PROBIOTICS

ADVANCED FOOD AND HEALTH APPLICATIONS

Edited by

ADRIANO BRANDELLI

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Preface

The rapid progress of scientific research documented in the last decades, culminating in a burst of novel methodologies for both laboratory and in vivo studies, resulted in a significant development of our understanding of the functions of gut microbiota and the potential of probiotics to maintain and improve human health. Significant advances in the field of food science also recognized foods as an important way to deliver beneficial microorganisms, offering a groundbreaking approach to the development of functional products and prevention of specific diseases. Thus, a diversity of food formulations and medical approaches based on the use of probiotics have been suggested to deal with gastrointestinal and other diseases through modulation of the intestinal microbiota.

During the preparation of this book, the world has been challenged by the difficulties imposed amid the COVID-19 pandemic. Although there is no definitive conclusion on the potential benefits of probiotics, many studies provide evidence that the acknowledged immunomodulatory and anti-inflammatory properties of some probiotic strains can be helpful to ameliorate the symptoms of COVID-19. This is summed with evidence that probiotics can be important auxiliary therapeutics for many diseases.

The book *Probiotics: Advanced Food and Health Applications* contains 24 chapters prepared by important specialists that have made significant impacts on the field of probiotics and related research sectors. This book addresses diverse points of both essential and practical aspects of probiotics, presenting the topic from fundamentals to state-of-the-art information. This approach allows the reader to start with essential information on probiotics, understanding their nature and functional properties, essential features, and related concepts such as prebiotics, synbiotics, and microbiota composition. The natural occurrence of probiotics in foods is addressed, and this book then discusses advanced technological aspects of food formulations, nutrition, and health implications. As the basic working mechanisms of probiotics are revealed, the importance of the complex relationship among the composition of the intestinal microbiota, gut-associated immune system, the fermentation of substrates (prebiotics), and probiotic metabolites on the health status is discussed. This comprehensive coverage provides up-to-date and highly organized material for undergraduate and postgraduate students in food science, nutrition, biomedical sciences, biotechnology, and related fields, but is also a valuable resource of recent scientific advances and applications of probiotics in the food sector to be used by researchers, professionals, and academics.

This book includes sections written by a diverse and highly qualified set of contributing authors, composed by a group of international experts from universities, research centers, and corporations around the world. The efforts of the contributing authors at assembling a large amount of recently published referred research into outstanding chapters are deeply acknowledged as the key factor that made this book possible. This book brings the most recent and innovative applications of probiotics in food and health sectors, and it will hopefully be a useful reference and resource for those requiring insight and current knowledge in the fascinating field of probiotics.

Chapter 10

Encapsulation of probiotics

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Abbreviations

BSH	bile salt hydrolases
CAGR	compound annual growth rate
CFU	colony-forming units
EMU	emulsification
EXT	extrusion
FAO	Food and Agriculture Organization
FBD	fluid bead drying
FD	freeze drying
GALT	gut-associated lymphoid tissue
GEL	ionic gelation
GI	gastrointestinal
IBD	inflammatory bowel disease
IMP	impinging aerosol
LAB	lactic acid bacteria
SD	spray drying
SPC	spray chilling
WHO	World Health Organization

10.1 Introduction

Elie Metchnikoff (1845–1916) is considered the grandfather of modern probiotics (*Greek*, "for life") science. In his book *The Overtime of Life* (Metchinkoff, 1908), he proposed that administering live beneficial microbes to humans through fermented dairy may result in better health and senility delay (Zendeboodi et al., 2020). However, to exert such effects on the host, enough live cells should be guaranteed during storage and gastrointestinal (GI) passage. The loss of cell viability in prepared foods (especially fermented ones) and harsh GI conditions (e.g., low pH/osmolarity) has encouraged researchers to find new protection methods (Mokhtari, Jafari, & Khomeiri, 2019), from which microencapsulation and nano-covering stand are the most studied. These methods protect viable cells from oxygen, light, temperature, osmolarity, and free radical damage (Corona-Hernandez et al., 2013). Modern omics sciences offer new perspectives on the differential modulation of probiotics' metabolism when delivered to the GI tract in free vs. entrapped or viable vs. nonviable cells. These and other relevant aspects of probiotic science are reviewed and discussed in the following sections.

10.2 Market and research trends

The global market of probiotic-based foods has shown a sustained expansion in the last two decades. Its size (in billion USD) was estimated at 32.1 in 2013 and 48.8 in 2018, with unstoppable growth to 57.4 billion in 2022; projections indicate a compound annual growth rate (CAGR) of 7.7% during 2016–22 (Dixit, Wagle, & Vakil, 2016; Allied Market Research, 2021). The global probiotic market is segmented by the type of microbial culture [bacteria (*Lactobacilli*, *Bifidobacterium*,

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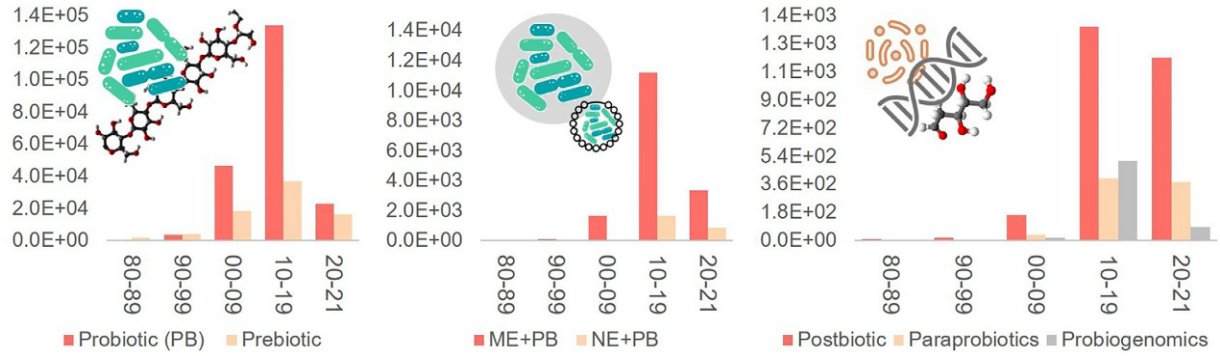

FIG. 10.1 Research trends in the probiotic arena: 1980-onward. *(Source: Google Scholar. No permission required.)*

and *Streptococcus*) > yeast (*Saccharomyces* spp.)], function (regular > preventive care > therapeutic), application (food and beverage > dietary supplement > animal feed), consumer (human > animal), and geography (Asia-Pacific > Europe > North America > Latin America, Middle East, and Africa), being probiotic supplements for human use the outstanding and most profitable trend.

Consumer awareness on digestive health and immunity-promoting foods have justified incremental investments in product innovations and novel food technologies by key probiotic market players, a fact that will increase their market share within the functional food and nutraceutical segments. Moreover, public sanitary emergencies such as the COVID-19 pandemic (2019-onward) caused an extraordinary boost in the segment of artisanal and industrialized fermented foods since probiotics have a tremendous potential to prevent SARS-CoV-2 infection (Anwar et al., 2020). However, the reliability and accuracy of any launched probiotic-based product can vary among the product category and geographical regions (Dixit et al., 2016; Sanders, Merenstein, et al., 2018; Sanders, Benson, et al., 2018). As if this were not enough, regulations on the use and labeling of probiotics for the nutraceutical, cosmetic, and dietary supplement markets still pose a challenge for regional and global markets (Dixit et al., 2016; Sanders, Benson, et al., 2018).

The search for scientific support to sustain health claims associated with beneficial microorganisms has taken a new course very recently. A brief inspection of scientific articles, academic documents (e.g., postgraduate thesis) indexed in *Google Scholar* reveals not only that research and development in probiotic science have both grown exponentially in the last decade but also that novel approaches based in "omics" sciences are gaining field (Castro-López et al., 2021; Mozzi et al., 2013; Papadimitriou et al., 2015; Sánchez et al., 2013). According to the information compiled in Fig. 10.1, the number of reports on probiotic > prebiotic (group of nutrients and xenobiotics that can be degraded by either gut microbiota or exogenous probiotics) and micro- (ME) > nano-encapsulation (NE) grew exponentially ($e^{1.2}$ to $e^{3.0}$, $R^2 \geq 0.96$) between 1990 and 2020, while new reports published between January 2020 and March 2021 were ~23,000, ~16,000, ~3300, and ~900, respectively.

Also, probiotic-related terms such as postbiotics, paraprobiotics, and probiogenomics (Aguilar-Toalá et al., 2018; Taverniti & Guglielmetti, 2011) have expanded the horizon for probiotic research from 2010 onward. Soon, probiotic market differentiation will be based on specific strains (beyond whether they are bacteria or yeasts) since these have a specific metabolomic signature. For example, *Immuse* is a novel paraprobiotic (*L. lactis* plasma) ingredient patented by Kyowa Hakko (USA) that improves immunity by activating plasmacytoid dendritic cells (DC) while Morinaga milk industries launched another ingredient called LAC-shield (heat-killed *Lactobacillus*—now *Lacticaseibacillus—paracasei* MCC1849) also with immunoenhancing activity (Maehata et al., 2021). Moreover, new trends in personalized nutrition and the advanced knowledge on the human microbiome project will drive new market launches for specific health situations (disease-specific prevention claims) as support of this is the number of patents of probiotic-based products which climbed from 360 to 1200 between 2000 and 2010, a fact that is partially explained by the scientific support for claims about the preventive action of probiotics in lipid metabolism disorders, obesity, immunocompromised illnesses, allergies, viral infections, and GI disorders, among others (Chavda et al., 2020; Dixit et al., 2016; Wilkins & Sequoia, 2017).

10.3 The gastrointestinal journey of probiotics

Many nutritional and functional benefits associated with beneficial microbes have held the attention of the world scientific community; their potential to prevent and even treat various diseases while achieving an optimal nutritional state has driven research exponentially (Fig. 10.1). However, health claims initially depend on the intake of an effective number of live

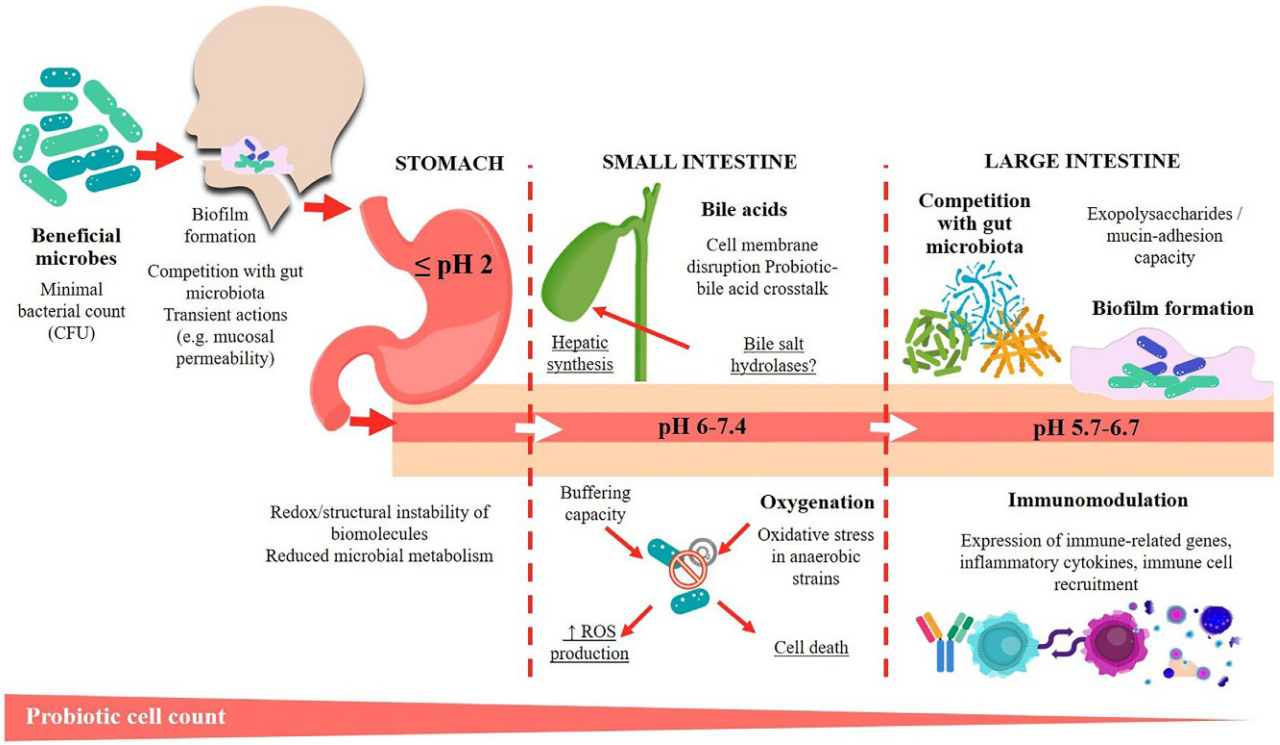

FIG. 10.2 Gastrointestinal barriers that compromise the viability of beneficial microbes. *(Source: The authors. No permission required.)*

microbial cells that must remain viable throughout the GI tract transit. However, the latter has a highly changing physical and biochemical environment that compromises the fate of these beneficial microbes. Probiotics must overcome many harsh conditions including sudden oxygen and pH changes, interaction with enzymes and mucin, nonspecific interactions, and competition with the host's colonic microbiota (Liu et al., 2019; Suez et al., 2019; Wilkins & Sequoia, 2017). Most of these factors are depicted in Fig. 10.2 and explained below.

The oral and gastric microenvironments represent the first two barriers to overcome. *Lactobacillus* (gram-positive, homofermentative, acid-tolerant, facultative anaerobic, thermophilic, and nonspore-forming rods/coccobacilli) (Huang et al., 2018), *Bifidobacterium* (gram-positive, heterofermentative, thermophilic, and nonmotile, nonspore-forming rods) (Duranti et al., 2019), and *Streptococcus* (gram-positive coccus, facultative anaerobic) (Spellerberg & Brandt, 2015) strains seem to colonize the oral cavity competing with biofilm-forming microorganisms (e.g., *C. albicans*, *C. glabrata*, *P. aeruginosa*, and *S. aureus*) for adhesion sites, inhibiting pathogen colonization and performing other bioactivities in caries and oral cancer prevention (Allaker & Stephen, 2017; Barzegari et al., 2020) but reducing their odds to complete their GI journey. Once probiotics leave the oral cavity, the second barrier is gastric pH (Fig. 10.2): evidence suggests that high H^+ levels (pH <2.0) promote denaturation of the cell wall and cytoplasmic macromolecules, including proteins, cholesterol, and nucleic acids that compromise microbial metabolism and viability (Liu et al., 2019). Conversely, probiotic counts remain constant at intestinal (pH 6.0–7.4) and colonic (pH 5.7–6.7) conditions. Thus, acid pH and macromolecular complexation are considered the main obstacles to probiotic colonization in the lower GI and so, personalized probiotic therapies must consider the use of pH-resistant strains, massive probiotic supplementation, and/or the use of immobilized probiotics in food-grade matrices to ensure their viability (Corona-Hernandez et al., 2013).

The nature of the intestinal microbiome (taxa and biomass) is related to host-microbiota redox homeostasis and O_2 microfluctuations (Friedman et al., 2018). High levels of oxygen in nontolerant bacteria initiate a cascade of events that begin with the production of oxidant molecules, such as superoxide anion ($O_2 \cdot^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO·), followed by damage to microbe macromolecules and finally cell death (Feng & Wang, 2020). It is noteworthy that *Lactobacillus* spp. (Huang et al., 2018) and *Streptococcus* spp. (Spellerberg & Brandt, 2015) are more tolerant of oxygen fluctuations and oxidative stress than *Bifidobacterium* spp. (Duranti et al., 2019).

Under intestinal conditions, probiotics are also susceptible to bile acid damage (Fig. 10.2). Chenodeoxycholic acid, cholic acid, and deoxycholic acid have detergent properties and can disrupt bacterial membranes and exert antibacterial activity (Prete et al., 2020). However, not all lactic acid bacteria (LAB) will be affected by bile acids as over time the

bacterial genome has acquired adaptive mechanisms to deal with these conditions. Certain *Lactobacillus* and *Bifidobacterium* members express bile salt hydrolases (BSH) which provide competitive advantages over other non-BSH producers (*L. lactis* and *S. thermophilus*) in terms of cell survival while decreasing the blood cholesterol levels in the host (Fiocco et al., 2020; Ruiz et al., 2012). At this point, bacteria capable of withstanding the above harsh conditions will be available to form communities composed of single as well as multispecies interacting through syntrophic relations in the form of biofilms (Fig. 10.2). It is noteworthy that *Lactobacillus mucosae* is not a major member of the gut microbiota; yet it poses a great mucin-adhesion capacity, good autoaggregation, and cell wall hydrophobicity that help in its interaction with human epithelial cells and other microorganisms, such as *Lactobacillus gasseri* and *B. brevis*, improving their colonization by quorum sensing and their interaction with intestinal epithelial cells (Pereira et al., 2018).

Certain *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and *Saccharomyces* (yeast) species survive the adverse conditions of the GI tract, colonizing it and improving the overall health status of the host. Furthermore, they interact with the gut-associated lymphoid tissue (GALT), exerting immunomodulatory and antiinflammatory actions associated with the improvement of acute infectious diarrhea, lactose intolerance, and inflammatory bowel disease (IBD), among other conditions (Leis et al., 2020; Wilkins & Sequoia, 2017), as it will be reviewed in other chapters. Some beneficial effects of probiotics are summarized in Table 10.1. Such probiotic-GALT crosstalk is partially due to the ability of probiotics to build biofilms that constitute an interchange bridge for biotic products (postbiotics and paraprobiotics) and a transient transmural transit in a strain-specific and dose-dependent manner (Maehata et al., 2021; Santiago-López et al., 2021).

In conclusion, several physicochemical and biochemical factors affect the fate of probiotics within the GI tract. This fact makes it necessary to establish high doses of habitual consumption of these organisms or to guarantee their viability through cell entrapment techniques. In support of this, Taverniti and collaborators compared the effect of administering two doses (F1 and F2, containing 7 and 70 billion colony-forming units (CFU) per capsule, respectively) of a shelf-stable industrially lyophilized multistrain formulation FlorMidabìl. This formulation is composed of *Bifidobacterium animalis* subsp. *lactis* Bl-04 and *Lactobacillus acidophilus* La-14 from the human fecal origin, *L. plantarum* SDZ-11 (from fermented vegetables) and *Lactobacillus paracasei* SDZ-22 (dairy origin). They demonstrated the cooperative survival of probiotics in the GI tract upon ingestion but F2 formulation caused a higher, earlier, and longer recovery of the probiotics in feces from healthy volunteers (Taverniti et al., 2019). As for cell entrapment techniques, they will be discussed in the following paragraphs.

TABLE 10.1 Gastrointestinal ameliorating effects of selected probiotics.

Illnesses	Probiotics	Health benefits
Antibiotic-associated diarrhea	*Lactobacillus rhamnosus/S. boulardii*	↓Stool frequency/diarrhea, ↑recovery rate
Colic	*Lactobacillus reuteri*	↓Abdominal pain, crying (vs. placebo)
Lactose intolerance	*L. bulgaricus, Lactobacillus acidophilus, Bifidobacterium longum*	↓Abdominal pain, bloating, diarrhea, flatulence
Chronic gastritis	*L. acidophilus/Lactobacillus casei*	Synergistic action with antibiotics against *Helicobacter pylori*
Irritable bowel syndrome	*B. longum*	↓Local/systemic symptoms, bloating, flatulence
Hepatic encephalopathy	*B. longum, B. breve, L. acidophilus, L. casei*	↓Plasma ammonia, ↑recovery rate
Constipation	*B. longum*	↑Stool frequency, recovery rate (vs. placebo)
Ulcerative colitis	*B. longum, B. breve, L. acidophilus, Bifidobacterium lactis*	↑Remission rates, same effect to 5-aminosalycilic acid (5-ASA)
Intestinal cancers	*Lactobacillus* spp.	Sequestration of mutagenic xenobiotics, ↑SCFA probiotic-GALT cooperation

GALT, gut-associated lymphoid tissue; *SCFA*, short-chain fatty acids.
Data source: Leis, R. et al. (2020). Effects of prebiotic and probiotic supplementation on lactase deficiency and lactose intolerance: A systematic review of controlled trials. *Nutrients. 12* (5), 1487. https://doi.org/10.3390/nu12051487 and Wilkins, T. and Sequoia, J. (2017). Probiotics for gastrointestinal conditions: A summary of the evidence. *American Family Physician, 96* (3), 170–178.

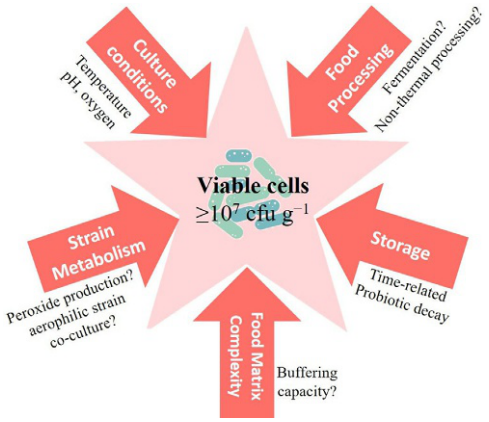

FIG. 10.3 Critical aspects for probiotic survival. *(No permission required.)*

10.4 Storage of probiotic-based foods and over-the-counter (OTC) formulations

As previously stated, the ability of probiotics to confer health benefits on the host depends upon the number of viable and active cells wherein the product preserving the survival of bacterial cells within food matrices during processing and storage is challenging and has been a topic of continued discussion by food scientists (Liu et al., 2019). Nevertheless, both natural (fermented goods) and artificial (microencapsulated bacteria) probiotication of foods are useful strategies to increase consumer acceptability and promote an adequate environment for maintaining bacterial viability ($\geq 10^7$ CFU g^{-1}) before consumption (Mokhtari et al., 2019). A good selection of the probiotic strain, culture conditions, chemical nature of food matrices, food processing, and/or the storage conditions depends on the number of live cells to be delivered in the intestinal lumen and their ultimate health effects (Corona-Hernandez et al., 2013; Frakolaki et al., 2021). The critical parameters for the maintenance of the viability of probiotic bacteria are summarized in Fig. 10.3.

Temperature is a cornerstone to preserving probiotics' survival. Several reports indicate that the thermal treatment above 45°C (e.g., baking and pasteurization) has a tremendous impact on the probiotics' viability (Dinkçi, Akdeniz, & Akalin, 2019). Fortunately, nonthermal technologies such as freeze drying (FD), emulsification (EMU), and impinging aerosol (IMP) increase the odds for cell survival. Once processed, food matrix components may also play a role in probiotics' viability. Food additives (e.g., nitrite, artificial sweeteners, or food-grade colorants) may be deleterious for certain probiotics. On the other hand, antioxidant compounds and protein hydrolysates could prevent oxidative damage, by modulating pH during storage and ensuring probiotic stability at a neutral pH (Corona-Hernandez et al., 2013; Liu et al., 2019). Additionally, it has been recognized that molecular oxygen reduces microbial growth and survival during storage. Thus, the assessment of oxygen tolerance by *Lactobacillus* and *Bifidobacterium* is an essential tool, but also the inclusion of high-oxygen consuming strains (such as *S. thermophilus*) has been recommended to protect strict anaerobic strains (such as *Bifidobacterium*) from oxygen toxicity (Ladero & Sánchez, 2017).

10.5 Selection of probiotic strains

After many changes to the original Metchnikoff's definition (Zendeboodi et al., 2020), a consensus to define probiotics was reached in 2001 by the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) expert panel as follows: "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). Such definition covers a broad range of microbes and applications, while capturing Metchnikoff's essence of beneficial microbes (Parker, Tindall, & Garrity, 2019; Zheng et al., 2020). According to this accepted definition, viability is an inherent property of probiotics. However, recent studies have evidenced that not all mechanisms nor clinical benefits are necessarily directly related to viable bacteria and new terms such as paraprobiotics (also defined as "inactivated" or "ghost probiotics"), nonviable microbial cells (intact or broken), or crude cell debris with complex chemical composition, and postbiotics (metabiotics, biogenics, or cell-free supernatants), byproducts of the fermentation process (soluble metabolites) or released after bacterial lysis, have been coined to provide a wider dimension to the original probiotic concept (Aguilar-Toalá et al., 2018; Vallejo-Cordoba et al., 2020).

Despite these emerging terms having been adopted rapidly in the last decade (Fig. 10.1), a new terminology based on a different notion has also been proposed, which consists of categorizing the probiotic concept into three classes according to

its functionality: true probiotic (viable and active cells), pseudo-probiotic (viable and inactive cells in the forms of vegetative cells or spores), and ghost probiotic (dead/nonviable cells, in the forms of intact or ruptured). In turn, each class is subclassified into internal (in vivo) or external (in vitro) based on their site of action and/or impact (Zendeboodi et al., 2020). Although all these valuable attempts to properly define the term probiotic to be used responsibly, it is worth mentioning that only those characterized strains with a scientifically demonstrated effect on health may correctly be named as probiotics; to determine whether a candidate strain (or combination of them) qualifies for probiotic status regardless of the final application, some key aspects of bacterial strains should be considered (Fig. 10.4). Thus, potential probiotic bacteria must be: (i) sufficiently characterized (taxonomically defined at genus, species, and strain level); (ii) safe for its intended use; (iii) scientifically substantiated (at least one positive human clinical trial) regarding its health benefit in the target host; and (iv) alive in the product at an efficacious dose throughout shelf life (Binda et al., 2020).

Evidence has shown that the nomadic bacteria (e.g., *L. plantarum*, *Lactobacillus casei*, and *Lactobacillus fermentum*) have a wider genome and more tools to adapt to different environments, while host-adapted species, such as *Lactobacillus reuteri*, *Lactobacillus johnsonii*, and *Lactobacillus acidophilus*, are more competitive when compared to bacteria that do not share an evolutionary history with the host. This may imply that the evolutionary history of bacteria determines how they interact with the host (Papadimitriou et al., 2015). For this reason, probiotics should be sufficiently characterized, and a key component of correct characterization is proper strain identification and naming, which requires the use of internationally accepted procedures, such as microbial culturomics techniques, and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS), and whole genome sequencing approaches (Castro-López et al., 2021). Then, identified bacteria should be named according to the currently valid bacterial nomenclature (Parker et al., 2019; Zheng et al., 2020).

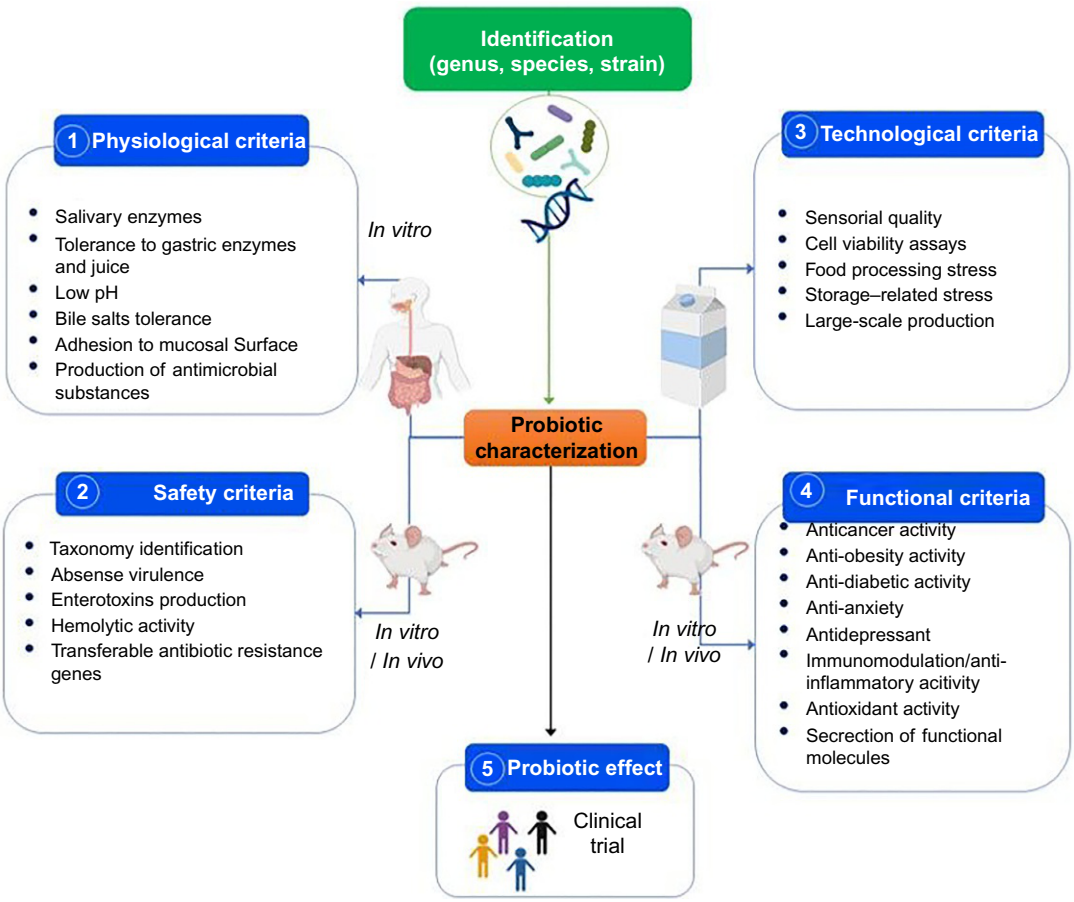

FIG. 10.4 Criteria to qualify potential probiotics in foods and over-the-counter (OTC) formulations. *(Data source: Binda, S., et al. (2020). Criteria to qualify microorganisms as 'probiotic' in foods and dietary supplements.* Frontiers in Microbiology, 11, *1662. https://doi.org/10.3389/fmicb.2020.01662. No permission required.)*

In addition to proper strain identification and documented historical data of safe use, safety tests should also be performed (Sanders et al., 2010). Particular focus can be placed in: i) the intrinsic bacterial properties such as bile salt deconjugation capacity, the presence of transferable antibiotic resistance genes, and mucin degradation properties; ii) pharmacokinetic properties (e.g., method of administration, level of exposure, the health status of the host); and iii) strain-host interactions (possible health and adverse effects) by animal models, clinical trials, and accurate surveillance and epidemiological studies (Gambaro et al., 2020; Žuntar et al., 2020). Although it has widely been accepted that probiotic effects are strain-dependent (Table 10.2), subspecies-specific, species-specific, or genus-specific probiotic effects may also exist (Sanders, Benson, et al., 2018; Sanders, Merenstein, et al., 2018). Hence, identification of genus or species-specific risk factors and testing at strain level are also required.

TABLE 10.2 Health-promoting mechanisms of postbiotics and paraprobiotics.

Beneficial microbe	Health benefits	Model	Paraprobiotic/ postbiotic	Bioactivities
Enterococcus faecium (WEFA23), *Lactobacillus gasseri* (FR4), *Lactobacillus fermentum* (E-3; E-18), *Pediococcus pentosaceus* (M41), *L. plantarum* (YML009, ZDY2013), *Lactobacillus casei* (BL23)	Antioxidant	Caco-2 and RAW 264.7 cells	Exopolysaccharides, SOD, catalase, glutathione	↓O_2^-, H_2O_2, ROS, RNS, ↑metal binding
Lactobacillus rhamnosus (GG), *L. gasseri* (FR4), *E. faecium* (WEFA23)	Antimicrobial	In vitro/in vivo *E. coli* K1 infection	Enterocins, reuterin, bacteriocins, reutericyclin, CO_2, diacetyl organic acids, exopolysaccharides, cell-free supernatant	Bacterial antagonism with *E. coli, Salmonella typhimurium, M. luteus, Salmonella enterica, Listeria monocytogenes* (MTCC 657); pore-forming and inhibition of cell wall/ nucleic acid/protein synthesis/activity
Bifidobacterium longum (BCRC 14634), *Bacillus licheniformis* (BioE-BL11), *Lactobacillus reuteri* Mh-001, *L. fermentum* (Lf2), *B. coagulans* (GBI-30), *L. casei* (Shirota)	Immunomodulation, antiinflammatory	Mouse splenocytes, monocytes, and macrophages, human/mice	Soluble peptides/ protein, butyric acid, cell-free supernatant, cell wall and cytosolic components, exopolysaccharides	↑B/T lymphocytes, natural killer cells, phagocytic/ mitogenic activity; ↓Signaling pathways (NF-kB, MAPK, pattern recognition receptors pathways); ↑IgM/IgG, cytokines (e.g., TNF-α, IL-1β, and IL-17), maturation of dendritic cells
P. pentosaceus (M41, FP3), *L. plantarum* (NCU116, JCM1149), *L. fermentum* (NCIMB 5221, 2797, 8829)	Anticancer	Colon cancer cells	Lipoteichoic acid, butyric acid, CLA	↑Binding/degradation of procarcinogens/ xenobiotics, ↑apoptosis
L. plantarum (RJF4, H31), *E. faecium* (F1), *B. longum* BL1	Hypolipidemic, antidiabetic, antiobesogenic	WT C57BL/6J mice	Cell wall-derived muramyl dipeptide, exopolysaccharides	↓Normolipidemic action (total and LDL-cholesterol, TAG), bile salt hydrolase activity; ↓adipose tissue inflammation, glucose intolerance
L. plantarum (LRCC5310)	Antiviral	BALB/c mouse model	Lipoteichoic acids	↑Viral inhibitory substances, ↑Th1-type, innate and adaptative immunity

Continued

TABLE 10.2 Health-promoting mechanisms of postbiotics and paraprobiotics—cont'd

Beneficial microbe	Health benefits	Model	Paraprobiotic/ postbiotic	Bioactivities
B. longum (NCC3001), *Bifidobacterium bifidum* (W23), *L. brevis* (W63), *L. casei* (W56), *L. helveticus* (R0052), *B. longum* (R0175), *Lactobacillus acidophilus* (La5)	Antianxiety/ depression	Randomized controlled trial (RCT)	Not determined	↓Anxiety and depressive symptoms, insulin resistance, HOMA-IR, CRP, glutathione, and inflammatory biomarkers
L. plantarum	Antiviral (SARS-CoV-2)	In silico protein docking	Plantaricin D, W, JLA-9	Strong affinity (11.1–8.0 Å) to the residual binding protein (RBD)

CLA, conjugated linoleic acid; *CRP*, C-reactive protein; *HOMA-IR*, homeostatic model of insulin resistance; *ROS*, reactive oxygen species; *RNS*, reactive nitrogen species; *SCFA*, short-chain fatty acids; *SOD*, superoxide dismutase.
Data source: Angelin, J. and Kavitha, M. (2020). Exopolysaccharides from probiotic bacteria and their health potential. *International Journal of Biological Macromolecules, 162*, 853–865. https://doi.org/10.1016/j.ijbiomac.2020.06.190; Vallejo-Cordoba, B. et al. (2020). Postbiotics and paraprobiotics: A review of current evidence and emerging trends. In Cruz, A. G. et al. (Eds.), *Advances in food and nutrition research. Vol. 94. Probiotic and prebiotics in foods: Challenges, innovations and advances*. Mexico: Academic Press Inc., pp. 1–34. https://doi.org/10.1016/bs.afnr.2020.06.001; Gambaro, E. et al. (2020). 'Gut–brain axis': Review of the role of the probiotics in anxiety and depressive disorders. *Brain and Behavior: A Cognitive Neuroscience Perspective, 10* (10), e01803. https://doi.org/10.1002/brb3.1803; Anwar, F. et al. (2020). Antiviral effects of probiotic metabolites on COVID-19. *Journal of Biomolecular Structure and Dynamics*, 2020, 1–10. https://doi.org/10.1080/07391102.2020.1775123; Aguilar-Toalá, J. E. et al. (2018). Postbiotics: An evolving term within the functional foods field. *Trends in Food Science and Technology, 75*, 105–114. https://doi.org/10.1016/j.tifs.2018.03.009; and Taverniti, V. and Guglielmetti, S. (2011). The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: Proposal of paraprobiotic concept). *Genes & Nutrition, 6* (3), 261–274. https://doi.org/10.1007/s12263-011-0218-x.

Once proven that a particular microorganism qualifies a probiotic, the next step is to determine whether such strain can be scaled up from laboratory to industrial scale to be successfully incorporated into edible goods. However, some points must be considered, for instance, although it is desirable for the bacteria to be viable in the GI tract when administered, this is not necessary; administration of probiotics is mainly through foods and/or dietary supplements. Nevertheless, other routes of administration are also possible (nasal spray, intravaginal, topical, etc.). Regarding the doses and delivery format, it is generally considered that daily doses between 10^6 and 10^{11} CFU day^{-1} (Taverniti et al., 2019) are required, but literature reviews indicate that different factors (e.g., dosing patterns, strain variations, and variation in the health endpoint being tested) may affect the outcome; thus, generalizing is difficult. Therefore, the adequate number of bacteria, at the end of the product's shelf life, can be assumed to be at least the dose that was documented in a clinical study to provide the claimed effect or benefit (Liu et al., 2019).

It is well known that delivery format has a significant influence on probiotic viability during shelf life and GI digestion (Corona-Hernandez et al., 2013). However, there is still scarce information regarding whether and how delivery matrices affect probiotic activity in terms of tolerability, efficacy, and safety (Binda et al., 2020). Considering that characterization of potential probiotic bacteria is a complex task, genomic-based approaches (also called probiogenomics) such as genomic, transcriptomic, proteomic, and metabolomic techniques (Fig. 10.5) have contributed to validate the real potential of probiotic cells while elucidating their physiological impact on the host (Castro-López et al., 2021; Sánchez et al., 2013).

Probiogenomics has not only been used to help identify the genetic and molecular markers associated with cell adaptation under stress conditions such as those occurring within the GI tract or during storage but has also allowed determining some specific mechanisms associated with benefits on hosts, including competitive exclusion, bacteriocin-mediated protection, modulation of the immune system (Papadimitriou et al., 2015). Also, transcriptomic and proteomic platforms have provided information on cell-surface proteins involved in membrane modification, protection, detoxification, and bile tolerance present in *Lactobacillus* and *Bifidobacterium* strains (Castro-López et al., 2021; Sánchez et al., 2013). Moreover, considering that probiotics may exert influence on the host through the production of metabolites, several metabolomic studies have been conducted to understand the cumulative health effect potentially delivered to the host by the different metabolites secreted by live bacteria or released after bacterial lysis (Castro-López et al., 2021). For instance, the metabolomic analysis of *L. plantarum* ATCC 14917 under initial acid and alkali stress revealed that the pH-mediated adhesion activity of bacteria is related to the metabolism of the related amino acids involved in energy expenditure (Wang et al., 2018). Moreover, metabolomics techniques have also been used in food science to determine the molecular fingerprints of fermented foods, including soy foods, cheeses, and wines (Mozzi et al., 2013).

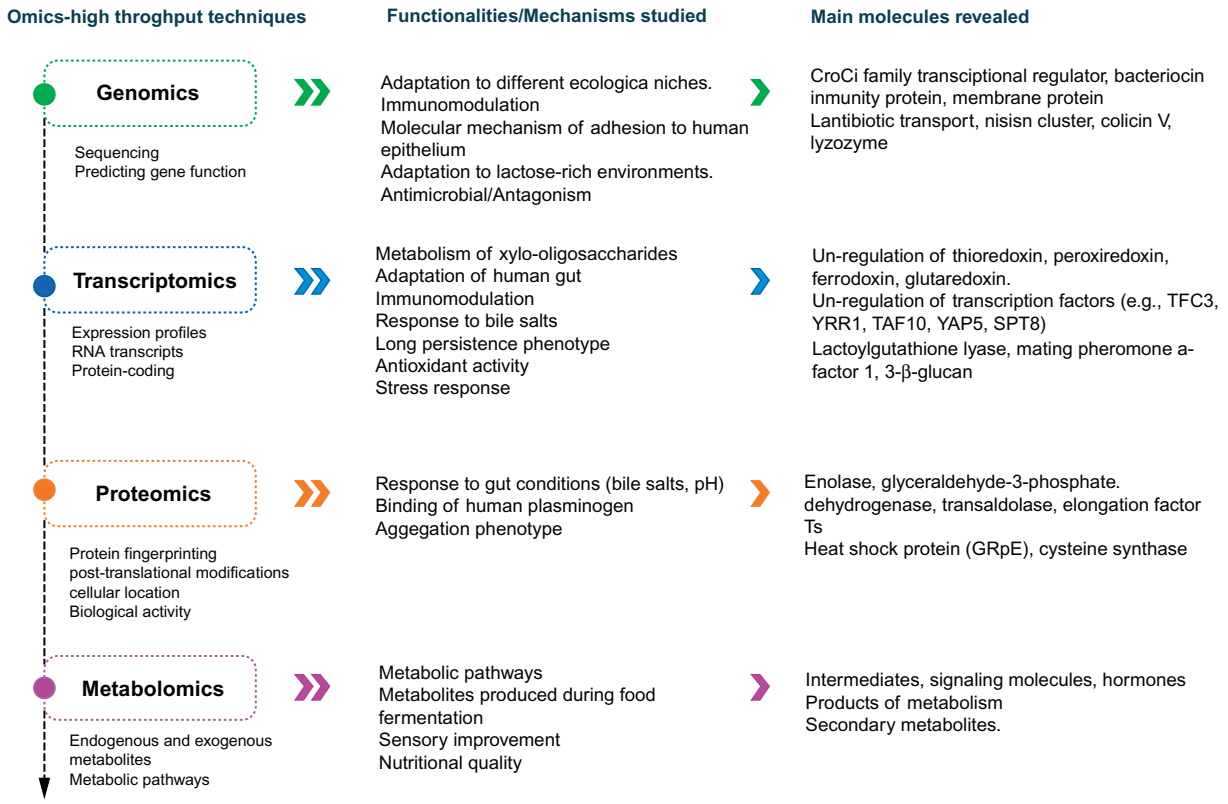

FIG. 10.5 Probiogenomics platforms to characterize probiotics. *(No permission required.)*

10.6 Micro-/nanoimmobilization of probiotics

As previously stated, to exert the biological effects of probiotic bacteria on the host, enough live cells should be guaranteed during storage and GI digestion. Thus, the loss of probiotic viability within food products (especially fermented ones) and in the acidic-bile conditions of the GI tract has encouraged researchers to find new efficient methods for improving bacterial viability. In this sense, it has been reported that microencapsulation and/or immobilization increase the probiotic viability and are an effective barrier against several environmental parameters, such as oxygen concentration, light, free radicals, and many others (Corona-Hernandez et al., 2013). The terms "immobilization" and "encapsulation" are both used as synonyms in many reported works (Frakolaki et al., 2021). However, a slight difference between immobilization and microencapsulation relies on the way bacteria are immobilized.

Microencapsulation is the process of forming a continuous coating around an inner matrix that is wholly contained within the capsule wall. The encapsulated material is usually called core, fill, active, internal, or payload phase, whereas the material used for encapsulation is called coating membrane, shell, capsule, carrier material, external phase, or matrix. On the other hand, immobilization refers to the trapping of materials or living cells in a particular matrix (e.g., spherical gel beads), but not necessarily inside it (Kavitake et al., 2018). However, both immobilization and microencapsulation have advantages and disadvantages. For instance, immobilization exhibits serious hurdles when used for the entrapment of cells in various food applications. Most of them are related to the physical-chemical properties of beads, such as particle size and adverse chemical interactions with the food environment. As for microencapsulation, it produces spherical particles having diameters to few nanometers (Frakolaki et al., 2021).

From a structural standpoint, three basic types of microcapsules can be used to protect probiotics during GI digestion (Fig. 10.6). In the matrix-type structure, the encapsulating polymer (e.g., protein, polysaccharide, or both) forms a continuous core through hydrogen bonds, van der Waals forces, or hydrophobic forces. In the cross-linked structures, polymeric biomaterials (i.e., alginate and chitosan) are cross-linked with divalent cations (e.g., Ca^{+2}) or enzymes (e.g., transglutaminase) induced by methods such as extrusion (EXT), ionic gelation (GEL), and IMP. Lastly, outer shell structures require additional cationic (e.g., xylan-rich hemicellulose and poly-L-lysine) or anionic (e.g., carrageenan and

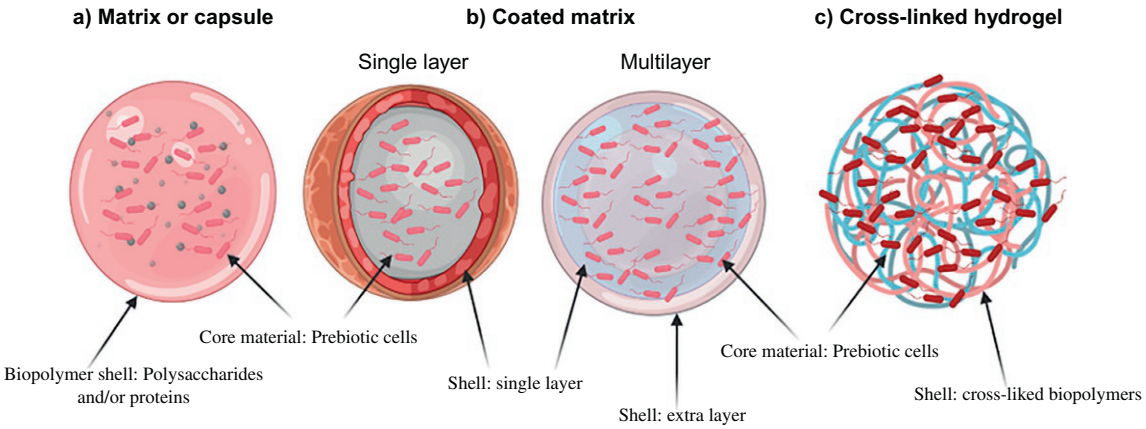

FIG. 10.6 Microencapsulation of viable probiotic cells: Type of structures. Matrix and capsules alone (A) coated with single (one core) or multiple (layer-by-layer core) layers (B) are vehicles in which probiotics are trapped in the inner cavity while in cross-linked hydrogels (C) produced by ionic/enzymatic gelation methods), probiotics are dispersed all over the carrier (inner core/surface). *(No permission required.)*

alginate) biopolymers (alternating cationic and anionic) to build additional covering layers (layer-by-layer) in monoencapsulated structures (Liu et al., 2019).

The incorporation of nanotechnology into probiotic science (also defined as nanoprobiotics) is an emerging field with great potential in the food, cosmetic, and pharmaceutical industries and the most studied nanostructures (Fig. 10.7) are nanoparticles (nanocoating), nanolayers (layer-by-layer, consisting of at least three layers of a charged polyelectrolyte), nanofibers (obtained by electrospinning, ELSPI), gold/silver/selenium particles, and nanoemulsions to produce anticancer, antimicrobial, antioxidant, and photoreactive products (Ashaolu, 2021; Durazzo et al., 2020). In a strict sense, unlike

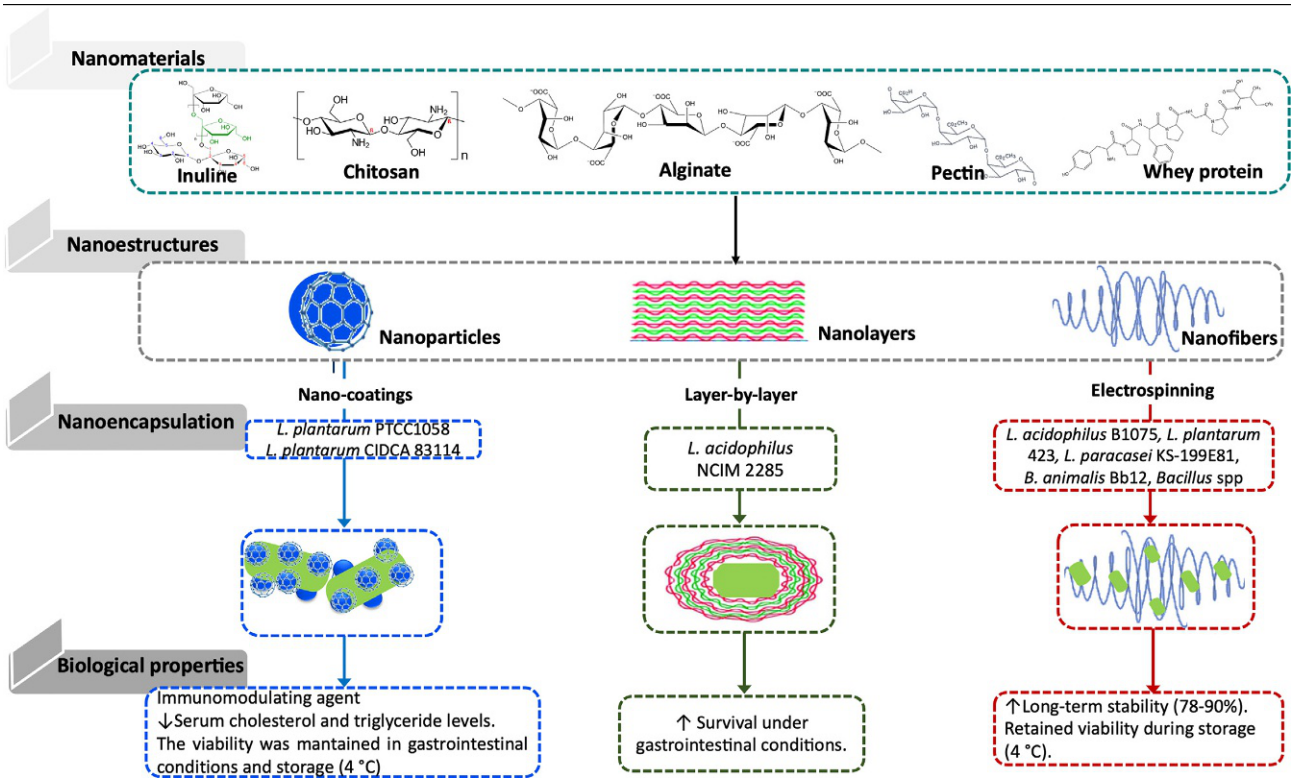

FIG. 10.7 Nanoprobiotics. *(Source: The authors; compiled from Ashalou et al. 2021; Pathak, K. and Akhtar, N. (2018). Nanoprobiotics: Progress and issues. In Singh, B., Hakkarainen, M., and Singh, K. K. (Eds.),* NanoNutraceuticals. *Boca Raton: CRC Press, pp. 147–164. https://doi.org/10.1201/9781351138949-9. No permission required.)*

microencapsulation, nanocoatings individually isolate and protect probiotics from various physical (e.g., pH and osmolarity) and biochemical agents (e.g., enzymes and mucin) and even from intercellular adhesion with other pathogenic microorganisms. For example, *Lactobacillus casei* protected with copper oxide nanoparticles increases its anticancer effect while exerting an antimicrobial effect on *P. aeruginosa* and *S. aureus* (Kouhkan et al., 2020). An interesting approach also includes the formulation of probiotics with nanoparticles of prebiotics, as described for phthalyl dextran and phthalyl inulin for *Pediococcus acidilactici*, and phthalyl pullulan for *L. plantarum*. The prebiotic nanoparticles enhance the production of antimicrobial peptides by the probiotic bacteria, improving the antimicrobial activity against gram-positive and gram-negative bacteria as compared with the probiotics alone (Durazzo et al., 2020).

These nanostructures protect the individual probiotic core even though originally it was thought that bacterial size (~1–5 μm) ruled out the nanotechnology as a protection strategy. Nowadays, nanoprobiotics are being developed to avoid GI obstacles (e.g., self-aggregation, partial digestion, and interaction with mucin), increasing the odds for microbial survival (Pathak & Akhtar, 2018). Moreover, novel nanoprobiotic-based foods are being developed at a laboratory scale (Salmerón, 2017), waiting to be launched in the probiotic market. Nanoprobiotics can be further transported along the GI tract and, while avoiding harsh conditions at this stage, they can interact with specific epithelial receptors or act as immune enhancers (Pathak & Akhtar, 2018). For example, *Pediococcus acidilactici* or *L. plantarum* carried in phthalyl-dextran or phthalyl-pullulan nanoparticles result in higher production of antimicrobial peptides and antibiotic activity against many pathogenic bacteria (Cui et al., 2018). Other probiotics efficiently packed in nanomaterials include *Lactobacillus acidophilus*, *Lactobacillus paracasei*, *Bifidobacterium animalis*, and *Bacillus* spp. (Fig. 10.7).

10.7 Coating materials

Coating materials behave in structurally different ways, as they are often based on sugars or amino acids for natural biopolymers and, therefore, their capacity to protect living microorganisms varies. The effectiveness of any material depends on its capsule-forming capability, chemical composition, mechanical strength, size, stability in gastric and intestinal fluids, and enhancing viability but also on its cheapness, availability, and biocompatibility. For instance, reduction in diameter can remove the protective effect, whereas, increasing capsule diameter decreases the digestibility by pancreatic enzymes (Afzaal, Khan, et al., 2019; Afzaal, Saeed, et al., 2019). On the other hand, coating materials with mucoadhesive ability (polymers with charged and/or nonionic groups) and desirable mechanical properties (surface roughness) are highly desirable for probiotic encapsulation and colon release (Li et al., 2020). Many biomaterials have been proposed for the microencapsulation of probiotic cells (Table 10.3), and some of them are also used to produce nanoprobiotics (Severino et al., 2013).

Polysaccharides, such as starch, alginate, chitosan, and carrageenan, and milk-derived proteins and gelatin have been the most reported, but emerging biomaterials such as microbial polysaccharides and pullulan have also been tested (Frakolaki et al., 2021). In general, these polymers contribute to increasing the stability and survival of various strains of probiotic bacteria in intestinal media, storage conditions, processing conditions, and food systems. However, accumulated evidence to date indicates that the selection of the most convenient coating material is strain-specific and method-specific, as will be discussed in the following paragraphs.

10.7.1 Natural and synthetic polysaccharides

Starch is a polysaccharide consisting of multiple glucose moieties joined by glycosidic bonds. Its basic units are amylose (linear) and amylopectin (branched), both dense with hydroxyl groups. Starch has moderate mucoadhesive properties that can be improved by replacing free hydroxyl groups with other functional groups (phosphate, carboxyl, amino, etc.) by esterification (phosphorylation, succinylation, acetylation, and oxidation) or when mixed with other ionic biopolymers. The efficiency of microencapsulation of *Lactobacillus*, *Bifidobacterium*, and *Pediococcus* with starch derivatives (e.g., hydrogels, resistant starch, and esterified starches) and in binary systems that include another biomaterial (e.g., carrageenan, protein, and chitosan) has been extensively documented (Afzaal, Khan, et al., 2019; Alfaro-Galarza et al., 2020; Ashwar et al., 2021, 2018; Chávarri et al., 2010; Cortés et al., 2014; Zhang et al., 2021). Starch-based microcapsules efficiently resist the harsh conditions found in the GI tract and are stable under prolonged storage in many food systems (Table 10.4).

For instance, porous maize and rice starch were efficient to encapsulate and promote survival, heat resistance, and GI stability of *L. plantarum* (Benavent-Gil, Rodrigo, & Rosell, 2018; Li et al., 2016). On the other hand, some starch derivatives (hydrolyzed, esterified, hydrogels) have been successfully combined with other polymeric systems of a lipid nature (e.g., cocoa butter), whey protein, and other polysaccharides (pectin and alginate) to increase the viability of many *Bifidobacterium and Lactobacillus* strains (Dafe, Etemadi, Zarredar, et al., 2017; Hernández-Barrueta et al., 2020).

TABLE 10.3 Coating materials and encapsulation methods.

	Spray drying	Electrospinning/ electrospraying	Emulsification	Extrusion	Freeze drying	Fluid bed drying	Spray chilling	Ultrasonic vacuum spray drying	Impinging aerosol
Milk and animal proteins	X	X	X	X	X	X			
Alginate	X	X	X	X		X			X
Vegetable proteins	X	X	X		X	X			
Natural/modified starches	X	X	X	X		X			
Gelatin	X	X	X	X					
Cellulose	X	X	X			X			
Maltodextrin/cyclodextrin	X		X		X			X	
Xanthan gum	X	X		X	X				
Chitosan	X	X	X	X					
Hydrophilic polymers	X			X			X		
Arabic gum	X		X		X				
Pectin	X	X		X					
Gellan gum	X			X	X				
Sugars	X				X			X	
Carrageenan		X	X			X			
Collagen		X							
Guar gum		X							
Dextran		X							
Acetate phthalate			X						
Carboxymethyl cellulose			X						
Amino acids					X				
Sorbitol					X				
Pullulan		X							
Hydrophobic polymers				X			X		
Waxes/fatty acids						X	X		

Data source: Frakolaki, G. et al. (2021). A review of the microencapsulation techniques for the incorporation of probiotic bacteria in functional foods. *Critical Reviews in Food Science and Nutrition, 61* (9), 1515–1536. https://doi.org/10.1080/10408398.2020.1761773.

TABLE 10.4 Gastrointestinal and shelf-life probiotic survivability in optimized microencapsulation conditions.

Biomaterial	Method	Probiotic	Findings	Reference
Oxidized high amylose starch macrogels	Extrusion	*Lactobacillus paracasei*	EC 88%, macrogels pH/GI resistant related to carboxyl groups	Zhang et al. (2021)
Type-4 resistant starch	Extrusion	*Lactobacillus casei*	↑Cell survival in cereal-based foods	Ashwar et al. (2021)
Taro/rice starch	Spray drying	*L. paracasei*	↑Cell survival under simulated GI conditions	Alfaro-Galarza et al. (2020)
Micro/nanosized particles	Emulsification	*Pediococcus acidilactici*	Starch micro > nanosized particles ↑cell survival	Ahmad, Gani, Hamed, and Maqsood (2019)
Type-4 resistant starch	Emulsification	*L. casei*, *L. brevis*, *L. plantarum*	EC 43%–48%, particle size 45–49 µm, thermal/GI resistant, ↑shelf life (2 months/4°C)	Ashwar et al. (2018)
Native, P-/Ac-/Suc-starch (amaranth)	Spray drying	*B. breve*, *L. casei*	All starches ↑cell survival during storage at 4°C, Suc-starch ↑resistant to GI conditions	Cortés et al. (2014)
Starch-alginate blend	Emulsification	*Lactobacillus acidophilus*, *Bifidobacterium lactis*	↑Cell survival under simulated GI conditions in a yogurt system	Kailasapathy (2006)
Alginate/starch	Emulsification	*L. brevis*	EC 89%, survival rate 94% as related to alginate component	Thangrongthong et al. (2020)
Alginate nanofibers	Electrospinning	*L. paracasei*	Particle size 305 nm, ↑thermal stability, pH (gastric) and GI resistance, ↑viability/survival in kefir	Yilmaz et al. (2020)
Alginate	Extrusion	*L. casei*	↑Cell survival under simulated GI conditions and storage (28 days) in fermented milk	Dimitrellou et al. (2019)
Alginate/carrageenan	Spray drying	*L. acidophilus*	EC 98%, particle size 715 µm, ↑GI resistance, alginate > carrageenan in cell releasability, ↑survival in ice cream	Afzaal, Khan, et al. (2019)
Alginate, chitosan, resistant starch	Extrusion	*L. acidophilus*	↑Cell survival under simulated GI conditions and storage	Etchepare et al. (2016)
Sugar beet, alginate, chicory oligosaccharides	Emulsification	*Staphylococcus succinus*, *Enterococcus faecium*	↑Cell survival under simulated GI conditions (88%–98%) and storage (30 days, 4°C)	Sathyabama, Ranjith Kumar, Bruntha Devi, Vijayabharathi, and Brindha Priyadharisini (2014)
Alginate/chitosan	Impinging aerosol	*Lactobacillus rhamnosus*, *L. acidophilus*	Particle size 10–40 µm, pH/GI resistant, chitosan-extended survival (90–120 min)	Sohail et al. (2011)
Chitosan, alginate, starch	Emulsification	*L. casei*, *Bifidobacterium bifidum*	Chitosan ↑particle size (>100 µm), shape, morphology, and pH/GI resistance of microcapsules associated with a ↓porosity	Zanjani, Tarzi, Sharifan, and Mohammadi (2014)
Chitosan, alginate, starch	Extrusion	*Lactobacillus gasseri*, *B. bifidum*	Chitosan ↑cell survival under simulated GI conditions	Chávarri et al. (2010)

Continued

TABLE 10.4 Gastrointestinal and shelf-life probiotic survivability in optimized microencapsulation conditions—cont'd

Biomaterial	Method	Probiotic	Findings	Reference
Chitosan, alginate	Extrusion	*L. plantarum*	↑Cell survival in microspheres (96%), pH/GI and temperature (65°C or 4°C) resistance	Trabelsi et al. (2013)
Chitosan, alginate	Extrusion	*L. rhamnosus*	Particle size 40 µm, pH/GI resistant, ↑survival in apple juice, chitosan ↑cell survival and stability associated with a ↓porosity	Gandomi et al. (2016)
k-Carrageenan, carboxymethyl cellulose	Extrusion	*L. plantarum*	Encapsulation yield (94%), pH/GI resistance; k-carrageenan increases stability of CMC particles	Dafe, Etemadi, Dilmaghani, et al. (2017) and Dafe, Etemadi, Zarredar, et al. (2017)
Carrageenan-soy protein isolate (SPI)	Spray drying	*Bifidobacterium longum*	1:3C/SPI ratio the most effective for ↑cell survival under simulated GI conditions and pasteurization (85°C)	Mao et al. (2018)
Carrageenan	Spray drying	*L. acidophilus*	EC 96%, particle size 726 µm, ↑cell survival in simulated GI conditions and yogurt	Afzaal, Saeed, et al. (2019)
Gellan gum	Ionic gelation	*L. paracasei*	↑Shelf life (7 days, 4°C), ↑inhibitory activity against Candida sp.	Ribeiro et al. (2020)
Xanthan gum, nonfat milk	Spray drying	*L. acidophilus*	Particle size 16–24 µm, xanthan gum substitution ↑cell survival up to 98%	Tantratian, Wattanaprasert, and Suknaisilp (2018)
Xanthan, pullulan and jamilan, gellan gums	Extrusion	*L. plantarum, L. rhamnosus*	Particle size 1.7–2.5 mm, pH/GI resistance, xanthan + gellan gums ↑resistance to bile	Jiménez-Pranteda et al. (2012)
Whey protein-pullulan	Electrospraying	*Bifidobacterium animalis*	↑Cell survival during storage	López-Rubio et al. (2012)
Whey protein	Extrusion	*L. acidophilus*	Particle size 107–233 µm, EC 82%–91%, whey protein multilayer ↑pH/GI/thermal resistance	Etchepare et al. (2020)
Gelatin, alginate	Emulsification	*Bifidobacterium adolescentis*	↑Cell survival under simulated GI conditions	Annan et al. (2008)

EC, encapsulation efficiency; *GI*, gastrointestinal.

Alginate is a biopolymer derived from brown algae that is mainly composed of β-D-mannuronic and α-L-glucuronic acids. Alginate is a hydrophilic polyanion that is biocompatible, inexpensive, and nontoxic and forms gel beads that envelop bacterial cells under mild processing conditions. Alginate has been used for the encapsulation of probiotics commonly after a gelling/cross-linking process with calcium ions. Usually, aqueous alginate solution dropped (by extrusion procedures) into a calcium-containing bath will form gel beads by rapid cross-linking between alginate guluronic units and calcium ions (Corona-Hernandez et al., 2013). Alginate poses a strong mucoadhesive property due to the large number of carboxyl and hydroxyl groups present in the molecular skeleton, which facilitate penetration and mucin interaction (Chen et al., 2013). However, alginate beads exhibit high porosity and degrade soon in acidic pH (Kavitake et al., 2018). To overcome such structural disadvantages, it can be mixed with other biopolymers such as starch, carrageenan, and chitosan to reduce its resistance and incrementing probiotic survival under GI conditions, storage, and addition to food systems such as fermented milk and kefir (Afzaal, Khan, et al., 2019; Dimitrellou et al., 2019; Thangrongthong et al., 2020; Yilmaz et al., 2020). Some examples are presented in Table 10.4. Alginate has been successfully combined with several encapsulating materials. Nine types of herbal-based polymers blended with alginate alone or mixed with psyllium and fenugreek were tested as a candidate for encapsulation matrix for the probiotic *L. plantarum* 15HN (Haghshenas et al., 2015).

The blend formulations were prepared by a simple extrusion method and all of them showed encapsulation efficiency values >98%. Besides high encapsulation efficiency, the high survival rate of probiotic cells in low pH and high bile salt concentration, and the sustained release rates of probiotic cells in colonic conditions during storage time were observed for the formulations with herbal polymers. The herbal biopolymers may offer additional advantages as prebiotic molecules toward the improvement of bacterial growth in the gastrointestinal environment.

Chitosan is a linear polysaccharide made up of glucosamine and *N*-acetylglucosamine units, which polymerize by cross-linking in the presence of anions and polyanions (polyphosphates and sodium alginate). The unique mucoadhesive properties of chitosan are due to the strong ionic interactions between the positively charged amino groups and the negatively charged structures of the intestinal mucosa (Chen et al., 2013). However, chitosan is not recommended for oral administration because it precipitates at pH values around 6.0, though it is useful as a coating material for other encapsulating biopolymers to be employed in multilayer approaches (Kavitake et al., 2018). Chitosan efficiently decreases the porosity of alginate beads, increasing the GI and storage stability of *Lactobacillus* spp. and *Bifidobacterium* spp. (Chávarri et al., 2010; Etchepare et al., 2016; Gandomi et al., 2016; Sohail et al., 2011; Trabelsi et al., 2013).

Carrageenan is a natural biopolymer with sulfated galactans and galactose repeats, extracted mainly from red marine macroalgae. Carrageenan gels at temperatures between 40 and 50°C with the addition of potassium ions, resulting in stable gelled microparticles that can successfully encapsulate probiotic cells (Kavitake et al., 2018). Regarding the mucoadhesive properties, the sulfate and hydroxyl groups are involved in the interaction with the GI mucosa. Pure carrageenan or in combination with other biopolymers such as cellulose and soy proteins has been documented to be efficient in increasing the survival of probiotic cells (*Lactobacillus* and *Bifidobacterium*) in GI media, thermal pasteurization methods, and in food systems such as yogurt (Afzaal, Khan, et al., 2019; Afzaal, Saeed, et al., 2019; Dafe, Etemadi, Dilmaghani, et al., 2017; Dafe, Etemadi, Zarredar, et al., 2017; Mao et al., 2018).

Microbial polysaccharides (also called exopolysaccharides) are polymeric carbohydrates plus proteins of microbial origin (Angelin & Kavitha, 2020). They can be found in both the cell surface and the extracellular medium (secreted by microorganisms). They protect microbial cells against severe environmental conditions like desiccation, osmotic stress, antibiotics, or toxic compounds. Those used to microencapsulate probiotics are gellan (from *S. elodea*), xanthan (from *Xanthomonas campestris*), and dextran (from *Leuconostoc* spp.), but pullulan (from *A. pullulans*) and jamilan (from *Paenibacillus jamilae*) are also gaining much attention (Liu, Xie, & Nie, 2020). Gellan is made up of repeating units of glucose, glucuronic acid, and rhamnose and produces a thermoreversible gel whose gelling temperature depends on its concentration, ionic strength, and the presence of cations (Kavitake et al., 2018). Gellan-based microcapsules are not easily degraded by GI enzymes and resist acidic environments (Liu et al., 2020). Xanthan consists of repeating units of glucose, mannose, and glucuronic acid (2: 2:1) and easily hydrates (cold water) forming a three-dimensional network, exhibiting pseudoplastic behavior due to its stiffness and viscosity. A great disadvantage of xanthan gum is that it has a high setting temperature (80–90°C), a fact that can be reversed in xanthan-gellan mixtures (Kavitake et al., 2018).

10.7.2 Proteins

Some important works that highlight the usefulness of various proteins for the encapsulation of probiotic cells are listed in Table 10.3. Whey protein (Etchepare et al., 2020; López-Rubio et al., 2012), soy protein (Mao et al., 2018), and gelatin (Annan, Borza, & Hansen, 2008) are the most used. These are commonly used as multilayer systems (layer-by-layer) and in binary systems with other biopolymers, such as microbial exopolysaccharides, alginate, and gums (Angelin & Kavitha, 2020; Annan et al., 2008; Tantratian et al., 2018). Milk contains about 3.5% of protein and the major milk proteins are casein (80%) and whey protein (rich in beta-lactoglobulin; the principal by-product of cheese manufacture). Milk proteins have good emulsifying, viscosity building and gelling, and film-forming properties. In hydrogel-based encapsulated systems, the ability of the milk proteins to form a gel phase is a useful property that can be capitalized to probiotic cells. Also, proteins interact with oppositely charged biopolymers to form a separate phase that encapsulates components.

Whey proteins have an amphoteric character, and thus, they can be easily mixed with negatively charged polysaccharides, such as alginate, carrageenan, or pectin to form cross-linked beads for probiotic cell encapsulation (López-Rubio et al., 2012). Some recent studies tested different combinations of whey proteins and polysaccharides for the encapsulation of probiotics. *L. acidophilus* was encapsulated into calcium alginate particles coated with up to three layers of whey proteins. The encapsulation efficiency observed for all multilayer treatments was higher than 80%, while the mean particle diameter ranged from 107 to 222 μm, among the different multilayered structures. The viability of encapsulated *L. acidophilus* was maintained during the simulated gastrointestinal conditions, and the treatment with a layer of whey proteins provided greater protection for the strain, showing final viable counts of 9.19 CFU g^{-1} after all the steps of the

analysis. The multilayer particles provided greater protection for encapsulated probiotic cultures in thermal resistance evaluations as free cells that did not resist the tested conditions. The storage for up to 120 days at refrigeration and freezing temperatures were more efficient for all types of multilayer microparticles when compared to free cells (Etchepare et al., 2020). In another study, a combination of whey proteins and maltodextrin was used to encapsulate probiotic *Enterococcus* strains of human origin by spray drying. The microcapsules showed good physicochemical and morphological characteristics regarding low moisture content with low water activity, mean particle size, thermophysical properties, and storage stability under room temperature conditions, with the maintenance of recommended viable counts of *Enterococcus* strains (Bhagwat, Bhushette, & Annapure, 2020). These studies reinforce that whey proteins are interesting coating materials for the development of microparticles as an effective way to improve probiotic viability during storage, thermal treatments, and gastrointestinal conditions.

On the other hand, gelatin is a complex mixture of single or multichain polypeptides used in probiotic encapsulation. The polymer is translucent, colorless, and odorless. Its amphoteric nature makes it an excellent candidate for its incorporation into anionic-gel-forming polysaccharides and the formation of reinforced microcapsules (Kavitake et al., 2018). The viability of commercial probiotic strains *L. acidophilus* LA-5 and *Bifidobacterium lactis* BB-12 was evaluated after encapsulation with ricotta whey supplemented with gelatin and hydrolyzed collagen as encapsulating material (Rama et al., 2020). The gelatin:collagen ratios were defined as 7:3 for *L. acidophilus* LA-5 and 9:1 for *B. lactis* BB-12, which had reduced bacterial counts of 0.46 and 1.26 log CFU g^{-1}, respectively. The survival rates of encapsulated LA-5 and BB-12 (89.91 and 95.83%, respectively) exposed to simulated gastric juice were higher than nonencapsulated probiotics (54.78% and 57.27%, respectively) under the same conditions. Although free BB-12 showed reduced inactivation rates in the presence of bile salts which is a characteristic of this strain, similar rates were observed for LA-5. After 30 days of storage at 25°C, the viability loss was 0.70 log CFU g^{-1} and 0.34 log CFU g^{-1} for LA-5 and BB-12, respectively, suggesting that the system formulated with ricotta whey associated with gelatin and hydrolyzed collagen can be considered for spray drying encapsulation of probiotic dairy bacteria.

The use of egg white proteins in alginate scaffolds has been also evaluated for the development of formulations to protect *Lactobacillus casei* and *L. acidophilus* against the stomach acidic environment (Jalilpour et al., 2017). The combination of egg white protein with alginate showed great potential in protecting the probiotics from acid conditions since a significant increase in the survival rate of bacteria was observed upon encapsulation. The viability of free *L. casei* and *L. acidophilus* was significantly reduced under acid exposure, but the encapsulation of the bacteria into the protein/alginate formulation significantly increased their survival. Swelling and shrinkage behavior of egg white proteins/alginate capsules at different pH values showed that the capsules had more swelling capacity in distilled water than a similar position in terms of gastric acidity, suggesting enhanced stability of these microorganisms in adverse gastric environments.

Finally, a number of studies report successful encapsulation of probiotic bacteria into biopolymer matrices prepared with legume proteins. Most studies describe encapsulation of lactic acid bacteria as compared to bifidobacteria strains and other probiotics such as *Saccharomyces* spp. (yeast). Several probiotic strains have been encapsulated into protein scaffolds composed of native and modified soy protein isolate, soy protein concentrate, and pea protein concentrate (Gharibzahedi & Smith, 2021). Soy protein microparticles have been employed to increase the tolerance of probiotics to gastric conditions and heat stresses, as demonstrated for *L. plantarum* CECT 220 formulations. The stability of the strain was evaluated during the shelf life of different formulated foods and the encapsulation with soybean protein showed significant improvement of *L. plantarum* viability from production to the end of the food shelf life (González-Ferrero et al., 2020). Although soy protein has been more extensively investigated, the increased tolerance of pea protein to mild heating processing (30–40 min at 60°C for example) qualifies it as a good candidate to fortify some food products (e.g., sausages, cheeses, chocolate bars, etc.) with a promising probiotic delivery property.

10.8 Encapsulation techniques

The selection of an entrapment method for probiotic microencapsulation depends on many factors, including the potential for large-scale production, cost, particle shape, resistance, and the resulting viable bacterial count (Corona-Hernandez et al., 2013). Today, most methods are based on drying and gelling procedures, as depicted in Fig. 10.8.

The main objective is to obtain probiotic powders with a decreased water activity. Drying technologies can be categorized into thermal, such as spray drying (SD), fluid bead drying (FBD), and nonthermal methods like freeze drying (FD), electrospinning (ELSPI), spray chilling (SPC) (Liu et al., 2019). Industrial methods often involve spray drying, emulsification, extrusion, or their combination, but other sophisticated electrodynamic methods such as impinged aerosol technique (IMP) have also been reported.

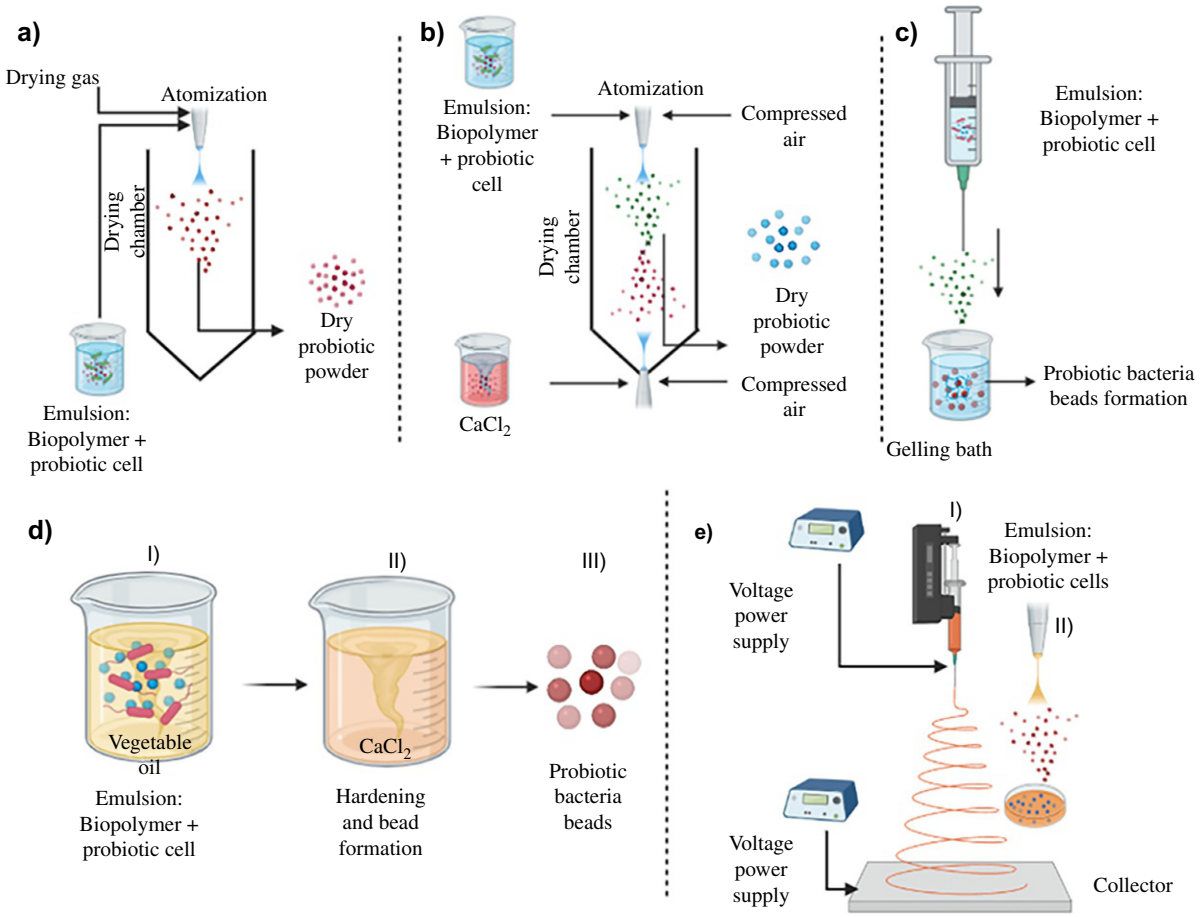

FIG. 10.8 Microencapsulation methods. (A) Spray drying (SD), (B) impinging aerosol (IMP), (C) extrusion (EXT), (D) emulsification (EMU), (E) Electrospinning (I; ELSPI)/electrospraying (II; ELSPA). *(No permission required.)*

10.8.1 Thermal methods

In spray drying (SD), a probiotic emulsion and/or suspension is instantly atomized by a tiny nozzle and dried by a stream of hot air (Fig. 10.8A; the emulsion contains the dispersed encapsulating polymer and probiotic cells and must meet specific viscosity criteria (Afzaal, Khan, et al., 2019; Afzaal, Saeed, et al., 2019; Alfaro-Galarza et al., 2020; Cortés et al., 2014; Mao et al., 2018; Tantratian et al., 2018). It is a fast process, easily industrially scalable, and 10 times less expensive than FD. However, it uses high temperatures, osmotic stress, and exposure to oxygen, which can be detrimental to the bacterial membrane and cytoplasmatic components. To overcome this, the concentration and type of coating polymer (often carbohydrates), inlet and outlet temperatures, flow rate, and air pressure can be adjusted to preserve microbial cells (Liu et al., 2019). Some heat protectants, such as granular starch, soluble fibers, trehalose, and lipophilic antioxidants, are added as a second encapsulating layer to increase cell viability (Frakolaki et al., 2021).

Due to the harsh process conditions of spray drying, formulations of dietary probiotics can be manipulated to maintain adequate viability and a good survival rate during transit through the gastrointestinal tract. Some studies describe direct spray drying from properly formulated cultivation media or food matrices. *Lactobacillus reuteri* was cultivated in a 20% (w/v) whey solution supplemented with 5% (w/v) yeast extract in submerged fermentation. The whey-based media containing 10^9 CFU g^{-1} was subjected to direct spray drying, and cell counts in the resulting product decreased by 2 log cycles after drying and 1 log cycle after 4 weeks of storage. A similar survival rate after the spray drying process at 55°C or 65°C outlet temperature was observed. The encapsulated *L. reuteri* showed a 32% higher survival rate as compared with free bacteria exposed to simulated digestive juice. Thus, the cultivation of probiotic strain in whey supplemented with yeast extract allowed direct spray drying of the culture, and the resulting microcapsules provided a suitable survival rate for *L. reuteri* (Jantzen, Göpel, & Beermann, 2013). In another study, a combination of probiotics, *Lactobacillus acidophilus*

NRRL B-4495 and *Lactobacillus rhamnosus* NRRL B-443, was encapsulated in raspberry juice by spray drying (Anekella & Orsat, 2013). Maltodextrin was included in the formulation. A sublethal thermal shock (50°C for *L. acidophilus* and 52.5°C for *L. rhamnosus*) was employed to circumvent the harmful effect of high temperatures on the probiotic strains. The survival rate of the probiotics increased by increasing the concentration of microencapsulated material. Considering the advantages of the spray drying process, the successful encapsulation of probiotics in fruit juices may represent an interesting alternative to offer nondairy probiotics in particular to individuals with lactose intolerance or allergies to dairy proteins.

Fluid bead drying (FBD) also uses high-temperature conditions. Probiotics in solid form are suspended in a flow of hot air with specific moisture content and a biopolymeric solution (fats, proteins, carbohydrates, and gums) is atomized with hot air on the surface of the probiotic bacteria; as fast as water evaporates, the biopolymer adheres to the cell surface. It is a low-cost process and is versatile to promote multilayer systems; however, it requires long processing times which can inactivate probiotic bacteria (Liu et al., 2019). The aggregation of particles due to the coalescence of the covering material is a characteristic disadvantage of FBD. Like SD, both temperature and air pressure are critical to maintaining bacteria survival. In addition, the use of protectants such as some sugars (trehalose, sorbitol, glucose, and sucrose) is common to stabilize cell membrane lipids and proteins (Frakolaki et al., 2021).

10.8.2 Nonthermal methods

In response to the disadvantages found in the high-temperature methods for encapsulating probiotic bacteria, some methods that use relatively low to very low temperatures such as ultrasonic vacuum spray drying (USP), SPC, ELSPI, supercritical methods, and FD (Liu et al., 2019). These and other methods are also employed to produce nanoprobiotics (Škrlec et al., 2019; Yilmaz et al., 2020). To overcome the oxidative and thermal stress inherent in SD, USP is proposed, which has the advantages of using the vacuum to evaporate water molecules at low temperatures (Frakolaki et al., 2021). Usually, a solution containing the probiotic bacteria is atomized using an ultrasonic atomizer that operates under vacuum conditions. The vacuum favors the evaporation of free water molecules at temperatures around 20–30°C. At the end of the process, fluidized nitrogen is applied until the required water activity is obtained in dry powders. Using this low-temperature technology, it has been possible to guarantee the survival of probiotic cells by up to 70% (Liu et al., 2019).

Spray chilling (SPC; also called spray cooling) consists of atomizing an emulsion containing the probiotic bacteria and the carrier material through a nozzle into a chamber containing cold air or nitrogen. The formed droplets get in contact with air refrigerated below the melting point of the carrier material (commonly lipid material), thus converted into fine solid lipid microbeads. SPC is regarded as the least expensive method that can be exploited industrially, as it exhibits high yields and can be applied under either continuous or batch processing modes. It is a process of real interest because it may widen the range of materials used as encapsulants and, also, be exploited to produce smaller beads (Frakolaki et al., 2021). In addition, the process has some advantages (low cost, low temperature, and easy scalability); however, it has a low encapsulation efficiency, and a considerable number of bacteria remains on the external surface of the beads, and consequently is exposed to the environment. It is important to mention that, under this technique, lipids can be used as wall materials to coat probiotic bacteria, which can increase their intestinal bioaccessibility (Liu et al., 2019). The viability of probiotic *L. acidophilus* and *Bifidobacterium animalis* subsp. *lactis* encapsulated by the spray chilling method was evaluated. The resulting powder was composed of smooth and continuous spheres with low moisture content and low water activity. The encapsulated bacteria showed at least 90 days of storage viability as both microparticles and in savory cereal bars, and their counts were superior to those resulting from other methods of adding lyophilized probiotics to savory cereal bars (Bampi et al., 2016). In a similar study, the same bacteria were encapsulated in cocoa butter using the spray chilling technique. The viability of the cells was not affected by microencapsulation. The encapsulated probiotics were unstable during storage at 20°C. The population of encapsulated *B. animalis* decreased 28% after 90 days of storage at 7°C, while the percentage of viable cells of *L. acidophilus* was only 20% after the same period of storage. Promising results were obtained when the microparticles were stored at −18°C, yielding 90 days of shelf life to the encapsulated cells (Pedroso et al., 2013).

Freeze drying (FD; also called lyophilization) is another most widely used technique to encapsulate probiotic cells since it promotes a high survival rate of probiotic microorganisms. The rationale is that probiotic cells are first frozen at extremely low temperatures and subsequently sublimated under vacuum. Precisely, the use of extremely low temperatures represents a significant disadvantage since it can cause inactivation and cell damage (cell wall, surface proteins) due to the formation of ice crystals. To overcome these disadvantages, low (glucose, lactose, mannose, trehalose, and sorbitol) and high molecular weight (polysaccharides and proteins) cryoprotectants can be used (Liu et al., 2019). In this sense, it has also been reported that certain probiotic strains exhibit high viability during FD, due to differences in the characteristics of each strain, such as surface area, cell wall, and membrane composition.

Extrusion (EXT) is a simple and inexpensive method and allows high cell viability. In general, the method consists of extruding-dripping (at high pressure) a hydrocolloid solution containing the probiotic bacteria and the biopolymer (i.e., alginate) through a syringe outlet, which is deposited in a gelling solution containing $CaCl_2$, which facilitates the formation of gelled spheres (Fig. 10.8C. Cells are trapped in a three-dimensional network that occurs due to the cross-linking of polymers. The beads display a very narrow size distribution, and the operational conditions are gentle enough to ensure high percentages of viable cells. However, its low scalability and the large size of the formed particles are the major challenges. Some important parameters to evaluate are the syringe outlet size, the viscosity of the hydrocolloid solution, and the distance between the syringe and the solution with the divalent cations (Frakolaki et al., 2021; Liu et al., 2019).

Emulsification (EMU) is achieved through the dispersion of a liquid into another immiscible liquid. For probiotic cell encapsulation, a hydrocolloid solution is emulsified in vegetable oil and then added into an ionic solution ($CaCl_2$) to induce coacervates (Fig. 10.8D (Sohail et al., 2011). The particles formed can be, finally, coated with a second layer, by immersion into another polymer solution, for additional protection or improvement of the sensory characteristics. The size of the beads produced can be modified by changing the agitation speed, the water/oil ratio, the additional rate of the cross-linking solution, and the surfactant concentration. The method offers some important characteristics, such as obtaining sizes smaller than 300 μm. However, a wide range of sizes is obtained, some oil residues remain on the surface of the capsules and a great consumption of time is required as the method is not continuous (Sohail et al., 2011). On the other hand, in the enzyme gelation technique, enzymes (rennet or transglutaminase), probiotic cells, and proteins (whey, casein, and caseinate) are mixed and added to an oil phase to form an emulsion. The gelling of the proteins occurs later by manipulating the temperature or by shaking for a long time to finally bring them to FD (Liu et al., 2019).

The impinging aerosol technique (IMP) could be considered relatively new for the encapsulation of probiotic cells. It is considered a continuous process with the potential to operate on a large scale. In general, an aerosol mixture with the encapsulating material (alginate) and the probiotic cells is sprayed on top of a chamber (Fig. 10.8B. A hardening mist ($CaCl_2$) is also sprayed on the upper part, which causes immediate gelation of the microcapsules, which are recovered in the lower part of the chamber. A disadvantage is the loss of materials due to their adhesion to the atomization chamber (Liu et al., 2019; Sohail et al., 2011). ELSPI and electrospraying (ELSPA) are techniques widely used in the biomedical area. It is possible to obtain polymeric fibers and microcapsules of submicron size through an external electric field. The basic setup for ELSPA and ELSPI includes a high voltage source (1–30 kV) usually operated in direct current mode, a blunt-ended stainless-steel needle or capillary, a syringe pump, and a grounded collector which can be either a flat plate or a rotating drum (Fig. 10.8E. The electric current is imposed on a biopolymeric solution, which causes the formation of fibrous structures. The basic way to control the size and morphology of fiber and droplets is through modifying the concentration of the polymeric solution. High concentrations aid the stabilization of the jet during its elongation and, consequently, the jet is converted into a fiber instead of breaking it into droplets. In the case of low concentrations, the jet gets destabilized and forms fine droplets. Nevertheless, both methods have many advantages, among which are room temperature, large-scale production, efficient encapsulation, and increased probiotic cell stability. In this sense, proteins and polysaccharides are the polymers most used to encapsulate probiotic bacteria (Frakolaki et al., 2021; Liu et al., 2019).

Lastly, supercritical technologies are also an alternative to overcome the disadvantages of the solvents used in other methods to disperse probiotic cells. In the supercritical technique, the probiotic cells are immobilized during the formation process of the interpolymer complex (plasticization) of the polymer with supercritical carbon dioxide and then the probiotic microcapsule is obtained when the carbon dioxide is gasified by depressurization. This technology has the advantage of being scalable, but the economic expense of the equipment must be considered (Liu et al., 2019). Commercial probiotic strains have been encapsulated under supercritical conditions in poly-(vinylpyrrolidone)-poly-(vinylacetate-*co*-crotonic acid) (PVP:PVAc-CA) interpolymer complex microparticles. *Bifidobacterium lactis* BB-12 and *Bifidobacterium longum* BB-46 were encapsulated using supercritical CO_2 and the samples analyzed during storage at 30°C maintained viable counts above the recommended minimum for 12 and 10 weeks, respectively, thus extending their shelf lives under elevated storage temperature (Thantsha, Labuschagne, & Mamvura, 2014).

10.9 Sensorial aspects of probiotic formulations

Determining the sensory properties of a finished food product is a necessary step. Table 10.5 shows the sensory properties of certain foods incorporating probiotics such as cheeses (Kadiya et al., 2014), green beer (Benucci et al., 2021), ice cream (Mohammadi et al., 2011), yogurt (Kailasapathy, 2006), juice (Gandomi et al., 2016), fermented milk (Dimitrellou et al., 2019), and dark chocolate (Mirković et al., 2018).

Some of the most used biopolymers for this purpose are alginate, carrageenan, milk proteins, and chitosan while the most reported methods are emulsification, extrusion, and spray drying. The addition of free and/or microencapsulated

TABLE 10.5 Sensory impact of free and encapsulated probiotics in selected food systems.

Food system	Probiotics	Coating material/ method	Findings	Reference
Quarg cheese	*Lactobacillus casei, Lactobacillus acidophilus*	Alginate/emulsification	Changes in flavor, taste, body, texture	Kadiya, Kanawjia, and Solanki (2014)
Green tea	*Lactobacillus rhamnosus*	Whey protein isolate-huauzontle's starch/ spray drying	25%–50% rejection after 28 d storage	Hernández-Barrueta et al. (2020)
Yogurt	*L. acidophilus*	Carrageenan-alginate/ emulsification	Encapsulated probiotics affected sensory properties	Afzaal, Saeed, et al. (2019)
Milk	*Lactobacillus delbrueckii, L. casei, S. thermophilus*	Alginate/extrusion	Encapsulated probiotic did not affect sensory properties	Dimitrellou et al. (2019)
Dark chocolate	*L. plantarum*	Skim milk/spray drying	Encapsulated probiotic did not affect sensory properties after 180 days	Mirković et al. (2018)
Ice cream	*L. acidophilus*	Carrageenan-alginate/ emulsification	Encapsulated probiotic did not affect sensory properties and quality	Afzaal, Khan, et al. (2019)
Cheddar cheese	*L. rhamnosus*	Alginate/impinging aerosol	No changes in pH and chemical composition but modified its texture	Hou, Hannon, McSweeney, Beresford, and Guinee (2017)
Apple juice	*L. rhamnosus*	Alginate, chitosan/ extrusion	Encapsulation improved sensory properties as related to gel beads	Gandomi et al. (2016)
Iranian Doogh beverage	*L. acidophilus, L. rhamnosus*	Eudragit S100-chitosan-alginate/ emulsification	Texture and flavor scores were free >microencapsulated 7 and 21 days	Pourjafar, Noori, Gandomii, Basti, and Ansari (2020)
Green beer	*Saccharomyces cerevisiae*	Alginate-chitosan/ extrusion	Free and encapsulated fermented beverages differ in sensory profile	Benucci et al. (2021)

probiotic cells affects the sensory profile of these foods in many ways. Bacterial metabolism results in higher production of short-chain alcohols, fatty acids, ketones, aldehydes, peptides, amino acids, carbonyls, and volatile flavor compounds, which may or may not contribute negatively to taste, aroma, and appearances, such as changes in color or turbidity. Therefore, the use of well-selected strains with lipolytic and/or proteolytic activity, able to generate high amounts of aroma components, could allow achieving improved sensory quality (Mohammadi et al., 2011). On the other hand, the type of hydrocolloid used for the encapsulation of probiotic cells has an important effect. Encapsulated bacteria powders can have an impact on texture due to granulation. Some biopolymers cross-linked with ions (sodium salts) can impart a gritty texture. Lastly, the selection of encapsulating matrices based on biopolymers with small particle size, white color, and mild flavor will impart attractive sensory characteristics.

10.10 Future trends

Much progress has been made in the field of probiotics in the last two decades. Scientific research has increased exponentially, and new market launches of probioticated foods have entered a stage of differentiation (Dixit et al., 2016; Allied Market Research, 2021). Such advances have been possible by exploiting new OMICtools to support the molecular taxonomy and metabolism of beneficial microbes (Castro-López et al., 2021; Mozzi et al., 2013; Papadimitriou et al., 2015) and their health-related claims (Koirala & Anal, 2021) while increasing the number of food vehicles and technological alternatives to produce them. Despite this, technological problems to preserve microbial viability and bioactivity within

the host persist and so, alternatives to micro- and nanoentrapment of viable cells will sustain R&D in the following decades. Particularly, nanoprobiotics have very promising applications in the pharmaceutical and food industries (Škrlec et al., 2019) and the interest of the scientific community is guaranteed for the next few years; however, studies on this new field are currently scarce, and more in vivo and randomized case control studies are needed to sustain health claims (Ashaolu, 2021).

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