion of bowl through and towards posterior part of the large intestine. Prebiotics also escalate the bioavailability of numerous essential minerals and annihilate various malefic bacterial strains. Host is profited via ample ways after these short chain fatty acids are absorbed by mucous membrane of intestine. Multitude of these SCFAs are imperious for cellular differentiation and regularization of lipogenesis hence desired by colonic organisms. In consort with amending the consistency of stool, it rises the frequency of bowl (Olveira and Molero, 2016). Bifidobacteria tend to thrive when present along with fructooligosaccharide (Chen *et al.*, 2017). During the treatment of liver malfunctioning lactic acid have been found assistant in combination with lactulose. Gall bladder stones are actively disintegrated and calcium metabolism is enhanced by inulin and galactooligosaccharide (Anadon *et al.*, 2016). GOS has also been extremely effective in reduction of bloating (Li *et al.*, 2014; Vandenplas *et al.*, 2015).

### 10. Synbiotics

Synbiotics are resulted owing to concomitant encapsulation of probiotics with prebiotics as encapsulation matrices. This shielding approach offers surplus defense to the core material (probiotic) and added protection against harsh GIT milieu (Ivanovska *et al.*, 2014). The amalgam of two Greek words constitute the term “synbiotic” whose essence is

‘together and life’. The idea was first conceptualize in 1995. In short, the term can be considerably elucidated as “the assortment of probiotics and prebiotics that productively advantage the consumer. Non digestible dietary constituents in synbiotics intensify the persistence and survivability of probiotics and ameliorate the overall health and wellness of the host (Kolida and Gibson, 2011; Xue *et al.*, 2017). Various synbiotic assimilations proclaim heaps of plenteous health benefits. For instance, FOS & GOS in combination with *Bifidobacteria* help to relieve constipation and curate the anguishes encountered by hardened feces (Illanes *et al.*, 2016). Two approaches to devise a symbiotic combination are commonly undertaken. One is familiar as “complementary” while other is termed as “synergistic”.

In former practice, probiotic strain is carefully chosen on account of desired remedial gains necessitated by the host. And an entirely independent selection is made for prebiotic constituent. Juxtaposed to aforementioned practice, synergistic approach demands a careful criteria for selection of prebiotic constituent as well *i.e.* selective escalation potential of probiotic’s strain of interest to impart salubrious remedial aspects to the host (Mugambi *et al.*, 2014). Patients with gastric dysfunction are prescribed synbiotics to arouse the progression of colonic microbes for instance, bifidobacteria (Patel *et al.*, 2014). Food engineers are undertaking unwavering struggles to explore numerous synbiotic combinations. One such exemplary development is “Synbiotic 2000” which is a mish mash of four probiotic species belonging to three distinguished genera *i.e.* *Pediococcus*, *Lactobacillus* and *Leuconostoc* each accompanying one of four commercially available prebiotics individually e.g. beta glucan, pectin, inulin and resistant starch (Magrone and Jirillo, 2013). The ingestion of synbiotic 2000 is associated with various healthier effects for instance, obvious decline in the probability of liver ailments, chronic pancreatitis, poly-trauma and irritable bowel syndrome (Bartosch *et al.*, 2005).

Accomplishment of customer’s demands to furnish value added food products in terms of high nutritional status and curative significance is a major challenge to food technologists. Synbiotics are conquering the zeniths of popularity gradually. *Aloe barbedensis* is a distinguished remedial plant. Taking into account the established curative and bioactive features, *Aloe vera* can serve as natural substrate for lactic acid bacterial fermentation as well as outstanding source of prebiotics. Hence a synbiotic drink developed from *Aloe vera* and *Lactobacillus* bacteria would represent an ideal functional beverage (Cuvas *et al.*, 2016).

### 11. Encapsulation

Microencapsulation is the entrapment of a microbe inside a shielding that allows protection and conservation of the isolate from severe gastric environment. Microcapsules are fabricated by two fundamental components i.e. core and coat. Core is the interior part that may be a sensitive phytonutrient, a bioactive ingredient or a probiotic. Core is enveloped by a coat which is chiefly of prebiotic origin. Bile as a result of its bactericidal characteristics annihilates integrity of probiotics via disrupting their cytoplasmic membrane. The principal objective is to aggravate as well as escalate the viability and target delivery of probiotic to colon while efficaciously surviving the gastric juice, intestinal fluids, and bile (Zhu, 2017). Encapsulation matrix preserves the sustainability of the cell and subsequently drops the probability of cellular disintegration. Likewise, it also safeguards the detained probiotic against phages, lethal factors and uncomplimentary ambiance encountered in stowage containers and GIT (Dogahé *et al.*, 2015; Martin *et al.*, 2015). This shielding stratagem catches ample usage in food and pharmaceutical sectors especially in case of bioactive components where the entire dimension of capsule ranges from few nanometers to few millimeters (Burgain *et al.*, 2011).

## 12. Encapsulation matrices

### 12.1. Alginate

Alginate is a biocompatible natural polysaccharide acquired from various kinds of algae and formulates hydrogels in abundance of alkali metal cations. The microbeads present extensive protection to the cored probiotic consequentially upholding its vitality and vigor (Cook *et al.*, 2012). Alginate catches preferences over other encapsulation matrices owing to its simplicity, non-toxic nature, and cost effectiveness (Mandal *et al.*, 2006; Trabelsi *et al.*, 2014).

### 12.2. Chitosan

Chitosan solely is incapable of providing anticipated defense to the probiotic hence typically manipulated for coating of microcapsules. Its combination with alginate has been employed by several researchers and conclusions of their trials signifies this mish mash as an idyllic stratagem for enteric conveyance of probiotics (Nualkaekul *et al.*, 2013). Juxtaposed to the cells in free form, chitosan coated beads offer superior protection and conserve the viability of bacterial cells (Chavarri *et al.*, 2010).

### 12.3. Resistant starch

When numerous glucose subunits adjoin by glycosidic linkage the giant structure fabricated is starch. Enzymes of human pancreas are incapable of dismantling this colossal edifice. Its fermentation only happens in certain portion of large intestine hence this matrix is regarded as an idyllic substrate for projected conveyance of probiotics. Owing to inclusive

adherence of probiotics to the resistant starch, it finds numerous applications in food sector (Mirzae *et al.*, 2012).

### 12.4. Milk proteins

Casein and whey are natural biocompatible carriers for projected conveyance of good bacteria owing to their outstanding gel founding features (Livney, 2010). These indigenous proteins of milk are extensively manipulated for microencapsulation of probiotic species. A study documented practically promising results for survivability (about 99%) when a strain of lactic acid bacteria *L. rhamnosus* GG was encapsulated using casein micelles in conjunction with whey (Burgain *et al.*, 2013). An analogous testing was conducted exploiting whey proteins to microencapsulate a bacterial species of probiotic origin. Persistence of probiotics was continued up to 3 hours and an apparent sustain in vitality was observed (Doherty *et al.*, 2011).

### 12.5. Carrageenan

Carrageenan is also a biopolymer with widespread applications in food industries and research sector. Although, the gel it develops has considerably less tolerance against stress yet the microcapsules conserve the vitality of probiotic to an appreciable extent (Burgain *et al.*, 2013). Carrageenan demonstrates exceptional binding with whey and casein hence these indigenous proteins of milk are broadly employed for amending the adhering attributes of carrageenan. When Carrageenan is moderately heated it transforms into solution form in which probiotic cells are introduced and the solution is again cooled to room temperature for gel formulation (Shi *et al.*, 2013).

### 12.6. Pectin

Pectin has natural occurrence in fruit peels and commonly employed in beverage industries as a thickening agent. Pectin tends to retain its intactness in harsh gastric ambiance of human body (Martin *et al.*, 2015). Various researchers have explored its potential as an encapsulation matrix for coating microbeads carrying probiotics and satisfactory outcomes have lifted its glory to a substantial status (Gebara *et al.*, 2013).

### 12.7. Gum Arabic

Gum Arabic is procured from the wood of *Acacia nelotica* and is regarded as a tremendous source of dietary fiber (Wang *et al.*, 2014). Its excellent miscibility with water and considerable tolerance in acidic milieu are the salient attributes which make it a good matrix for encapsulation purposes (Kuck and Norena, 2016). Even though merged in excess concentration with food articles, yet it preserves genuine organoleptic characteristics (Hadzeiva *et al.*, 2017). It is either employed solely or in amalgamation with inulin to impart exceptional

defensive facets to the cored probiotic (Fernandes *et al.*, 2014).

### 12.8. Other materials of prebiotic origin

Apart from aforementioned matrices, numerous other materials are also utilized experimentally and commercially for imparting intended safety to the probiotic being encapsulated. Out of these isolates of lentil and soy proteins are extensively operated (Karaca *et al.*, 2013; Khan *et al.*, 2013). Usage of isolates from pea proteins are also attaining considerable popularity in probiotication (Klemmer *et*

*al.*, 2011).

## 13. Encapsulation techniques

### 13.1. Spray drying

This cost effective stratagem of encapsulation is primarily exploited for shielding bioactive components however owing to thermo-sensitivity concerns of probiotics and other heat sensitive objects, this technique has voluminous hindrances. Firstly, dispersion of core is accomplished in emulsified encapsulation matrix followed by thorough homogenization and drying via.

**Table 3. Relative efficiency of different biopolymers as encapsulation matrices**

Biopolymer	Source	Efficiency as encapsulation matrix	GRAS status	Reference
Alginate	Brown seaweed	Strong or insoluble gel in presence of Ca <sup>+2</sup> ions. Provides extensive protection to entrapped active ingredient	Yes	Cook <i>et al.</i> (2012)
Chitosan	From chitin deacetylation	Coating of chitosan with alginate is an excellent mish mash for release of probiotics.	Yes	Chavarrri <i>et al.</i> (2010)
Resistant starch	Potato, rice, corn, wheat	Fermentation of colossal structure of starch occurs in large intestine for probiotics delivery.	Yes	Mirzae <i>et al.</i> (2012)
Carrageenan	Red seaweed	Capsules are formed at room temperature due to helical configuration resulting in solid stable films. The gel has less stability against stress.	Yes	Shi <i>et al.</i> (2013)
Pectin	Apple and citrus peel	Microbeads have excellent barrier properties against moisture and oxygen. Higher elastic modulus and low extensibility rate.	Yes	Martin <i>et al.</i> (2015)
Gum arabic	Acacia plant	Highly soluble in water and tolerant in acidic environment.	Yes	Kuck and Norena (2016)
Milk proteins	Milk	Better optical and mechanical properties but limited barrier effect against moisture.	Yes	Burgain <i>et al.</i> (2013)
Gelatin	Acidic hydrolysis of collagen	Microcapsules have excellent antimicrobial and antioxidant properties.	Yes	Phan <i>et al.</i> (2005)
Agar	Red algae	Makes gel at low concentration and viscosity of film matrix is less.	Yes	Hanani <i>et al.</i> (2014)
Xanthan gum	Bacterium <i>Xanthomonas campestris</i>	Act as an emulsifier preventing solution form separation. Used at an amount of 0.5-1.0% and inhibits enzymatic degradation.	Yes	Jain <i>et al.</i> (2016)

atomization (Martin *et al.*, 2015).

### 13.2. Extrusion

The most reasonable, reliable and gentle technique of encapsulation is extrusion. In this methodology, beads are acquired via extrusion of probiotic and encapsulation mixture through a needle in CaCl<sub>2</sub> solution (Cook *et al.*, 2012; Ying *et al.*, 2016). Microencapsulation executed via this manner offers utter protection and astounding propensity to shielded probiotic for evasion of all the obstacles of harsh gastric milieu (Musikasang *et al.*, 2009).

### 13.3. Emulsification

This scheme of encapsulation primarily circles around the nexus between two phases i.e. continuous

and discontinuous (Shima *et al.*, 2006). A solution is formulated by blending altered concentrations of probiotic culture and sodium alginate. This liquefied blend is decanted in vegetable oil already supplied with tween-80 which essentially serves to improve the process and offers creamy texture. After subsequent settling of beads to the bottom of beaker, the solution is centrifuged to harness the beads and oil layer is refined (Durante *et al.*, 2012).

## 14. Viability of encapsulated probiotics

*Bifidobacterium pseudocatenulatum* was encapsulated within alginate based gastroprotective microgels. The internal pH of capsulaes was

maintained owing to the presence of antacid agents. In vitro trials were conducted to monitor the stress response and cell viability of these microbeads on exposure to simulated GIT models. The absence of antacid was coupled with complete inability to detect any live probiotic cell after a certain holding span in simulated gastric environment. Contrariwise, the presence of antacid lead to a minimal loss of viability after an identical incubation span. When Mg (OH)<sub>2</sub> was alternated with CaCO<sub>3</sub> as antacid, considerable quantities of viable cells were still detected on equal incubation period. Overall, these findings significantly suggested that alginate in combination with Mg (OH)<sub>2</sub> can be efficiently manipulated to confer maximal integrity and safety to *Bifidobacterium pseudocatenulatum* during their voyage in human gut (Gu *et al.*, 2019). Two bacterial strains with astonishing probiotic potential namely *Enterococcus fecium* and *Staphylococcus succinus* were coencapsulated with corresponding prebiotics on alginate matrix to monitor the effects on survival rate and viability in simulated gastric prototypes. Outcomes demonstrated a significant rise in persistence of coencapsulated cells while exposure to low pH and bile. Survivability rates were also amazingly elevated ranging from 95.32 to 98.75% (Sathyabama and Vijayabharathi, 2014).

A synbiotic ice-cream was developed by exploiting microencapsulated *Lactobacillus acidophilus* as probiotic and fructooligosaccharide as accompanying prebiotic. Some physicochemical aspects of ice-cream including overrun and firmness were analyzed. Higher levels of FOS resulted in raised total solid contents and lower pH values. The viable counts were significantly reduced for free probiotics contrary to encapsulated ones which merely dropped to a negligible extent after 2 months of frozen storage (Ahmadi *et al.*, 2014). Fructooligosaccharide as coating material was employed to microencapsulate *Lactobacillus plantarum*. Study demonstrated that the major drawback of exploiting FOS as wall material is stickiness during spray drying owing to the little glass transition temperature of FOS. The supplementation of whey protein isolates in combination with denatured whey protein isolates mitigated this interruption to a great deal. Capsules of 1-1.5 core-to-coating ratio exhibited finest results in term of improved stability profile. FTIR confirmed the existence of FOS and whey proteins after spray drying in microcapsules (Rajam *et al.*, 2015). Carrageenan owns an innate inclination towards milk proteins. The blend of carrageenan, milk and locust bean gum was utilized to develop synbiotic microspheres that sheltered *L. bulgaricus* against punitive in vitro gastric simulations (Shi *et al.*, 2013).

*Bifidobacterium lactis* and *Lactobacillus*

*plantarum* were coencapsulated with inulin as complementary prebiotic in alginate-chitosan microcapsules by EHDA. Probiotic assay was conducted to evaluate the release profile from aforementioned encapsulation matrix. Shielding potential of polysaccharide was also investigated under simulated gastric models. Encapsulation yield was estimated to be 98, 79 and 99 percent for inulin, bacteria and resistant starch correspondingly. After 24 hours of incubation, merely 10% of inulin was excluded from aforementioned matrix with no release of resistant starch at all. While in case of chitosan, the release of inulin was slashed up to 5%. Viability losses were also profoundly reduced after 90 days of storage at room temperature for microcapsules containing inulin (Zaeim *et al.*, 2019). Duly coated microcapsules consisting of pectin and chitosan manifested enhanced protection to *B. longum* and *L. plantarum* in comparison to chitosan-alginate beads. Free cells were unable to survive after 4 weeks of storage while coencapsulated cells were still viable and exhibited better growth even after 6 week storage span. The fascinating amendment was directly attributed to dual shielding comprising of pectin and chitosan (Nualkaekul *et al.*, 2013).

#### Conclusion:

The discovery of probiotics fetched an infinite revolution in numerous arenas of biological sciences including the food science and technology. Their application is enormously prevalent in several overlapping sectors and unquestionably they have managed to achieve an irrevocable distinction in the lives of mankind via conferment of numerous pragmatic advantages. The ultimate objective of blending traditional foods with these marvelous bacteria is to convey them safely to the lateral part of digestive tract where they function to their maximal extent. Encapsulation matrix preserves the sustainability of the cell and subsequently drops the probability of cellular disintegration. Likewise, it also safeguards the detained probiotic against phages, lethal factors and uncomplimentary ambiance encountered in storage containers and GIT. There is a dire need for commercialization and large scale production of synbiotic products owing to their therapeutic and curative significance.

#### Authors contributions.

All the authors listed in this manuscript contributed and approved the manuscript.

#### Conflict of Interest

Authors listed in the manuscript have no contending interest.



**Corresponding Author:**

Dr. Muhammad Saeed

National Institute of Food Science &amp; Technology

University of Agriculture, Faisalabad

Email: [drmsaeed@uaf.edu.pk](mailto:drmsaeed@uaf.edu.pk)**References**

- Ahmadi, A., E. Milani, A. Madadlou, S.A. Mortazavi, R.R. Mokarram and D. Salarbashi. 2014. Synbiotic yogurt-ice cream produced via incorporation of microencapsulated *Lactobacillus acidophilus* and fructooligosaccharide. *Journal of Food Science and Technology*. 51: 1568-1574.
- Aimmo, M.R., P. Mattarelli, B. Biavati, N.G. Carlsson and T. Andlid. 2012. The potential of bifidobacteria as a source of natural folate. *Journal of Applied Microbiology*. 112: 975-984.
- Amara, A. and A. Shibl. 2015. Role of probiotics in health improvement, infection control and disease treatment and management. *Saudi Pharmaceutical Journal*. 23: 107-114.
- Anadon, A., M.M. Larranaga, I. Ares and M. Martinez. 2016. Prebiotics and probiotics: An assessment of their safety and health benefits. In: Watson, R.R. and V.R. Preedy (Ed.). *Probiotics, prebiotics, and synbiotics: Bioactive foods in health promotion*. Academic Press. Amsterdam, Netherlands. pp. 3-23.
- Arbolea, S., P.R. Madiedo, A. Margolles, G. Solis, S. Salminen, G. Clara and M. Gueimonde. 2011. Characterization and in vitro properties of potentially probiotic *Bifidobacterium* strains isolated from breast-milk. *International Journal of Food Microbiology*. 149: 28-36.
- Aron, N.M., M. Boev and G. Bahrim. 2015. Probiotics and therapeutic effect in clinical practice-Review. *Romanian Biotechnological Letters*. 20: 10162-10175.
- Asahara, T., K. Shimizu, K. Nomoto, T. Hamabata, A. Ozawa and Y. Takeda. 2004. Probiotic bifidobacteria protect mice from lethal infection with Shiga toxin-producing *Escherichia coli* O157: H7. *Infection and Immunity*. 72: 2240-2247.
- Bartosch, S., E.J. Woodmansey, J.C. Paterson, M.E. Mcmurdo and G.T. Macfarlane. 2005. Microbiological effects of consuming a synbiotic containing *Bifidobacterium bifidum*, *Bifidobacterium lactis*, and oligofructose in elderly persons, determined by real-time polymerase chain reaction and counting of viable bacteria. *Clinical Infectious Diseases*. 40: 28-37.
- Bertelsen, R.J., E.T. Jensen and T.R. Kulka. 2016. Use of probiotics and prebiotics in infant feeding. *Best Practice & Research Clinical Gastroenterology*. 30: 39-48.
- Bianchi, F., E. Rossi, R. Gomes and K. Sivieri. 2015. Potentially synbiotic fermented beverage with aqueous extracts of quinoa (*Chenopodium quinoa* Willd) and soy. *Food Science and Technology International*. 21: 403-415.
- Botes, M., B. Loos, C.A. Reenen and L.M. Dicks. 2008. Adhesion of the probiotic strains *Enterococcus mundtii* ST4SA and *Lactobacillus plantarum* 423 to Caco-2 cells under conditions simulating the intestinal tract, and in the presence of antibiotics and anti-inflammatory medicaments. *Archives of Microbiology*. 190: 573-584.
- Boyle, R.J., R.M.R. Browne and M.L. Tang. 2006. Probiotic use in clinical practice: What are the risks? *The American Journal of Clinical Nutrition*. 83: 1256-1264.
- Braegger, C., A. Chmielewska, T. Decsi, S. Kolacek, W. Mihatsch, L. Moreno, M. Piescik, J. Puntis, R. Shamir and H. Szajewska. 2011. Supplementation of infant formula with probiotics and/or prebiotics: A systematic review and comment by the ESPGHAN committee on nutrition. *Journal of Pediatric Gastroenterology and Nutrition*. 52: 238-250.
- Burgain, J., C. Gaiani, M. Linder and J. Scher. 2011. Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of Food Engineering*. 104: 467-483.
- Burgain, J., C. Gaini, G. Francius, A.M.R. Junelles, C.C. Grimal, S. Lebeer, H.L.P. Tytgat, J. Vanderleyden and J. Scher. 2013. In vitro intercation between probiotic bacteria and milk proteins probed by atomic free microscopy. *Colloides and Surfaces B: Biointerfaces*. 104: 153-162.
- Cani, P.D., S. Possemiers, T.V.D. Wiele, Y. Guiot, A. Everard, O. Rottier, L. Geurts, D. Naslain, A. Neyrinck and D.M. Lambert. 2009. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut*. 58: 1091-1103.
- Chavarri, M., I. Maranon, R. Ares, F.C. Ibanez, F. Marzo and M.D.C. Villaran. 2010. Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. *International Journal of Food Microbiology*. 142: 185-189.
- Chen, H., D. Ma, Y. Li, Y. Liu and Y. Wang. 2017. Effect of microencapsulation on survival and stability of *Bifidobacterium bifidum* BB01 Exposed to simulated gastrointestinal conditions

- and in different food matrices. *Acta Universitatis Cibiniensis. Series E: Food Technology.* 21: 23-34.
19. Chua, K.J., W.C. Kwok, N. Aggarwal, T. Sun and M.W. Chang. 2017. Designer probiotics for the prevention and treatment of human diseases. *Current Opinion in Chemical Biology.* 40: 8-16.
  20. Cleusix, V., C. Lacroix, S. Vollenweider, M. Duboux and G.L. Blay. 2007. Glycerol induces reuterin production and decreases *Escherichia coli* population in an in-vitro model of colonic fermentation with immobilized human feces. *FEMS Microbiology Ecology.* 63: 56-64.
  21. Cook, M.T., G. Tzortzis, D. Charalampopoulos and V.V. Khutoryanskiy. 2012. Microencapsulation of probiotics for gastrointestinal delivery. *Journal of Controlled Release.* 162: 56-67.
  22. Cousin, F.J., D.D. Mater, B. Foligne and G. Jan. 2011. Dairy propionibacteria as human probiotics: A review of recent evidence. *Dairy Science & Technology.* 91: 1-26.
  23. Cronin, M., M. Ventura, G.F. Fitzgerald and D.V. Sinderen. 2011. Progress in genomics, metabolism and biotechnology of bifidobacteria. *International Journal of Food Microbiology.* 149: 4-18.
  24. Cruz, A.G.D., W. Castro, J.A.F. Faria, H.M.A. Bolini, R.M.D.S. Celeghini, R. Raices, C.F.D. Oliveira, M. Freitas, C.C. Junior and E. Marsico. 2013. Stability of probiotic yogurt added with glucose oxidase in plastic materials with different permeability oxygen rates during the refrigerated storage. *Food Research International.* 51: 723-728.
  25. Cuvas, L.R., M.S. Julio, C.E.J. Carlos, C.H. Mario, S.I. Mussatto and B.C. Ruth. 2016. *Aloe vera* and probiotics: A new alternative to synbiotic functional foods. *Annual Research & Reviews in Biology.* 9: 11-15.
  26. Dogahe, M.K., K.K. Darani, A. Tofighi, M. Dadgar and A.M. Mortazavian. 2015. Effect of process variables on survival of bacteria in probiotics enriched pomegranate juice. *British Biotechnology Journal.* 5: 37-50.
  27. Doherty, S., V. Gee, R. Ross, C. Stanton, G. Fitzgerald and A. Brodkorb. 2011. Development and characterisation of whey protein micro-beads as potential matrices for probiotic protection. *Food Hydrocolloids.* 25: 1604-1617.
  28. Durante, M., M.S. Lenucci, B. Laddomada, G. Mita and S. Caretto. 2012. Effects of sodium alginate bead encapsulation on the storage stability of durum wheat (*Triticum durum* Desf.) bran oil extracted by supercritical CO<sub>2</sub>. *Journal of Agricultural and Food chemistry.* 60: 10689-10695.
  29. Elli, M., R. Zink, A. Rytz, R. Reniero and L. Morelli. 2000. Iron requirement of *Lactobacillus* spp. in completely chemically defined growth media. *Journal of Applied Microbiology.* 88: 695-703.
  30. Eshaghi, M., M.H. Bibalan, M. Rohani, M. Esghaei, M. Douraghi, M. Talebi and M.R. Pourshafie. 2017. *Bifidobacterium* obtained from mother's milk and their infant stool; A comparative genotyping and antibacterial analysis. *Microbial Pathogenesis.* 111: 94-98.
  31. Esmerino, E., A. Cruz, E. Pereira, J. Rodrigues, J. Faria and H. Bolini. 2013. The influence of sweeteners in probiotic Petit Suisse cheese in concentrations equivalent to that of sucrose. *Journal of Dairy Science.* 96: 5512-5521.
  32. Ezema, C. 2013. Probiotics in animal production: A review. *Journal of Veterinary Medicine and Animal Health.* 5: 308-316.
  33. FAO/WHO, 2002. Guidelines for the Evaluation of Probiotics in Food. Food and Agriculture Organization of the United Nations and World Health Organization. Working Group Report.
  34. Fernandes, R.V.B., S.V. Borges and D.A. Botrel. 2014. Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. *Carbohydrate Polymers.* 101: 524-532.
  35. Filannino, P., L. Azzi, I. Cavoski, O. Vincentini, C.G. Rizzello, M. Gobbetti and R.D. Cagno. 2013. Exploitation of the health-promoting and sensory properties of organic pomegranate (*Punica granatum* L.) juice through lactic acid fermentation. *International Journal of Food Microbiology.* 163: 184-192.
  36. Floch, M.H. 2018. The role of prebiotics and probiotics in gastrointestinal disease. *Gastroenterology Clinics.* 47: 179-191.
  37. Florowska, A., K. Krygier, T. Florowski and E. Dłuzewska. 2016. Prebiotics as functional food ingredients preventing diet-related diseases. *Food & Function.* 7: 2147-2155.
  38. Freire, A.L., C.L. Ramos and R.F. Schwan. 2015. Microbiological and chemical parameters during cassava based-substrate fermentation using potential starter cultures of lactic acid bacteria and yeast. *Food Research International.* 76: 787-795.
  39. Freitas, A.C. and J.E. Hill. 2017. Quantification, isolation and characterization of *Bifidobacterium* from the vaginal microbiomes of reproductive aged women. *Anaerobe.* 47: 145-156.
  40. Gandhi, A. and N.P. Shah. 2015. Effect of salt on cell viability and membrane integrity of

- Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium longum* as observed by flow cytometry. *Food Microbiology*. 49: 197-202.
41. Gebara, C., K.S. Chaves, M.C.E. Ribeiro, F.N. Souza, C.R. Grosso and M.L. Gigante. 2013. Viability of *Lactobacillus acidophilus* La5 in pectin-whey protein microparticles during exposure to simulated gastrointestinal conditions. *Food Research International*. 51: 872-878.
  42. Ghosh, K., M. Ray, A. Adak, S.K. Halder, A. Das, A. Jana, S. Parua, C. Vagvolgyi, P.K.D. Mohapatra and B.R. Pati. 2015. Role of probiotic *Lactobacillus fermentum* KKL1 in the preparation of a rice based fermented beverage. *Bioresource Technology*. 188: 161-168.
  43. Gogineni, V.K., L.E. Morrow, P.J. Gregory and M.A. Malesker. 2013. Probiotics: History and evolution. *Journal of Ancient Diseases & Preventive Remedies*. 1: 1-7.
  44. Gu, M., Z. Zhang, C. Pan, T.R. Goulette, R. Zhang, G. Hendricks, D.J. McClements and H. Xiao. 2019. Encapsulation of *Bifidobacterium pseudocatenulatum* G7 in gastroprotective microgels: Improvement of the bacterial viability under simulated gastrointestinal conditions. *Food Hydrocolloids*. 91: 283-289.
  45. Hadzieva, J., K. Mladenovska, M.S. Crcarevska, M.G. Dodov, S. Dimchevska, N. Geskovski, A. Grozdanov, E. Popovski, G. Petrusovski and M. Chachorovska. 2017. *Lactobacillus casei* encapsulated in soy protein isolate and alginate microparticles prepared by spray drying. *Food Technology and Biotechnology*. 55: 173-186.
  46. Hanani, Z.A.N., Y.H. Roos and J.P. Kerry. 2014. Use and application of gelatin as potential biodegradable packaging materials for food products. *International Journal of Biological Macromolecules*. 71:94-102.
  47. Hassan, M., M. Kjos, I. Nes, D. Diep and F. Lotfipour. 2012. Natural antimicrobial peptides from bacteria: Characteristics and potential applications to fight against antibiotic resistance. *Journal of Applied Microbiology*. 113: 723-736.
  48. Hess, P., A. Altenhofer, A.S. Khan, N. Daryab, K.S. Kim, J. Hacker and T.A. Oelschlaeger. 2004. A *Salmonella fim* homologue in *Citrobacter freundii* mediates invasion in vitro and crossing of the blood-brain barrier in the rat pup model. *Infection and Immunity*. 72: 5298-5307.
  49. Iannitti, T. and B. Palmieri. 2010. Therapeutical use of probiotic formulations in clinical practice. *Clinical Nutrition*. 29: 701-725.
  50. Illanes, A., C. Guerrero, C. Vera, L. Wilson, R. Conejeros and F. Scott. 2016. Lactose-derived prebiotics (1<sup>st</sup> Ed.). Academic Press. Amsterdam, Netherlands.
  51. Ivanovska, T.P., L.P. Tozi, A. Grozdanov, R. Petkovska, J. Hadjieva, E. Popovski, T. Stafilov and K. Mladenovska. 2014. From optimization of synbiotic microparticles prepared by spray-drying to development of new functional carrot juice. *Chemical Industry and Chemical Engineering Quarterly*. 20: 549-564.
  52. Jain, A., D. Thakur, G. Ghoshal, O.P. Katare and U.S. Shivhare. 2016. Characterization of microcapsulated  $\beta$ -carotene formed by complex coacervation using casein and gum tragacanth. *International Journal of Biological Macromolecules*. 87:101-113.
  53. Karaca, A.C., M. Nickerson and N.H. Low. 2013. Microcapsule production employing chickpea or lentil protein isolates and maltodextrin: Physicochemical properties and oxidative protection of encapsulated flaxseed oil. *Food chemistry*. 139: 448-457.
  54. Khan, N.H., D.R. Korber, N.H. Low and M.T. Nickerson. 2013. Development of extrusion-based legume protein isolate-alginate capsules for the protection and delivery of the acid sensitive probiotic, *Bifidobacterium adolescentis*. *Food Research International*. 54: 730-737.
  55. Klemmer, K.J., D.R. Korber, N.H. Low and M.T. Nickerson. 2011. Pea protein - based capsules for probiotic and prebiotic delivery. *International Journal of Food science & Technology*. 46: 2248-2256.
  56. Kolida, S. and G.R. Gibson. 2011. Synbiotics in health and disease. *Annual Review of Food Science and Technology*. 2: 373-393.
  57. Kuck, L.S. and C.P.Z. Norena. 2016. Microencapsulation of grape (*Vitis labrusca* var. Bordo) skin phenolic extract using gum Arabic, polydextrose, and partially hydrolyzed guar gum as encapsulating agents. *Food Chemistry*. 194: 569-576.
  58. Lau, C.S. and R.S. Chamberlain. 2015. Probiotic administration can prevent necrotizing enterocolitis in preterm infants: A meta-analysis. *Journal of Pediatric Surgery*. 50: 1405-1412.
  59. Li, F., X. Jin, B. Liu, W. Zhuang and D. Scalabrin. 2014. Follow-up formula consumption in 3-to 4-year-olds and respiratory infections: An RCT. *Pediatrics*. 133: 1533-1540.
  60. Livney, Y.D. 2010. Milk proteins as vehicles for bioactives. *Current Opinion in Colloid & Interface Science*. 15: 73-83.
  61. Mackowiak, P.A. 2013. Recycling Metchnikoff: Probiotics, the intestinal microbiome and the quest for long life. *Frontiers in Public Health*. 1: 52-66.

62. Magrone, T. and E. Jirillo. 2013. The interaction between gut microbiota and age-related changes in immune function and inflammation. *Immunity & Aging*. 10: 31-37.
63. Mahony, L., M. Feeney, S. Halloran, L. Murphy, B. Kiely, J. Fitzgibbon, G. Lee, G. Sullivan, F. Shanahan and J. Collins. 2001. Probiotic impact on microbial flora, inflammation and tumour development in IL - 10 knockout mice. *Alimentary Pharmacology & Therapeutics*. 15: 1219-1225.
64. Mandal, S., A. Puniya and K. Singh. 2006. Effect of alginate concentrations on survival of microencapsulated *Lactobacillus casei* NCDC-298. *International Dairy Journal*. 16: 1190-1195.
65. Mantegazza, C., P. Molinari, E. Auria, M. Sonnino, L. Morelli and G.V. Zuccotti. 2018. Probiotics and antibiotic-associated diarrhea in children: A review and new evidence on *Lactobacillus rhamnosus* GG during and after antibiotic treatment. *Pharmacological Research*. 128: 63-72.
66. Martin, M.J., F.L. Villoslada, M.A. Ruiz and M.E. Morales. 2015. Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. *Innovative Food Science & Emerging Technologies*. 27: 15-25.
67. McFarland, L.V. 2010. Systematic review and meta-analysis of *Saccharomyces boulardii* in adult patients. *World Journal of Gastroenterology*. 16: 2202-2222.
68. McFarland, L.V. 2015. From yaks to yogurt: The history, development, and current use of probiotics. *Clinical Infectious Diseases*. 60: 85-90.
69. Mirzaei, H., H. Pourjafar and A. Homayouni. 2012. Effect of calcium alginate and resistant starch microencapsulation on the survival rate of *Lactobacillus acidophilus* La5 and sensory properties in Iranian white brined cheese. *Food Chemistry*. 132: 1966-1970.
70. Mugambi, M.N., T. Young and R. Blaauw. 2014. Application of evidence on probiotics, prebiotics and synbiotics by food industry: A descriptive study. *BMC Research Notes*. 7: 754-763.
71. Musikasang, H., A. Tani, A.H. Kittikun and S. Maneerat. 2009. Probiotic potential of lactic acid bacteria isolated from chicken gastrointestinal digestive tract. *World Journal of Microbiology and Biotechnology*. 25: 1337-1345.
72. Myllyluoma, E., A.M. Ahonen, R. Korpela, H. Vapaatalo and E. Kankuri. 2008. Effects of multispecies probiotic combination on *Helicobacter pylori* infection in vitro. *Clinical and Vaccine Immunology*. 15: 1472-1482.
73. Nieuwboer, M., E. Claassen, L. Morelli, F. Guarner and R.J. Brummer. 2014. Probiotic and synbiotic safety in infants under two years of age. *Beneficial Microbes*. 5: 45-60.
74. Nualkaekul, S., M.T. Cook, V.V. Khutoryanskiy and D. Charalampopoulos. 2013. Influence of encapsulation and coating materials on the survival of *Lactobacillus plantarum* and *Bifidobacterium longum* in fruit juices. *Food Research International*. 53: 304-311.
75. Oelschlaeger, T.A. 2010. Mechanisms of probiotic actions-A review. *International Journal of Medical Microbiology*. 300: 57-62.
76. Olveira, G. and I.G. Molero. 2016. An update on probiotics, prebiotics and synbiotics in clinical nutrition. *Endocrinologia, Diabete & Nutricion*. 63: 482-494.
77. Orgil, O., L. Spector, D. Holland, J. Mahajna and R. Amir. 2016. The anti-proliferative and anti-androgenic activity of different pomegranate accessions. *Journal of Functional Foods*. 26: 517-528.
78. Ossowski, I., R. Satokari, J. Reunanen, S. Lebeer, S.C.D. Keersmaecker, J. Vanderleyden, W.M.D. Vos and A. Palva. 2011. Functional characterization of a mucus-specific LPXTG surface adhesin from probiotic *Lactobacillus rhamnosus* GG. *Applied and Environmental Microbiology*. 77: 4465-4472.
79. Pakbin, B., S.H. Razavi, R. Mahmoudi and P. Gajarbeygi. 2014. Producing probiotic peach juice. *Biotechnology and Health Sciences*. 9: 17-30.
80. Parker, E.A., T. Roy, C.R. Dadamo and L.S. Wieland. 2018. Probiotics and gastrointestinal conditions: An overview of evidence from the Cochrane Collaboration. *Nutrition*. 45: 125-134. e11.
81. Patel, P.J., S.K. Singh, S. Panaich and L. Cardozo. 2014. The aging gut and the role of prebiotics, probiotics, and synbiotics: A review. *Journal of Clinical Gerontology and Geriatrics*. 5: 33-41.
82. Perricone, M., M.R. Corbo, M. Sinigaglia, B. Speranza and A. Bevilacqua. 2014. Viability of *Lactobacillus reuteri* in fruit juices. *Journal of Functional Foods*. 10: 421-426.
83. Perricone, M., A. Bevilacqua, C. Altieri, M. Sinigaglia and M.R. Corbo. 2015. Challenges for the production of probiotic fruit juices. *Beverages*. 1: 95-103.
84. Phan, T.D., F. Debeaufort, D. Luu and A. Voilley. 2005. Functional properties of edible agar-based and starch-based films for food quality preservation. *Journal of Agricultural and Food Chemistry*. 53:973-981.



85. Possemiers, S., M. Marzorati, W. Verstraete and T.V.D. Wiele. 2010. Bacteria and chocolate: A successful combination for probiotic delivery. *International Journal of Food Microbiology*. 141: 97-103.
86. Prado, F.C., J.D.D. Lindner, J. Inaba, V.T. Soccol, S.K. Brar and C.R. Soccol. 2015. Development and evaluation of a fermented coconut water beverage with potential health benefits. *Journal of Functional Foods*. 12: 489-497.
87. Prasanna, P., A.S. Grandison and D. Charalampopoulos. 2014. Bifidobacteria in milk products: An overview of physiological and biochemical properties, exopolysaccharide production, selection criteria of milk products and health benefits. *Food Research International*. 55: 247-262.
88. Rabah, H., R.D. Carmo, F. Luiz and G. Jan. 2017. Dairy propionibacteria: Versatile probiotics. *Microorganisms*. 5: 24-41.
89. Rajam, R. and C. Anandharamakrishnan. 2015. Microencapsulation of *Lactobacillus plantarum* (MTCC 5422) with fructooligosaccharide as wall material by spray drying. *LWT-Food Science and Technology*. 60: 773-780.
90. Ranadheera, C.S., C. Evans, M. Adams and S. Baines. 2012. Probiotic viability and physico-chemical and sensory properties of plain and stirred fruit yogurts made from goat's milk. *Food Chemistry*. 135: 1411-1418.
91. Rasmussen, H.E. and B.R. Hamaker. 2017. Prebiotics and inflammatory bowel disease. *Gastroenterology Clinics*. 46: 783-795.
92. Roos, S. and H. Jonsson. 2002. A high-molecular-mass cell-surface protein from *Lactobacillus reuteri* 1063 adheres to mucus components. *Microbiology*. 148: 433-442.
93. Rouhi, M., S. Sohrabvandi and A. Mortazavian. 2013. Probiotic fermented sausage: Viability of probiotic microorganisms and sensory characteristics. *Critical reviews in Food Science and Nutrition*. 53: 331-348.
94. Sagar, S., A.P. Vos, M.E. Morgan, J. Garssen, N.A. Georgiou, L. Boon, A.D. Kraneveld and G. Folkerts. 2014. The combination of *Bifidobacterium breve* with non-digestible oligosaccharides suppresses airway inflammation in a murine model for chronic asthma. *Molecular Basis of Disease*. 1842: 573-583.
95. Salmeron, I., K. Thomas and S.S. Pandiella. 2015. Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. *Journal of Functional Foods*. 15: 106-115.
96. Sanchez, B., M.C. Urdaci and A. Margolles. 2010. Extracellular proteins secreted by probiotic bacteria as mediators of effects that promote mucosa-bacteria interactions. *Microbiology*. 156: 3232-3242.
97. Santos, C.C.A., B.D.S. Libeck and R.F. Schwan. 2014. Co-culture fermentation of peanut-soy milk for the development of a novel functional beverage. *International Journal of Food Microbiology*. 186: 32-41.
98. Sathyabama, S. and R. Vijayabharathi. 2014. Co-encapsulation of probiotics with prebiotics on alginate matrix and its effect on viability in simulated gastric environment. *LWT-Food Science and Technology*. 57: 419-425.
99. Saulnier, D.M., S. Kolida and G.R. Gibson. 2009. Microbiology of the human intestinal tract and approaches for its dietary modulation. *Current Pharmaceutical Design*. 15: 1403-1414.
100. Savilahti, E., K. Kukkonen and M. Kuitunen. 2009. Probiotics in the treatment and prevention of allergy in children. *World Allergy Organization Journal*. 2: 69-76.
101. Savino, F., L. Cordisco, V. Tarasco, E. Palumeri, R. Calabrese, R. Oggero, S. Roos and D. Matteuzzi. 2010. *Lactobacillus reuteri* DSM 17938 in infantile colic: A randomized, double-blind, placebo-controlled trial. *Pediatrics*. 126: 526-533.
102. Shi, L.E., Z.H. Li, Z.L. Zhang, T.T. Zhang, W.M. Yu, M.L. Zhou and Z.X. Tang. 2013. Encapsulation of *Lactobacillus bulgaricus* in carrageenan-locust bean gum coated milk microspheres with double layer structure. *LWT-Food Science and Technology*. 54: 147-151.
103. Shima, M., Y. Morita, M. Yamashita and S. Adachi. 2006. Protection of *Lactobacillus acidophilus* from the low pH of a model gastric juice by incorporation in a W/O/W emulsion. *Food Hydrocolloids*. 20: 1164-1169.
104. Siezen, R.J. and G. Wilson. 2010. Probiotics genomics. *Microbial Biotechnology*. 3: 1-9.
105. Simone, M., C. Gozzoli, A. Quartieri, G. Mazzola, D.D. Gioia, A. Amaretti, S. Raimondi and M. Rossi. 2014. The probiotic *Bifidobacterium breve* B632 inhibited the growth of *Enterobacteriaceae* within colicky infant microbiota cultures. *BioMed Research International*. 2014: 11-17.
106. Singh, P., B. Medronho, L. Alves, G.D. Silva, M. Miguel and B. Lindman. 2017. Development of carboxymethyl cellulose-chitosan hybrid micro- and macroparticles for encapsulation of probiotic bacteria. *Carbohydrate Polymers*. 175: 87-95.
107. Solis, G., C.G.R. Gavilan, N. Fernandez, A. Margolles and M. Gueimonde. 2010.

- Establishment and development of lactic acid bacteria and bifidobacteria microbiota in breast-milk and the infant gut. *Anaerobe*. 16: 307-310.
108. Taipale, T., K. Pienihakinen, E. Isolauri, C. Larsen, E. Brockmann, P. Alanen, J. Jokela and E. Soderling. 2011. *Bifidobacterium animalis* subsp. *lactis* BB-12 in reducing the risk of infections in infancy. *British Journal of Nutrition*. 105: 409-416.
109. Tellez, G., L.R. Fragoso, V. Kuttappan, G. Kallapura, X. Velasco, A. Menconi, J. Latorre, A. Wolfenden, B. Hargis and J.R. Esparza. 2013. Probiotics for human and poultry use in the control of gastrointestinal disease: A review of real-world experiences. *Alternative and Integrative Medicine*. 2: 11-16.
110. Trabelsi, I., D. Ayadi, W. Bejar, S. Bejar, H. Chouayekh and R.B. Salah. 2014. Effects of *Lactobacillus plantarum* immobilization in alginate coated with chitosan and gelatin on antibacterial activity. *International Journal of Biological Macromolecules*. 64: 84-89.
111. Tripathi, M.K. and S.K. Giri. 2014. Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods*. 9: 225-241.
112. Vandenplas, Y., I. Zakharova and Y. Dmitrieva. 2015. Oligosaccharides in infant formula: More evidence to validate the role of prebiotics. *British Journal of Nutrition*. 113: 1339-1344.
113. Wang, J., D.R. Korber, N.H. Low and M.T. Nickerson. 2014. Entrapment, survival and release of *Bifidobacterium adolescentis* within chickpea protein-based microcapsules. *Food Research International*. 55: 20-27.
114. Xue, Z., J. Yu, M. Zhao, W. Kang and Z. Ma. 2017. Effects of synbiotics on intestinal mucosal barrier in rat model. *Clinical Nutrition Experimental*. 13: 12-21.
115. Yang, X., X. Hang, J. Tan and H. Yang. 2015. Differences in acid tolerance between *Bifidobacterium breve*.
116. Ying, D., S. Schwander, R. Weerakkody, L. Sanguansri, C.G. Demarchi and M.A. Augustin. 2013. Microencapsulated *Lactobacillus rhamnosus* GG in whey protein and resistant starch matrices: Probiotic survival in fruit juice. *Journal of Functional Foods*. 5: 98-105.
117. Yoon, K.Y., E.E. Woodams and Y.D. Hang. 2005. Fermentation of beet juice by beneficial lactic acid bacteria. *LWT-Food Science and Technology*. 38: 73-75.
118. Yoon, K.Y., E.E. Woodams and Y.D. Hang. 2006. Production of probiotic cabbage juice by lactic acid bacteria. *Bioresource Technology*. 97: 1427-1430.
119. Zaeim, D., M.S. Jamab, B. Ghorani and R. Kadkhodae. 2019. Double layer co-encapsulation of probiotics and prebiotics by electro-hydrodynamic atomization. *LWT-Food Science and Technology*. 110: 102-109.
120. Zhu, F. 2017. Encapsulation and delivery of food ingredients using starch based systems. *Food Chemistry*. 229: 542-552.
121. Zoumpopoulou, G., B. Pot, E. Tsakalidou and K. Papadimitriou. 2017. Dairy probiotics: Beyond the role of promoting gut and immune health. *International Dairy Journal*. 67: 46-60.