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# Polysaccharides systems for probiotic bacteria microencapsulation: mini review

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**Abstract**

Probiotic bacterial encapsulation systems have proven useful in protecting the bacteria from gastric acids, bile salts and other drastic conditions present in the gastrointestinal tract. In addition, daily intake of probiotic products has shown positive therapeutic effects on gastrointestinal and autoimmunity problems. Polysaccharides have aroused great interest in probiotic food applications due to their non-toxicity, biocompatibility, and the fact that they can be digested by enzymes in the gastrointestinal tract. The proper selection of an encapsulation system through the adequate combination of matrices and methods shows increased viability and provides a very promising shield for probiotic against various stress factors during processing, digestion, and storage conditions. Although most research has been conducted on simulated digestion, it is suggested to undertake systematic in vivo investigations of encapsulation efficacy where both the method and the encapsulation system are studied. The focus of this review is to provide an overview of the evolution of traditional encapsulation methods and the use of polysaccharides as efficient encapsulation systems. A second topic briefly reviewed are trends in encapsulation strategies and microencapsulation systems for non-dairy probiotic products. Finally, a new generation of probiotics as a preventive and therapeutic tool for different diseases, is showed.

**Keywords:** probiotics; encapsulation; polysaccharides; therapeutic effects.

**Practical Application:** Encapsulation of probiotic bacteria for food applications.

## 1 Introduction

Probiotics are "[...] living microorganisms that, when administered in the proper quantities, improve the health of the hosts [...]" (Food and Agriculture Organization of the United Nations, 2006, p. 2-4). Probiotics adhere easily to the human mucous membrane or epithelial cells and show antimicrobial activity against pathogenic bacteria and enterobacteria adhesion to cell surface reduction. They also secrete hydrolase and regulate immune activity (Anal & Singh, 2007; Parvez et al., 2006). However, the probiotics show low tolerance to both gastric acids and bile salts, and its stability is the major difficulty during administration to the colon when ingested orally. Hence, proper selection of the encapsulation system is required to protect against various stress factors and to preserve the potential of the probiotic throughout their shelf life.

The market for probiotics is estimated at USD54.77 billion in 2020 and is expected to grow approximately 7.2% by 2028 as a result of the consumers and clients growing more aware of the benefits that these microorganisms bring to their diets (Grand View Research, 2021).

Daily consumption of probiotics has beneficial effects such as the reduction of inflammation in the gastrointestinal tract (Bruzzese et al., 2016; Viramontes-Hörner et al., 2017; Simon et al., 2021), improving the immune and allergic response (Li et al., 2019a; Du et al., 2019: Zhang et al., 2018a; Simon et al., 2021), faster recovery from colitis (Jang et al., 2021; Barbieri et al., 2017), obesity and diabetes (Cai et al., 2019), skin diseases and eczemas (Sun et al., 2021), cancer (Zhang et al., 2005; Serban, 2014), improvement in slow bowel movements and stool formation (d'Ettorre et al., 2015). Thus, for example, clinical trials in nursing home residents demonstrated reduction of antibiotic (amoxicillin/clavulanic acid) associated diarrhea upon administration of probiotics containing *multispecies probiotics Ecologic® AAD* (van Wietmarschen et al., 2020).

Recently, probiotic intake has been associated with two possible mechanisms of immunity against Covid-19, the first one increases T cell activity, and the second one promotes lymphocyte maturation, differentiation, and reproduction (Hu et al., 2021). Among the beneficial effects on health, it has been reported to compare with COVID, regulate blood glucose, decrease oxidative stress of the cell, immunomodulation, among others. The level of delivered probiotics is mainly in intestine and colon.

Table 1 summarizes the main probiotic bacterias used according to the evaluation group and the main health effects of probiotic consumption.

Probiotics are present in various supplements and foods, mainly in milk and dairy products, which can become a limitation for mass consumption or people with some type of intolerance

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**Table 1**. main health effects of the consumption of probiotics.

| Experimental Groups | Probiotic Bacteria | Health effects | Reference |
|---|---|---|---|
| Human | *L. rhamnosus* GG | A decrease (50%) in episode of infections and lower bad days of illness in the probiotic group | Bruzzese et al. (2016) |
| Mouse | *L. rhamnosus* CRL1505 | Reduced alteration on CD4+ T cells in the bone marrow, thymus, spleen and lung. Increase IL-10 and IL-4 | Barbieri et al. (2017) |
| Human | *L. rhamnosus* GG *B. animalis* Bb-12 *L.acidophilus* La-5 | The proportion of Th22 cells was reduced in children in the probiotic group compared to the placebo group | Rø et al. (2017) |
| Human | *Lactobacillus spp. Bifidobacterium spp. Pediacoccus pentosaceus E. coli* Nissle *Leuconostoc mesenteroide* | Probiotics increased beneficial microflora and decreased pathogenic bacteria and endotoxemia compared with placebo/no treatment | Viramontes-Hörner et al. (2017) |
| Human | *L.s casei* 431 *L. paracasei L. fermentum* PCC | The result showed significantly higher level of IFN-y in the serum and IgA in the gut comparison with placebo group. | Zhang et al. (2018a) |
| Human | *Lactobacillus sp.* | The use of *Lactobacillus sp.*during prenatal and postnatal period showed a significantly reduced the incidence of atopic dermatitis | Li et al. (2019a) |
| Mouse | *Pediococcus acidilactici Lactobacili Streptococcus thermophilus* | P. acidilactici 004 and L. plantarum 152 could lower T2D blood glucose level more effectively and prevent the development of hyperglycemia in T2D. | Cai et al. (2019) |
| Human | *L. rhamnosus* GG | Reduction to the occurrence of asthma with *Lactobacillus rhamnosus* GG supplementation | Du et al. (2019) |
| Human | *Ls rhamnosus* GG | A significant difference in the HbA1c level where they remained stable in people who received the probiotic compared to the placebo group that increased | Sanborn et al. (2020) |
| Canine | *B. longum subsp. Longum* CACC517 *L.s plantarum* CACC558 | Mixed strains exhibited antibiosis, antibiotic activity, acid and bile tolerance and relative cell adhesion to the HT-29 monolayer cell line decreased oxidative stress in DH82 | Jang et al. (2021) |
| Human | *B. infantis L. acidophilus Bacillus cereus B. longum L. bulgaricus S.thermophiles Bacillus subtilis* | A decrease the CRP levels and the secondary infection in severe Covid-19 patient while total T lymphocytes, NK cells and B lymphocytes were increased in probiotics-treated patients. | Li et al. (2021) |
| Human | *L. rhamnosus GG B. longum* | The mixed probiotics suggesting a prevent eczema in children under 3 years of age compared to the placebo. | Sun et al. (2021) |

(Espitia et al., 2016). The main probiotic foods and supplements products generally come from the bacteria genera *Lactobacillus* sp and *Bifidobacterium* sp, known as lactic acid bacteria (LAB). Other microorganisms considered as probiotics are non-lactic microorganisms (NLAC), including *Escherichia coli*, Saccharomyces yeasts (cerevisiae and *boulardii*) and *Previtell*a *sp*., as a biomarker for intestinal disease (Precup & Vodnar, 2019).

Nowadays, incorporating healthy foods into our diets has become a popular trend among consumers because they provide benefits and help fight disease. One of these healthy foods is probiotics. This review will address the main studies that show the effects of probiotics on human health, the main encapsulation methods or techniques, and the main polysaccharide-based polymeric matrices that have demonstrated higher efficiency in the transport and administration of these microorganisms (Figure 1).

## 2 Traditional encapsulation methods

Probiotics must be capable of tolerating stomach pH conditions, bile salts in intestinal fluid, environmental stress, mechanical damage, interaction with foodstuffs, storage conditions, as well as oxygen and redox levels in the digestive system. These bioactive cells must be encapsulated to increase their viability, and the method chosen will depend on particle diameter, encapsulating agent, encapsulated substance, applications of the encapsulated material, release mechanism, and processing costs (García-Ceja et al., 2015; Călinoiu et al., 2016; Menezes et al., 2019).

### *2.1 Chemical methods for probiotics encapsulation*

Chemical methods for probiotic encapsulation are mainly ionic gelation, coacervation, and emulsion.

Ionic gelation occurs when an aqueous solution of negatively charged polyelectrolytes interacts with divalent ions, such as $Ca^{+2}$ and $Mg^{+2}$, forming a stable gel. According to Pedroso-Santana & Fleitas-Salazar (2020), ionic gelation is a simple, low-priced, and faster (< 10 hours) process with a high-efficiency rate (> 95%). Nevertheless, particle size heterogeneity can only be achieved with a polydispersity of up to 0.5, which might affect the quantity of the encapsulated bioactive compound and limit interaction with biological structures. There are two types of ionic gelation methods: internal and external (ionotropic). In the former, $Ca^{+2}$ or $Mg^{+2}$ ions migrate from the outside to the interior of the core fluid, prompting a structural reorganization, while in the latter, divalent ions migrate from the interior to the surface (Menezes et al., 2019; Silva et al., 2018). Kim et al. (2017) found higher stability against acidic change between pH 1.5 and 2 when *L. acidophilus* was encapsulated through ionotropic gelation between phytic acid (PA) and chitosan (CS) with

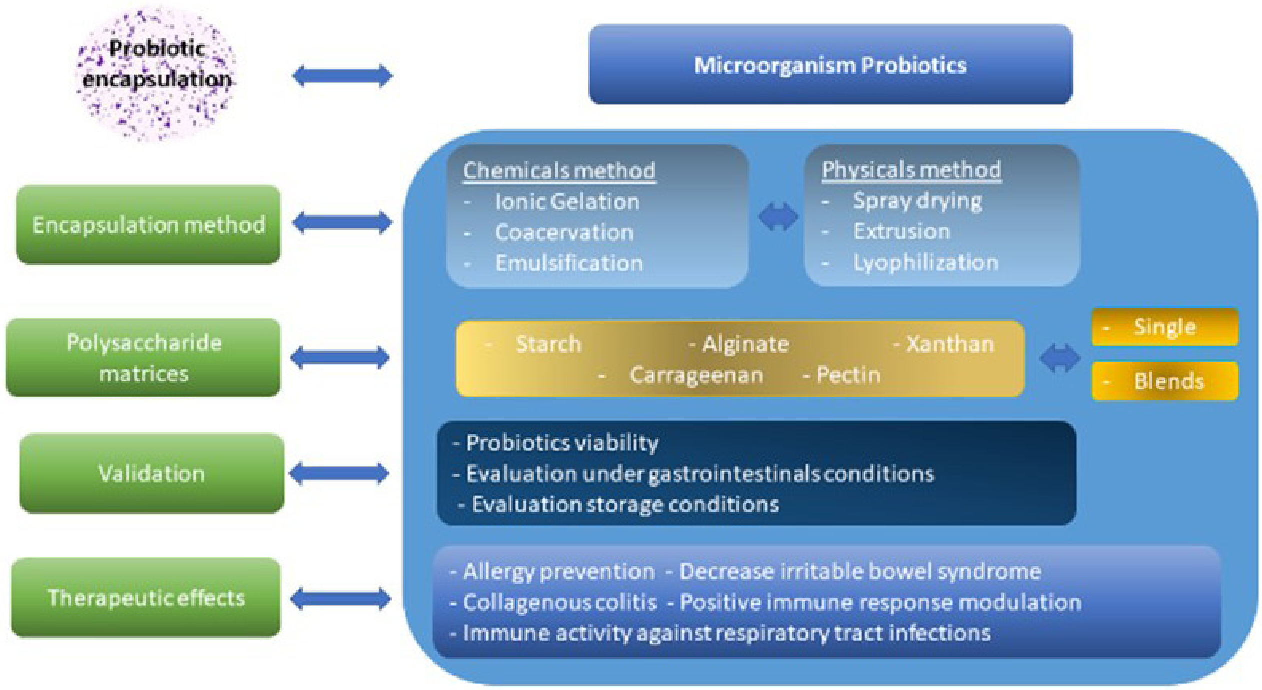


**Figure 1**. Main polysaccharides for probiotic bacteria microencapsulation.

$CaCO_3$ electrostatic extrusion, as well as higher microorganism survival in comparison with the PA-CS encapsulated bacteria without $CaCO_3$.

The coacervation method involves phase separation of a macromolecular solution to form two separate or immiscible liquid phases: a polymer-rich phase or colloideal solute such as chitosan, starch, gelatine, and a polymer-depleted phase, termed coacervate and equilibrium solution, respectively (Chadha, 2021). The process can be simple (only uses one polymer) or complex (requires two or more polymers with opposite charges). This is a relatively simple and low-priced method, it does not require high temperatures, nor organic solvents, presents high encapsulation rates (up to 99%). Nonetheless, it can only occur at certain pH levels and depends on the colloid and/or electrolyte concentration (Comunian et al., 2013; Huang et al., 2012; Piacentini et al., 2013). Complex coacervation has attracted more interest in studies of probiotics encapsulation. Eratte et al. (2015) encapsulated *L. casei* with omega-3 fatty acids in a whey-gum Arabic matrix through complex coacervation and found that probiotic viability increased significantly in comparison with those encapsulated without fatty acids.

Emulsification is another common chemical method encapsulation has the advantage of an efficiency >70%, high reproducibility, easy mass production, and similar size distribution. However, efficiency can decrease when the emulsions are dispersed in the aqueous phase, and large quantities of water are removed (Girija & Sakthi Kumar, 2016). Ma et al. (2020) proved *L. plantarum* LIPl encapsulation high efficiency through its emulsification system of skim milk/water/oil/chymosin matrices, achieving an 87% with a 1:10 water-oil ratio and survival rates of 55% in comparison with free bacteria (17%).

### *2.2 Physical methods for probiotic encapsulation*

***Physical methods for probiotic encapsulation are mainly spray drying, lyophilization, and extrusion***

The Spray Drying method (SD) consists of atomizing or spraying a liquid in fine droplets in a drying chamber with hot air flow operated between 60 °C to 150 °C. The liquid is composed of probiotics together with the encapsulating wall material or protection matrix (Haffner et al., 2016). This method is the most used one by the food industry and for research purposes, as it is fast, low-priced (less energy consumed), easy to adapt to industrial equipment, monodisperse; in addition, it helps in producing probiotic powders with higher stable shelf-life, powder properties such as size distribution bulk density, flowability, and lower transportation cost. The selection of wall material plays an important role in the system encapsulation as it directly links to encapsulation efficiency, stability, and release. Nunes et al. (2018) encapsulated *L. acidophilus* LA5 through SA, using different matrices based on gum Arabic, inulin, resistant starch (Hi-maize), and trehalose. They found that microparticles of trehalose matrix achieved higher protection and heat resistance while starch matrix showed more protection against stomach fluids.

Lyophilization (LI) is the separation of water from a solution through ice freezing and later sublimation at reduced pressure. This method requires a high energy intake and long periods of time; the bacteria might be cut by ice crystals or under stress due to high osmotic concentration. It also prevents oxidation, presents little dispersity, and is easy to use for the industry (Haffner et al., 2016). Shu et al. (2018) encapsulated *L. acidophilus* through a modified lyophilization method using cryoprotectant agents.

The results demonstrated a high survival rate after LI (93.9%) in comparison with the control group, which only reached 36.6%. Regarding the storage conditions of the encapsulated bacteria, the highest viability was 11 log CFUg$^{-1}$ at -18 °C, while at higher temperatures, it dropped under 10 log CFUg$^{-1}$.

Extrusion as a physical encapsulation method consists of producing small drops of encapsulating material through the forced stream of fluid from a syringe needle or nozzle, adding the microorganisms to the hydrocolloid solution, and then dripping its suspension over a drying solution (de Vos et al., 2010). Although extrusion is considered a simple method that does not use harmful solvents and that can protect the encapsulation at high temperatures, it has disadvantages such as slow particle formation, limited selection of coating or wall material, and high dispersion of samples (Burgain et al., 2011). A study conducted by Seth et al. (2017) confirm the efficiency of extrusion as a microencapsulation method to protect of *S. thermophilus* and *L. bulgaricus* at high temperatures (148 °C), allowing encapsulation in new products

Table 2 shows probiotics bacteria and encapsulation methods used in both in *in vitro* and *in vivo* studies. Most studies are performed under simulated or *in vitro* conditions. This is attributed to the fact that *in vitro* conditions are an excellent tool that helps to select and predict the possible behavior of

**Table 2**. Main probiotics bacteria and encapsulation methods of probiotic for food and target delivery application.

| Probiotics | Type / Encapsulation Method (EM) | In Vivo / In Vitro | Results | Application | References |
|---|---|---|---|---|---|
| *Bifidobacterium animalis* | Physical / Spray Drying | In Vitro | Enhanced viability (30%) at 5 °C for 30 days | Food / Acerola Nectar | Antunes et al. (2013) |
| *Lactobacillus rhamnosus LGG* | Physical / Spray Drying | In Vitro | WP favored a higher (38%) probiotic survival compared with free cells | Food / Apple Juice | Ying et al. (2013) |
| *Saccharomyces cerevisiae boulardii* | Physical / Extrusion | In Vitro | Improved cell survival (35%) through extrusion method | Food / Berry Juice | Fratianni et al. (2014) |
| *Lactobacillus acidophilus LA-5* *Lactobacillus casei 01* | Physical / Extrusion | In Vitro | Encapsulated system improved 43% cell survival and evidence higher encapsulation efficiency | Food / Orange Juice - Yogurt | Krasaekoopt & Watcharapoka (2014) |
| *Lactobacillus casei/ atun oil* | Complex Coacervation/ Spray drying (SD) or Freeze drying (FD) | In Vitro | Enhanced viability to bond to epithelial cells of the intestine and better structural integrity. | Delevery probiotic/ Colon | Eratte et al. (2015) |
| *Lactobacillus acidophillus LA-5* | Chemical / External Gelation | In Vitro | Encapsulated system improved 33% when storage along the 28 days. | Food / Yogurt | Silva et al. (2018) |
| *Lactobacillus plantarum HM47* | Physical / Spray Drying | In Vivo | Microencapsulated probiotic showed survival up to 180 days at 25°C and supressed the pathogenic bacterial in the intestine | Food / Milk Chocolate | Nambiar et al. (2018) |
| *Saccharomyces boulardii* *Lactobacillus acidophilus LA-5* *Bifidobacterium bifidum BB-12* | Physical / Spray Drying Spry Chilling | In Vitro | S. boulardii (67.44%) and L. acidophilus (70.73%) survival after baking with P/GS microcapsule | Food / Cake | Arslan-Tontul et al. (2019) |
| *Lactobacillus rhamnosus* | Physical / Spray Aerosol | In Vitro | Encapsulated probiotic led to a firmer and thicker cream cheese | Food / Cream cheese | |
| *Lactobacillus plantarum* | Chemical / Ionic Gelation | In Vitro | The beads showed successful resistant to thermal condition | Food / Mango Juice | |
| *Lactobacillus plantarum NCIM2083* | Physical / Spray Drying (SD) Spray-Freeze-Drying (SFD) | In Vitro | Encapsulation efficiency for SD was 89.21% and for SFD was 96.16% After the in vitro treatment of SGF and SIF, the probiotics encapsulated by SFD their viability by 15.7% and 35.79% for SD. | Delevery Probiotics / Intestinal | Yoha et al. (2020) |
| *Lactobacillus rhamnosus* ATCC 7469 | Physical / Freeze-Drying | In Vitro | Viability increased by compared to uncapsulated bacteria up to 90%. Allows stability of the matriz when passing through the gastric fluid to the intestinal fluid | Delevery Probiotics / Intestinal | Maleki et al. (2020) |
| *Lactobacillus paracasei* ATC334 | Chemical / Gelification | In Vitro / In Vivo | The release of probiotic-like biofilms showed a reduction in inflammation, in colon tissues and in tissue damage | Delevery Probiotics / Intestinal | Heumann et al. (2020) |

**Table 2**. Continued...

| Probiotics | Type / Encapsulation Method (EM) | In Vivo / In Vitro | Results | Application | References |
|---|---|---|---|---|---|
| *Lactobacillus plantarum* A7 | Physical / Electrospray | In Vitro | Inulin-containing and Starch-containing microcapsules could enhance the survival probiotic bacteria during the storage for 90 days. Starch-containing microcapsules showed a better viability in ice cream (93.43%) | Food / Ice Cream | Zaeim et al. (2020) |
| *Lactobacillus acidophilus* LA-5 | Physical / Spray Drying (SD) | In Vitro | The microcapsules provided better protection of *L. acidophilus* when compared with free cells with a reduction of 10.84% and 24.54%, respectively. Resulting in 93.95% maximum encapsulation efficiency and 48,36% maximum production efficiency | Food / Yogurt | Leylak et al. (2021) |
| Lactiplantibacillus plantarum 299v | Co-Extrusion | In Vitro | Encapsulated *L. plantarum* 299v with inulin showed higher survivability (>107 CFU/mL) than free cells and encapsulated *L. plantarum* 299v without inulin under simulated gastrointestinal conditions and after four (4) weeks of storage in roselle juice at 4 °C. | Food / Roselle Juice | Chean et al. (2021) |

bacteria in vivo. On the other hand, it is attractive given its low-cost and easy implementation, obtaining a response in a short time, and estimating the effect of probiotics; however, they cannot accurately simulate the human gut.

*In vivo* probiotic inoculation remains a challenge. For this reason, different strategies are being studied to improve their survival, avoid unpleasant changes, or improve their performance in new applications. Preliminary results suggest that probiotic resistance could be increased by cross-adaptation/adaptive evolution or by bioengineering (Fareez et al., 2015; Speranza et al., 2020).

## 3 Polysaccharides used for probiotic encapsulation

Encapsulation is considered one of the best methods to obtain a symbiotic and synergistic effect of probiotic bacteria subjected to gastro-intestinal conditions. Hence, the growing interest in the microencapsulation of probiotics in biopolymeric matrices can reduce their loss of viability and offer adequate protective barrier conditions. There are numerous polysaccharides that meet these conditions, such as starch, pectin, alginate, carrageenan, and xanthan. Table 3 shows a summary of the main polysaccharide-based matrices used for probiotic encapsulation.

### *3.1 Starch*

Is a polysaccharide composed of two main biopolymers, including amylose and amylopectin (Ismail et al., 2013). In addition, starch is considered the foremost glucose source for humans and is also low-priced raw material and renewable.

Starches are easily hydrolyzed by pancreatic enzymes, which is why they cannot reach the large intestine intact, as this might affect the viability of the probiotic.

Recently studies demonstrated starch blends are encapsulation efficient than alone starch. For example, pectin/starch blends form an interconnected network stabilized by a combination of weak intermolecular forces, hydrogen bonds, and hydrophobic interactions, which is an attractive alternative for the encapsulation of probiotics. According to Agudelo et al. (2014), incorporating pectin into native tapioca starch offers more thermal and mechanical stability. Dafe et al. (2017a) studied *L. plantarum* ATCC13643 viability when encapsulated in a pectin/starch blend under SGC and storage at 4 °C for 30 days. The results showed cell death after continuous exposure to SGC for 2 h in free bacteria, while the survival rate for those encapsulated in the pectin and pectin/starch matrices was, respectively, 5.15 log CFU $g^{-1}$ and 6.67 log CFU $g^{-1}$. Zanjani et al. (2018) found chitosan-starch and alginate-starch blends efficiency reached over 97% and increased the viability and storage condition. Besides, microorganism addition through the matrices did not affect the organoleptic parameters of the ice cream.

### *3.2 Pectin*

Is a heteropolysaccharide with D-galacturonic acid bound by α(1-4) glycosidic bonds with some methylated carboxylic groups. They have two forms: i) high methoxyl (HM) and ii) low methoxyl (LM) pectin. Gel formation depends on pectin structure, the presence of cross-linking agents, temperature, and pH. To form a gel with high methoxyl pectins (HM), a pH < 3.5 and high sugar concentrations are needed (Gawkowska et al., 2018; Martău et al., 2019), while low methoxyl pectins (LM) can form gels in the presence of divalent cations such as $Ca^{+2}$ at a pH between 2 and 6.

Li et al. (2019b) studied *B. breve* CICC6182 encapsulation efficiency in LM pectin and the viability both in storage at three different temperatures (-20 °C, 4 °C and 25 °C) for 13 weeks and in exposure to simulated gastrointestinal fluids. The encapsulation efficiency was 99%. After treatment under SGC, the viability of the encapsulated probiotics decreased only by 1.76 log CFU $g^{-1}$ versus 4.82 log CFU $g^{-1}$ of free bacteria. Stored under low-temperature conditions, encapsulated bacteria showed

**Table 3.** Polysaccharide-based matrices used for probiotic encapsulation.

| Matrix encapsulation | Probiotics | Method encapsulation | Encapasultion Efficiency | Application | Reference |
|---|---|---|---|---|---|
| Alginate / chitosan | *S. thermophilus L. delbrueckii* | Geletion | 99.8% | Delivery sytem | Vodnar et al. (2010) |
| Green tea / alginate /chitosan | *B. infantis* ATCC 15697 *B. breve* ATCC 15700 | Geletion/ coating | 36.15% - 38.24% | Delevery system | Vodnar & Socaciu (2012) |
| Selenium-green tea / alginate / chitosan | *L. plantarum L. casei* | Biotech encapsulator ® / coating | 38.01% - 38.33% | Delivery system | Vodnar & Socaciu (2014) |
| Chitosan / alginate | *L. rhamnosus* | External gelation | 92% | Delevery system | Cheow et al. (2014) |
| FOS / protein isolate | *L.acidophilus* NCDC 016 | Spray drying | 70% - 73% | Functional Food | Rajam & Anandharamakrishnan (2015a) |
| Xanthan / chitosan / xanthan | *Bifidobacterium spp.* BB01 *L. acidophilus* | Extrusion | 85.08% | Delevery system | Chen et al. (2017) |
| Maltodextrin | *L. casei* Shirota | Spray drying | 62% | Delevery system | Gul & Atalar (2019) |
| Maltodextrin / reconstituted skim milk | | Freeze drying | 68% | | |
| Maltodextrin / reconstituted skim milk / gum arabic | | | 74% | | |
| Gum arabic / maltodextrin / Hi-maize Trehalose | *L. acidophillus* LA-5 | Spray drying | 95% | Delevery system | Nunes et al. (2018) |
| Maltodextrin / inuline | *B. animalis* BB12 | Spray drying | 88% | Food | Dias et al. (2018) |
| Whey protein / whey protein | *L. lactis subsp. cremoris* LM0230 | Ionotropic gelation | 94% | Food | |
| Gelatin / gum arabic | *L. plantarum* ST-III | Complex coacervation | 102.8% | Delevery system | Zhao et al. (2018) |
| Waxy cassava starch Nomal cassava starch | *L. pentosus* | Spray drying | 94% | Delevery system | Cruz-Benítez et al. (2019) |
| FOS / skimmed milk | *L. acidophilus* LA-5 | Spray drying | 98.2% | Food | dos Santos et al. (2019) |
| Alginate / inulin / lecithin | *L. reuteri* | Extrusion | 94% | Food | Qaziyani et al. (2019) |
| Pectin | *B. breve* | Lyophilized | 99% | Food | Li et al. (2019b) |
| Alginate | *L. rhamnosus* | Spray aerosol | 90% | Food | |
| Calcium protein | *Lactococcus lactis subsp. cremoris* LM0229 | Ionotropic gelation | 96% | Food | Afzaal et al. (2020) |
| Whey protein | | | 94% | | |
| Alginate / chitosan | *L. plantarum* | Extrusion | 97.26% | Delevery system | Mahmoud et al. (2020) |
| Alginate / whey protein | | | 94.94% | | |
| Alginate / dextrin | | | 98.11% | | |
| Whey protein / microcrystalline cellulose / inulin | *L. rhamnosus* ATCC 7469 | Lyophilized | 90% | Delevery system | Maleki et al. (2020) |
| Whey powder / gum arabic | *L. acidophilus* LA-5 | Spray drying | 94% | Food | Leylak et al. (2021) |
| Alginate | *L. plantarum* 31F *L. plantarum* 25F | Extrusion | 93% - 94% | Delivery system | Pupa et al. (2021) |
| | *L. plantarum* 22F | Emulsion | 93% - 94% | | |
| | *P.pentosaceus* 77F *P.acidilactici* 72N | Spray drying | 74% - 75% | | |

a decrease of 1.5 log CFU g-$^{1}$ in comparison with unencapsulated probiotics (4 log CFU g-$^{1}$). Gebara et al. (2013) studied pectin-encapsulated (PEC) *L. acidophilus* LA5 and pectin/milk serum (P-S) viability under two simulated gastrointestinal conditions, SGC1 (pH between 1.2 and 7) and SGC2 (pH between 3 and 7), as well as after exposure to heat treatment (80 °C for 15 min). The results showed that viability after encapsulation was 8 log CFU g-$^{1}$. Furthermore, the viability of cells encapsulated in PEC after SGC1 treatment (5.45 log CFU g-$^{1}$) was higher than in those encapsulated with P-S (5.22 log CFU g-$^{1}$), while for SGC2 the viability was higher in free bacteria (3.55 log CFU g-$^{1}$), so adding pectin into the polymer matrix benefits probiotic survival rate.

### *3.3 Alginate*

Is a polysaccharide obtained from brown algae consisting of D-mannuronic acid (M) and L-guluronic acid (G) units that are linked linearly by 1.4-glycosidic bonds. High G-blocks percentages tend to generate more fragile and rigid gels, while those with more M-blocks produce less rigid and more fragile gels (Rodrigues et al., 2020). Alginates are biocompatible, non-toxic, low-priced, require mild processing conditions (up to 65 °C-70 °C), can form hydrogels with divalent ions, and are digested in the intestine. However, the gels obtained are very porous and susceptible to acidic environments, so they need to be applied with another polymer for probiotic encapsulation (Burgain et al., 2011), such as pectin, protein, and chitosan (Mahmoud et al., 2020). Thus, García-Ceja et al. (2015) demonstrated higher viability to encapsulate *L. acidophilus* and *L. reuteri in* Al-CH systems, stored at 5 °C for one month in different foods such as milk, peach juice, and yogurt. In this study was achieved viability of 11 log CFU $g^{-1}$ after storage in system matrix while viability in free cells was 7 log CFU $g^{-1}$.

Table 3 show polysaccharide matrices such as alginate, starch, chitosan, pectin, among other, offers adequate protection to encapsulated probiotics, independent of the encapsulated microorganism. This protection is reflected in the increased viability during the transport of the probiotic in the GI system. Furthermore, it can be observed that co-encapsulation favors this increase.

### *3.4 Carrageenan*

Is a linear anionic polysaccharide consisting of alternating β-galactose and 3.6-anhydro-α-galactose units linked by α(1.3) and β(1.4). There are three types: κ-carrageenan, ι-carrageenan, and λ-carrageenan, where the first of them is the most used one for probiotics encapsulation. κ-carrageenan can gel in the presence of monovalent or divalent cations, resulting in a heat-sensitive hydrogel that sustains reversible volume transitions in response to heat stimuli, making it suitable for probiotic administration with a temperature-controlled release (Gbassi & Vandamme, 2012; Kwiecień & Kwiecień, 2018). Soukoulis et al. (2017) found that the κ-carrageenan/carob bean gum showed greater stabilization of *L. rhamnosus* GG during 25 days of storage. However, studies by Zainal-Ariffin et al. (2014) and Shang et al. (2017) showed controversial results when using κ-carrageenan due to induced colitis in rats and inhibition in human (Caco-2 and FHs 74 Int) and liver (HepG2 and Fa2N-4) cell lines. The use of other polymers to enhance the benefits of carrageenan as a probiotic encapsulation matrix is recommended. Thus, Dafe et al. (2017b) reported a new system κ-carrageenan and carboxymethyl cellulose-based transport system to deliver *L. plantarum* ATCC13643 into the colon. After 2 hours of gastric juice incubation at pH 2 and bile at pH 7, the survival of the probiotics increased by 7.3 log CFU $g^{-1}$ and 7.48 log CFU $g^{-1}$, respectively, while free bacteria did not survive.

### *3.5 Xanthan*

is a branched polysaccharide consisting of β(1.4)-D-glucose units attached to D-glucuronic acid sidechains located between two D-mannose units. They are produced by bacteria that ferment agro-industrial waste and form hydrogels interacting with bivalent cations (Kwiecień & Kwiecień, 2018). Given the properties of xanthan, it needs to be mixed with other polymers to achieve optimal encapsulation applications. Alginate-xanthan matrix evidenced a higher survival rate of *L. plantarum* LAB12 after being incubated in gastric acid at pH 1.8 was higher (95%) than in free bacteria (46.15%) Fareez et al. (2015). In addition, the authors reported improvements in probiotic survival after exposure to SGC when coating the encapsulated material with chitosan. However, Chen et al. (2017) and Shu et al. (2018) found that *B. bifidum* BB01 and *L. acidophilus* encapsulation in xanthan/chitosan and xanthan/chitosan/xanthan matrices, respectively, showed significant improvements ($p < 0.05$) in probiotic survival when stored in yogurt for 21 days at 4 °C (8 log CFU $g^{-1}$), at 25 °C (5 log CFU $g^{-1}$), and after exposure to SGC (8 log CFU $g^{-1}$).

### *3.6 Chitosan*

Is a linear cationic natural biopolymer (amino polysacaccharide) that contains glycosidic linkages between monosaccharide units produced by the deacetylation of the naturally occurring chitin under high alkaline conditions (Dumitriu, 2004). Phuong Ta et al. (2021) suggest that incorporation of prebiotics into alginate-chitosan matrix encapsulation could lead to increase the survival of probiotics and their delivery to the target sites of action in human body. The first study in which a double coat of alginate and chitosan was used for the encapsulation of *L.plantarum* and *L. rhamnosus*, resulting in a higher encapsulating efficiency (98%) and promising improvement in the survival capacity of probiotics were reported by Padhmavathi et al. (2021).

## 4 Recent advances

Despite the numerous methods that have been used for oral administration of probiotics, the success rate achieved thus far remains limited. Hence, different strategies have been studied and proposed to increase or preserve the viability and stability of probiotics by combined encapsulation technologies (Table 4). Likewise, have alternatives of products of mass consumption different from milk or derivatives

### *4.1 Microencapsulation systems*

Microencapsulation of probiotic cells is now under special attention because it is considered as the best method for improving the survivability of probiotics (Padhmavathi et al., 2021). The viability of probiotics can be improved by embedding technologies within microgels or other types of microcapsules (Yao et al., 2020). Simple microgels, core-shell microgels, biopolymer-complex microgels, and nutrient-doped microgels, constitutes the main embedding technologies. Pectin, starch, gelatin, chitosan, and alginate are polysaccharide used in preparing microgels (Yao et al., 2020). Microgels are small spherical particles that form a network of cross-linked biopolymers inside, with the pores completed by an aqueous solution (Holkem et al., 2016).

The functional performance of core-shell microgels can be further enhanced by coating them with one or more layers of biopolymer molecules (de Araujo Etchepare et al., 2020). Chitosin is the most widely used polysaccharide in the formation of microgels due to its positive charge, whereas most other polysaccharides have a negative charge (Trabelsi et al., 2014). Biopolymer-complex microgels improve the viability of encapsulated probiotics under

**Table 4.** Encapsulation strategies emerging.

| Matrix | Probiotic | New Encapsulation Strategies | Main Result / Health Effect | Reference |
|---|---|---|---|---|
| WPI / SA WPI / FOS DWPI / SA DWPI / FOS | *L. plantarum* MTCC 5422 | SFD | Microcapsules exhibited good flowability and lower hygroscopicity. The method did not affect the cell viability. | Rajam & Anandharamakrishnan (2015b) |
| FOS / WP FOS / MD FOS / WP / MD | *L. plantarum* NCIM 2083 | SFD | SFD method demonstrated higher encapsulation efficiencies (96.16%) than spray drying (89.21%) and showed better survivability during digestion. | Yoha et al. (2020) |
| WPC / skimmed milk | *L. plantarum* CECT 748T | Electrospraying | Encapsulation efficiencies depend on voltage, surfactant and prebiotic concentration. Enhanced protection during storage at high relative humidity was observed and the method showed similar protection during digestion as freeze drying. | Gomez-Mascaraque et al. (2016) |
| WPC / gelatin | *L. plantarum* CECT 748T | Coaxial Electrospraying | The combination of high voltage with acetic acid showed severe impact on the probiotic, not only decreasing initial viability also negatively affecting the survival of probiotic during storage and their resistance. | Gómez-Mascaraque et al. (2017) |
| Poly (ethylene oxide) (PEO) / sucrose PEO / trehalose | *L. plantarum* ATCC 8014 | Electrospinning | The concentration of probiotic was reported as the most critical parameter for its high viability after electrospinning. the applied electric voltage and relative humidity. electrospinning demonstrated did not affect the viability | Škrlec et al. (2019) |
| Okara oil PPP12 Alginate | *L. plantarum* CIDCA 83114 | Microfluidic Freeze drying | Increased viability of bacteria under gastric conditions was observed with the use of PPP12 as the only dispersed phase | Quintana et al. (2021) |
| non-showed | *Lactobacillus jensenii* 1153 | Engineering | Expression of mCV-N with anti-HIV activity conserved in epithelial cell lines, expression of higher immunomodulatory potential by recombinant *L. jensenii* activity compared with control strains of *L. jensenii* 1153. | Yamamoto et al. (2013) |
| non-showed | *Lactobacillus gassei* ATCC 33323 | Engineering | Diabetic rats fed GLP-1–secreting bacteria showed significant increases in insulin levels and, additionally, were significantly more glucose tolerant than those fed the parent bacterial strain. | Duan et al. (2015) |
| Skim milk powder / trehalose β-lactoglobulin-propylene glycol alginate Soybean oil | *L. rhamnosus* GG | HIPEs | High resistance against pasteurization and demonstrated a significant reduction in the death of LGG (7.91 log cfu $cm^{-3}$) | Su et al. (2021) |
| WPI / EGCG FOS / skim milk | *L. plantarum* | HIPEs | Encapsulation of *L.s plantarum* powder was successful to enhance the viable cell count after 14 days storage and GIT digestion | Qin et al. (2021) |
| Wheat flour calcium caseinate | *L. plantarum* WCFS1 | 3D Printing | The viable counts of probiotics in the "honeycomb" structure exceeded 6 log cfu $g^{-1}$ when the end point of baking (at 145 °C) | Zhang et al. (2018b) |
| Green gram Barnyard millet Fried gram Ajwain seeds | *L. platarum* NCIM 2083 | 3D Printing | 3D printing offering benefits for the incorporation of probiotics. No significant loss of probiotic viability was observed during the 3D printing process | Yoha et al. (2021) |
| Alginate Inulin Rice bran Resistan starch | *L. acidophilus* | Co-encapsulation | Microcapsules showed higher protection for probiotics into the simulated GIT. The microencapsules containing prebiotics maintained viable probiotics for 4 months, but only inulin-treated demostrated to be more stable. | Poletto et al. (2019) |
| Calcium alginate / chitosan Inulin Resistant starch | *L. plantarum* *B. adolescentis* | Co-encapsulation | Inulin-treated were able to hinder the viability loss of probiotics | Zaeim et al. (2019) |

WPI: whey protein isolate; SA: sodium alginate; FOS: Fructooligosaccharide; DWPI: denatured whey protein isolate; WP: whey protein; MD: maltodrextrin; WPC: whey protein concentrate.

gastric conditions involve controlling the pore size and internal pH of microgels (Yao et al., 2017)). Nutrient-doped microgels Nutrient doped microgels that encapsulate probiotics within the nucleus of microgels have been studied to increase their viability (Liao et al., 2019)

### *4.2 Emerging encapsulation strategies*

*Spray-freeze-drying (SFD)*

In this method, the probiotics together with the encapsulating wall material (liquid feed) are atomized, forming fine droplets with a high interfacial area that come into contact with a cryogenic medium such as liquid nitrogen (Rajam & Anandharamakrishnan, 2015b; Rajam & Anandharamakrishnan, 2015b). Allows obtaining highly porous particles with excellent reconstitution capacity. However, its application on an industrial scale requires evaluating the high time consumed, adequate osmotic/ atomization pressure control, and the handling of cryogens (Meng et al., 2008; Semyonov et al., 2010).

*Electrohydrodynamic*

Electrohydrodynamic processes involve the use of electrostatic force to produce polymeric materials in the form of fibers (electrospinning) or powders electrospraying (Yoha et al., 2020). Electrospinning and electrospraying are well known within electrospinning processes. Several studies confirm that the electrospray coating improved the survivability and thermal stability of probiotics (Gomez-Mascaraque et al., 2016, Gómez-Mascaraque et al., 2017; Feng et al., 2018; Škrlec et al., 2019). This method is considered a promising process to protect microbial cells under various stress conditions. However, it requires adequate control of high voltage as this can be harmful to cells and affect their viability (Moayyedi et al., 2018; Phuong Ta et al., 2021)

*Microfluidics*

Very recent studies are finding synergy when applying microfluidic techniques for the individual cultivation of bacteria within double-layer emulsions (Yoha et al., 2021). Double water-in-oil-in-water (MDE) microfluidic emulsion is a relatively new class of soft solid, particularly in system encapsulation. MDE is considered a "deep functional profiling" technique, with the advantages of providing a single-celled functional characterization of the strain (Chen et al., 2018; Villa et al., 2019)

*Genetic engineering*

Engineering or genetically modified microorganisms (GMOs) is considered a promising way to achieve better performance of probiotic strains. It consists of the manipulation or design of a gene with specific properties or focused, for example, to improve tolerance to stress, extreme temperatures, oxygen, and acidification during food production, and or to treat metabolic diseases and cancer, and/or increase survival of probiotics under gastro-intestinal conditions (Appala Naidu et al., 2019)

Recently, the results of an animal study with GMOs have been reported, which proved to be very promising as they were able to treat diseases such as diabetes and colitis by having a metabolic pathway that efficiently produces and secretes various proteins (Speranza et al., 2020). However, they are classified as genetically modified organisms (Mathipa & Thantsha, 2017) and contain additional elements that could affect metabolic pathways and safety (Kumar et al., 2016).

*High-internal-phase emulsions (HIPEs)*

HIPEs are commonly defined as highly concentrated emulsions with an internal phase volume fraction of more than 74% or 64% for hexagonal close packing or random close packing of the droplets, respectively (Xu et al., 2020). HIPEs process integrated with Co-encapsulation technical exhibited a significantly higher physical stability as well as better protecting effects on strain and bioactive agent against pasteurization treatment (Su et al., 2021)

*3D Printing*

3D printing is an emerging technology and has promising food and encapsulation applications (Nachal et al., 2019; Pereira et al., 2021). Recent studies have reported the printing process integrated with encapsulation has no adverse effect on the viability of probiotic cells (Zhang et al., 2018b; Yoha et al., 2021)

*Co-encaspulation*

This technique consists of taking advantage of the same delivery vehicle or matrix to incorporate more than two active components that will lead to increased efficiency in the stability and/or viability of probiotics. Rashidinejad et al. (2022) assure that probiotic/prebiotic co-encapsulation is an effective method of administration of probiotic live cells and that a greater survival efficiency of probiotics can be achieved during the encapsulation process and the manufacture and storage of food. Likewise, Raddatz & Menezes (2021) and Youssef et al. (2021) reported different studies with an increase in the survival of probiotic cells by co-encapsulating.

Table 4 Summarizes the main encapsulation strategies emerging with the potential to increase the viability of probiotics in real application conditions.

### 4.3 Thermal resistance

Through modern encapsulation systems it has been possible to respond to one of the main challenges of a decade ago, and that was to improve the viability of probiotics during manufacturing processes, particularly thermal processing (Solanki et al., 2013). Table 5 describes the components of the matrices and results obtained with better behavior or thermal resistance. The use of carbohydrate as protectants of bacterial cell can alternatively be explained by the water replacement hypothesis, which envisages the function of carbohydrate as water substitutes when the hydration shell of proteins, as well as water molecules around polar residues in membrane phospholipids, are removed (Tantratian & Pradeamchai, 2020). The ability to protect bacterial cells of carbohydrate is related to the difference in their glass-forming tendencies, which is reflected in their glass transition temperatures (Tg) and indicates the efficacy of a protective agent to protect the bacterial cell during drying. Glass transition temperature (Tg) of selected protective agents in decreased order starch > maltodextrina >carbomethycellullose>lactose >sucrose >glucose.

Recent studies confirm that the combination of different methods such as: extrusion, emulsion, and spray drying methods used in the encapsulation of probiotics give a better heat resistance performance (greater viability) than that achieved with the one alternative method (Silva et al., 2018; Pupa et al., 2021). In a review article by Călinoiu et al. (2019), the importance of chitosan coating in encapsulation was investigated for improve the survival of the probiotic. The conclusion suggested that the

**Table 5**. Protective encapsulation systems and methods encapsulation that improve the thermal behavior of probiotic strains.

| Matrix | Probiotic | Method | Reference |
|---|---|---|---|
| Chitosan / alginate | *L. rhamnosus* GG | External gelation / Freeze drying | Cheow et al. (2014) |
| Maltodextrin Whey protein D-mannose | *L. plantarum* KLDS1.0344 | Spray drying | |
| Milk-derived | *B. lactis* INL1 | Spray drying | Burns et al. (2017) |
| Whey protein / maltodextrin | *L. rhamnosus* | Spray drying | Agudelo et al. (2017) |
| Chia seed (*Salvia hispanica* L.) and flaxseed (*Linum usitatissimum* L.) mucilage | *L. plantarum* ATCC8014 *B. infantis* ATCC15679 | Spray drying | Bustamante et al. (2017) |
| Trehalose Hi-maize Inulin | *L. acidophilus* LA5 | Spray drying | Nunes et al. (2018) |
| Alginate / denatured protein | *L. acidophilus* | Emulsification / internal gelation | Fang et al. (2018) |
| Maltodextrin / reconstituted skim milk / gum arabic | *L. casei* Shirota | Spray drying / freeze drying | Gul & Atalar (2019) |
| Alginate / Hylon starch-chitosan/ poly-L-lysine | *L. casei* ATCC 39392 *B. bifidum* ATCC 29521 *L. rhamnosus* ATCC 7469 *B. adolescentis* ATCC 15703 | Emulsification / freeze drying | Khosravi Zanjani et al. (2018) |
| Starch / Pulque | *L. pentosus* | Spray drying | Hernández-López et al. (2018) |
| Arabinoxilan gel | *B. longum* ATCC 15708 *B. adolescentis* ATCC 15703 | Electrospray | Paz-Samaniego et al. (2018) |
| Carboxymethylcellulose Hydroxypropylmethylcellulose Methylcellulose | *L. paracasei* LPC37 | Spray drying | Tao et al. (2019) |
| Glucose Maltodextrin DE10 Lactose Sucrose soluble Starch | *L. plantarum* FT35 | Spray drying | Tantratian & Pradeamchai (2020) |
| Modified corn starch (acid treated) Acacia gum Maltodextrin Carboxymethylcellulose Methylcellulose | *Saccharomyces cerevisiae* var. Boulardii | Coating | Singu et al. (2020) |
| Sodium alginate /carrageenan | *L. acidophilus* ATCC 4356 | External gelation | Afzaal et al. (2020) |
| Alginate / chitosan | *L. plantarum* 31F *L. plantarum* 25F *L. plantarum* 22F *Pediococcus pentosaceus* 77F *P. acidilactici* 72N | Extrusion Emulsion Spray drying | Pupa et al. (2021) |
| Resistant starch / D-mannose / maltodextrin / whey protein | *L. acidophilus* KLDS 1.1003 | Spray drying | Muhammad et al. (2021) |

chitosan coating provided the probiotic with greater survival at high temperatures.

#### *4.4 Next generation probiotics*

The administration of traditional probiotics does not aim against specific diseases. Based on these situations, identification and characterization of novel and disease-specific next-generation probiotics (NGP) are urgently needed (Olveira & González-Molero, 2016). Through analyses using the next generation sequencing and bioinformatics platforms, many potential NGP are currently under intensive development (Chang et al., 2019).

*Christensenella minuta*, *Parabacteroides goldsteinii, Prevotella copri,* and *Akkermansia muciniphila* are selectively as NGP potential against obesity and associated metabolic disorders (Everard et al., 2013; Plovier et al., 2017; Cani & de Vos, 2017) while *Bifidobacterium* spp and Bacteroides fragilis present in a systematic amelioration of inflammation-related diseases such as abscess, neuro-inflammations and good outcomes of anticancer therapies (Round & Mazmanian, 2010; Huang et al., 2011). Studies with *L. johnsonii* BS15 demonstrated that this type of microorganism prevents psychological stress-induced memory dysfunction in mice by modulating the intestinal environment (Wang et al., 2020a). In addition, it was possible to recover or repair the intestinal physiology (microbiota) and reverse the memory deficit in mice that were stressed and exposed to fluorides by inoculating *L. johnsonii* BS15 (Xin et al., 2021).

A higher molecular weight polysaccharide fraction (>300 kDa) isolated from the water extract not only lowers body weight by 50% but it also reduces intestinal permeability, metabolic endotoxemia, inflammation, and insulin resistance (Chang et al., 2015).

## 5 Trends and challenges

One of the main challenges in the encapsulation of probiotics is the development of manufacturing processes for products with greater tolerance to high temperatures. The opportunity exists to develop commercial probiotic products that are stable at higher temperatures. Consequently, future research should be focused on producing heat-resistant probiotic microorganisms (natural or engineered or recombinant) and developing encapsulation systems that act effectively as an "insulating material". Another challenge is focused on generating a greater number of in vivo studies of human nutrition to demonstrate the effectiveness and impact on health and anti-inflammatory diseases (Călinoiu et al., 2019; Simon et al., 2021). In addition, to know the results of different clinical trials on the effect and mechanism of action of probiotics in patients with Covid that evaluate the efficacy and safety of *Clostridium butyricum* capsules and live *Bacillus coagulans* tablets for the treatment of patients affected by pneumonia. by the new coronavirus and to study its mechanism of action (Gao et al., 2020; Vodnar et al., 2020).

## 6 Conclusions

Health benefits of probiotics have significantly increased their use and encouraged the food industry to develop alternative, non-dairy probiotic products. The regular use of probiotics poses challenges to the study of other associated therapeutic effects. Encapsulation of probiotics with highly digestible and naturally compatible polysaccharides has been found to be a promising approach. Polysaccharides can be used to provide protection for encapsulated probiotic cells under adverse conditions. Combining two polysaccharides or polysaccharides and non-polysaccharides can have a synergistic effect on the properties of the encapsulated materials, aiding encapsulation viability and improving protection against harsh conditions that reduce stability, storage, and consumption, compared to matrices based on only one polysaccharide. Pectin and starch-based blends are of increasing interest as a low-cost alternative for encapsulation of probiotics with a high viability rate, as well as probiotic and immunostimulatory capacity. Microfluidics, HIPE, double coating, coencapsulation, 3D printing, coaxial electrospinning or combinations ot these techniques show promise for the encapsulation of non-dairy probiotic products. These techniques are characterized by high yield and (probiotic) protection, as well as improved preservation and stability for longer shelf life

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