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MODERN ASPECTS OF PROBIOTIC MICROORGANISMS' MICROENCAPSULATION

Numerous studies in recent years have shown that the gut microbiome plays an important role in maintaining various physiological processes in the body, including digestion, metabolism, immune system function, defense against pathogens, biosynthesis of unique metabolites, elimination of toxins, and regulation of the function of the gut-brain axis. The gut microbiota is influenced by the way of birth, child's feeding, genetic background, and lifestyle, including diet, exercises, medication, stress, and general host's health. Intestinal microbial populations can vary significantly from person to person, including healthy individuals. Unfavorable changes in the microbial composition and in its functions are characteristic of dysbiosis and indicate pathological disorders in the body [1]. The introduction of pro-, pre-, synbiotics and their other derivatives into the body, as well as transplantation of fecal microbiota, can restore the disturbed microbiota of the gastrointestinal tract (GIT). There is now a growing interest in functional innovative foods as ideal carriers for probiotics. However, many commercial probiotic products are ineffective because the beneficial bacteria they contain do not survive food processing, storage, and passage through the upper GIT. Therefore, modern effective strategies are needed to improve the stability of probiotic microorganisms. One of the such strategies is a modern microencapsulation method. Using this technology in the manufacture of functional foods allows maintaining the stability of probiotic microorganisms during storage, protects them from the aggressive conditions of the GIT, and promotes their colonization on the mucous membrane of the large intestine. To achieve better protection and controlled release of probiotics, alginate microgels are most widely used as microcapsule shells.

Keywords: *probiotic microorganisms, intestinal microbiota, microbiome, encapsulation, functional foods.*

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In recent decades, interest in the development of functional foods containing probiotic microorganisms has increased. However, the stability and viability of these microorganisms in various food products that can be used as carriers are limited by their properties (pH, type of packaging, storage requirements, etc.). In addition, the acidic environment of GIT negatively affects the survival of probiotic bacteria. Innovative methods of probiotics' microencapsulation offer a new approach to the development of functional foods containing probiotic microorganisms with increased viability and stability [2]. Modern approaches make it possible to increase not only the shelf life and viability but also the activity of probiotic microorganisms by various encapsulation methods in various compositions.

Many studies have shown the importance of the gut microflora in maintaining human health. Dysbiosis is a violation of the microbiota's quantitative and qualitative composition of the human large intestine, which is associated with various chronic and acute diseases, including inflammatory bowel diseases, allergies, obesity, diabetes, autism, rheumatoid arthritis, and even cancer [3, 4]. Numerous strategies to correct the gut microbiota's composition have been developed. Thus, fecal microbiota transplantation has been proven to be an effective treatment for *Clostridium difficile* infection, inflammatory bowel disease, and some other gastrointestinal diseases [5, 6]. However, it is not a convenient method for general use, and researchers are exploring alternative approaches to managing the gut microbiota, including oral delivery of pre-, pro-, syn-, postbiotics and their derivatives, as well as functional foods enriched with probiotic microorganisms [7, 8].

There is some concern that many commercial probiotic products are ineffective because the beneficial bacteria they contain do not survive food processing, storage, and passage through the upper GIT [9]. Moreover, even if they do reach the colon, they cannot establish themselves as part of the gut microbiome and may simply pass out transiently with the stool.

Most modern probiotic microorganisms belong to the genera *Lactobacillus* or *Bifidobacterium,* which are particularly sensitive to the aggressive conditions of the human intestine. Potential candidates for the next generation of probiotics are *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*. However, these species are also extremely sensitive to oxygen, hydrochloric acid, and bile salts, which limit the effectiveness of their use to improve human health [10]. Therefore, modern effective strategies to improve the stability of probiotic microorganisms in food are needed. One of the such strategies is microencapsulation as an effective means of protecting probiotics from the aggressive conditions of the human GIT [11].

The purpose of this review is to analyze recent advances in the development of optimal systems for the probiotic microorganisms' oral delivery to the digestive tract, specifically designed to increase their viability and metabolic activity in functional foods.

Aggressive conditions affecting the efficiency and viability of probiotic microorganisms. The development of an effective probiotic delivery system depends on understanding the nature of the harsh conditions to which it is exposed before and after ingestion. A number of physicochemical factors affect the viability of probiotics during the production of functional foods based on them, as well as their storage, transportation, and passage through the GIT.

During passing through the GIT, the main aggressive conditions for probiotic microorganisms are:

- in the oral cavity: mineral ions, mucin, enzymes (amylase), chewing process;
- in the stomach: hydrochloric acid (pH 1—3), mineral ions, enzymes (lipase, protease, pepsin);
- in the small intestine: bile acids, enzymes (lipase, protease, amylase), peristalsis;
- in the large intestine: intestinal microbiota, enzymes, anaerobic conditions, peristalsis [12—34].

Consider in more detail the features of the influence of each of the stress factors presented above.

Production, storage and transportation of functional food products. The main environmental factors that negatively affect the viability of probiotics during the production, storage and transportation of functional foods are oxygen, relative humidity (RH), temperature, osmotic stress and pH. High oxygen levels, relative humidity, osmotic stress and temperature are detrimental to many types of probiotics. Microorganisms as representatives of the human intestinal microbiota are mainly microaerophiles or anaerobes, so exposure to high oxygen levels can compromise their viability. The absence of an electron transport chain in intestinal bacteria leads to an incomplete reduction of oxygen to hydrogen peroxide. The accumulation of toxic oxygen metabolites in cells eventually leads to the cell death, which is called oxygen poisoning. Moisture is also a huge problem for probiotic products, as moisture activates bacteria and, in fact, starts the process of their degradation. Gut microorganisms are evolutionarily adapted to the temperature in a given biotope of the human body, so they can die when exposed to elevated temperatures associated with the processing of many foods, in particular during pasteurization, sterilization, dehydration, or heat treatment during preparation [35]. Most commercial probiotic strains are inherently thermolabile and therefore must be protected from overheating. The exception is representatives of the genus *Bacillus*, which are resistant to negative environmental influences [36].

Gastrointestinal tract. Once ingested, probiotics are exposed to harsh environmental conditions in the upper GIT, especially the stomach and small intestine. In the mouth, they mix with saliva containing mucin, mineral ions, and amylase. After a few seconds, they are swallowed, pass through the esophagus, and reach the stomach. Probiotic microorganisms are usually able to survive in the pH (6—7) conditions that exist in the human colon [37]. However, gastric pH is usually very low (between 1 and 3), which can significantly affect the survival of many types of probiotics. In particular, low pH values in the GIT cause a decrease in cytoplasmic pH in probiotic cells. High levels of hydrogen ions (H^+) and reduced activity of glycolytic enzymes inside probiotic cells affect the F_1F_0-ATPase proton pump, which is responsible for the survival of probiotics in acidic conditions [38]. Other potentially unfavorable conditions in the stomach include high ionic strength, as well as digestive enzymes (pepsins), which affect the viability of some probiotics.

In the small intestine, bile acids and digestive enzymes (lipase, proteases, amylase, etc.) influence the viability of probiotics. One of the main functions of bile acids in the lumen is to enhance the digestion and absorption of lipids. However, they also have antibacterial properties, act as detergents that destroy cell membranes, and also act as DNA-damaging agents. Some probiotic microorganisms are able to synthesize bile salt hydrolases (BSH), which hydrolyze bile acids into unconjugated bile acids and glycine. The presence of high levels of bile acids in the small intestine, especially after a fatty meal, can reduce the viability of many probiotics. Having reached the colon, probiotic microorganisms must compete for adhesion sites to the intestinal mucosa with bacteria from the intestinal microbiota, attach to the intestinal mucosa, and then colonize it and multiply [39]. For example, *Lactobacillus rhamnosus* GG expresses an adhesive protein in the colon that promotes its binding to mucin and collagen on host epithelial cells [40].

Basic requirements for probiotic microorganisms' delivery systems. The most important challenges in designing optimal probiotic delivery systems are as follows.

▪ In the preparation process, the use of ingredients or processes that adversely affect the viability of probiotics (organic solvents, strong acids or bases, surfactants, excessive heat, intense me-

chanical stress, and aeration) should be avoided. For many foods, heat treatment is used to inactivate pathogens and spoilage organisms, but these processes can also inactivate probiotic microorganisms. Therefore, it is necessary to select or create heat-resistant strains of probiotic microorganisms for their further microencapsulation.

▪ Many colloidal delivery systems designed to encapsulate small molecules (such as vitamins, nutraceuticals, colors or flavors) cannot be used for probiotics because the particles are too small to contain bacteria. Microbial cell sizes typically range from 1 to 10 μM, while many colloidal delivery systems contain particles smaller than 1 μM, such as microemulsions, nanoemulsions, and biopolymer nanoparticles. Moreover, the concentration of viable probiotic microorganisms present in commercial products should typically be greater than 6—7 log10 CFU/g, which means that the loading capacity of any colloidal delivery system should be higher. Probiotics can be encapsulated in tablets or capsules that are large enough to encapsulate a large quantity of probiotics. However, probiotics included in tablets or capsules may not enter the human colon in a viable form because they are too large to pass directly through the pyloric sphincter. Instead, they break down and release probiotic microorganisms in the stomach, where they are susceptible to degradation due to harsh conditions. Moreover, if probiotic microorganisms are encapsulated in too large colloidal particles, they can adversely affect the sensory and textural properties of products.

▪ Many delivery systems previously developed to encapsulate probiotic microorganisms do not provide adequate protection during storage and passage through the gut. For example, biopolymer microgels are highly porous and allow gastric acid and enzymes to enter where they degrade encapsulated probiotic microorganisms. In addition, many colloidal delivery systems developed in research laboratories are not suitable for commercial use due to their high cost, complex processing requirements, or the use of ingredients unsuitable for the food industry [41—62].

Finally, it should be noted that any probiotic microorganism delivery system must be designed so that the probiotics are released in the colon to fully realize their health benefits. In addition, probiotic microorganisms must adhere to and colonize the colonic mucosa, otherwise, they will transit through the human body.

Microencapsulation of probiotic microorganisms. A number of studies have shown that the viability of probiotics can be improved by encapsulating them in microgels or other types of microcapsules [41—43].

Microencapsulation (encapsulation) is a process in which the smallest particles of a liquid, gaseous or solid ingredient are «packed» in a material that protects them from environmental influences. Microcapsules are miniature containers that protect the contents from evaporation, oxidation, and destruction before they are released.

Microencapsulation of probiotics in polymeric microcapsules successfully protects them from the harsh and changing conditions of the GIT. Microcapsules deliver live cells of probiotic microorganisms without losing their functionality to the target biotope of the host organism. Microcapsules can protect them against environmental hazards during transit through the GIT and promote their release at a controlled rate under certain conditions, usually in the colon. Microencapsulation also protects probiotic cells during storage over a wide range of temperatures and can also significantly increase their stability. In addition, microencapsulation of biologically active substances, designed to improve their low bioavailability in the host body, masks the unpleasant taste and also expands the range of their application [12].

The main requirements for biopolymers used for encapsulation are: the biopolymer must be permeable for nutrients and metabolites in order to maintain cell viability and show no cytotox-

ic and antimicrobial activity to ensure that the host and its microbiota are not affected. The efficiency of encapsulation, as well as the delivery of probiotic microorganisms with the desired viability and bioactivity to the site of exposure, depends on the composition and structure of the microcapsule shell material and the correct choice of co-encapsulation technology.

The main biocompatible and food carriers for the encapsulation of probiotics are alginate [13], chitosan [14], pectin [15], gelatin [16], starch [17], gum arabic [18], whey protein [19], and lipids [20], as well as various mixtures of these materials. Numerous studies have shown that the inclusion of prebiotics (inulin, corn, trehalose, resistant starch, etc.) as a shell material during encapsulation increases the stability and maintains the viability of probiotics under extreme conditions of the GIT [21—23]. The type of encapsulation technology is also important. The main methods for co-encapsulation of probiotic microorganisms with bioactive substances in a single delivery format are: spray drying [24], freeze drying [25], spray cooling [20], emulsification [26], extrusion [27], and coacervation [28, 29].

There are different types of microcapsules:

- ordinary microcapsules;
- microcapsules with a double shell;
- microcapsules in a microcapsule with different properties;
- a plurality of coated microcapsules in one liquid medium.

Microencapsulation has a number of advantages: it allows slowing down the release of the active principle, which leads to the prolongation of the drug action and more efficient use of it . Microcapsules allow a programmed release of probiotics under certain conditions.

An efficient microencapsulation system maintains the stability of probiotic microorganisms during storage, protects them from aggressive conditions in the upper GIT, releases them in the large intestine, and then promotes their colonization on mucosal surfaces [30]. Many modern reviews are devoted to various types of oral delivery systems designed to encapsulate probiotics [31—34]. However, many of these systems cannot adequately protect probiotics from degradation within the gut due to their inherent limitations (permeability for acids, enzymes, or bile salts).

Delivery systems for probiotic microorganisms to improve their viability can be developed in a variety of ways. First, they can form a physical barrier that protects probiotics from any problematic environmental components (gastric juice, bile salts, and digestive enzymes). Second, they can be designed to co-encapsulate probiotics with specific nutrients (easily digestible carbohydrates, dietary fiber, proteins, lipids, minerals) that help probiotic microorganisms survive [44]. Third, they may contain additives that provide favorable conditions (antacids to control local pH) for probiotic microorganisms [45]. Finally, the microparticles may contain, in addition to probiotic microorganisms, products secreted by them that contribute to their survival. For example, some probiotic microorganisms secrete bile salt hydrolyzing enzymes that protect probiotics from bile salts in the small intestine. Other additives are also used, for example, those that regulate the level of oxygen or osmotic stress inside microparticles [46—62].

There are several types of microgels for the delivery of probiotic microorganisms.

Simple microgels usually consist of small spherical particles containing a network of crosslinked biopolymers inside, with pores filled with an aqueous solution [37, 54, 57, 58]. The materials used for the manufacture of microgels are mainly biopolymers (starch, alginate, carrageenan, gelatin, xanthan gum) and proteins with good thermal stability, high biocompatibility, low toxicity, and low cost. It is also possible to use microgels based on polysaccharides as probiotic delivery systems [63]. The most widely used for the creation of microgels is alginate, which is a polysaccharide isolated from seaweed. Such microgels are formed by

electrostatic binding of anionic carboxyl groups of alginate with calcium cations. Encapsulation of *Lactococcus lactis* in alginate microgels improves its resistance to aggressive environmental conditions, leading to an increase in its viability compared to the unencapsulated form [46]. Encapsulation of probiotics in alginate microgels has also been used to enable the co-administration of probiotics and antibiotics [48]. To achieve better protection and controlled release of probiotics, alginate microgels containing for example *Bifidobacterium* BB-12 are also used, which are formed by emulsification/internal gelation followed by freeze drying [47]. Although alginate is the most widely used biopolymer for the manufacture of microgels, other biopolymers can also be used [48]. In addition, an increase in the thermal stability of probiotics and stability under aggressive conditions of the GIT due to their encapsulation in microgels formed from starch with high amylose content has been proven [64]. Protein-based microgels (soy protein) have also been developed [44].

The ability of microgels to improve the survival of probiotics depends on their size. If the microgels are large, they can adversely affect the organoleptic characteristics (mouthfeel) of commercial products, as well as make them difficult to pass through the gastric biotope. Conversely, small sizes do not allow probiotics to be encapsulated or decompose too quickly due to the large open surface area [48]. It has been experimentally shown that microgels should have a diameter of less than 200 μM to ensure good passage through the GIT [65]. Other studies have shown that they should have a diameter of about 500 μM [3, 48].

Core-shell microgels. The performance of microgels can be improved by coating them with one or more layers of biopolymer. Chitosan is one of the most commonly used polysaccharides for this purpose because it has a positive charge whereas most other polysaccharides have a negative charge. Core-shell microgels, consisting of a calcium alginate core and a chitosan coating, improve the viability of encapsulated probiotic microorganisms in the GIT [66]. Such alginate-chitosan systems are promising for the delivery of probiotics to the large intestine, since chitosan and alginate are degraded by the microflora of the large intestine, thereby releasing probiotics in this biotope. In addition, the chitosan coating affects the viability of bacteria, since chitosan is an effective antimicrobial agent [67].

Core-shell microgels, consisting of a cellulose core and a calcium alginate shell, retain probiotics in the stomach and then release them in the small intestine [68]. The survival rates of probiotic microorganisms increase when they are encapsulated in alginate microgels coated with zein [69], as well as when they are enclosed in microparticles with a polyalginate core coated with multiple layers (layer thickness 20 nm) of polyelectrolyte [48]. Another type of microparticles is based on whey protein and alginate with a diameter of 107 to 222 μM [53].

Biopolymer complex microgels. It is possible both to use one biopolymer to create microgels and to combine two or more biopolymers to improve their stability or functionality. Microgels can be made by combining biopolymers using complex coacervation (mixing a negatively charged biopolymer with a positively charged one). The positive aspects of complex coacervation are: high cell capture efficiency, improved performance, and good scalability. The following complex microgels are used: whey protein/gum arabic [70], whey protein/-carrageenan, whey protein/gum arabic/alginate, gelatin/gum arabic [71], gelatin/alginate [43], and starch/alginate [54]. For example, encapsulation of probiotics in alginate-gelatin microgels formed by electrostatic complexation improves the viability of *L. salivarus* Li01 during high-temperature treatments, long-term storage, and passage through the GIT [43]. The protective properties of these microgels are explained by the facts that: (i) gelatin is a protein with some buffer-

ing capacity, which can increase the stability of probiotics in the stomach; (ii) the biopolymer network in the alginate-gelatin microgel effectively slows down the molecular diffusion of digestive enzymes into the gel [43]. Alginate-starch microgels used to increase the viability of *Lactobacillus casei* in the GIT have also been demonstrated [54]. Another type of microcapsule with probiotics was developed using the enzyme transglutaminase with the formation of intra- and intermolecular covalent bonds between two amino acid residues of gelatin, which significantly improves the persistence and viability of *Lactobacillus acidophilus* [72].

Microgels resistant to aggressive conditions of the GIT. An interesting approach to increase the viability of encapsulated probiotics during the passage through the GIT has been developed. It consists in controlling the pore size and internal pH of the microgels. Many probiotics are inactivated when exposed to gastric juice due to its high acidity and the presence of digestive enzymes. This effect can be reduced if the internal pH of the microgels remains neutral in the stomach, and the impossibility of penetration of digestive enzymes into the microgel is ensured. Simple microgels are not very effective in protecting probiotics in the GIT because small hydrogen ions (H^+) and digestive enzymes can easily diffuse into them due to the relatively large pore size of the biopolymer network [37, 50]. For example, alginate microgels with a pore size of 17 nm [15, 57], which is much larger than the size of hydrogen ions (<1 nm) or enzymes (<5 nm). Therefore, H^+ ions and digestive enzymes can easily diffuse into them and promote the degradation of encapsulated probiotics. Studies have shown that the incorporation of an insoluble antacid (magnesium hydroxide $Mg(OH)_2$) into calcium alginate microgels can significantly improve the gastric stability of encapsulated probiotics by creating a neutral internal pH around them [57]. Similar results were also obtained using another insoluble antacid (magnesium oxide MgO) included in calcium alginate microgels [45]. These antacids are insoluble in water at neutral pH but dissolve under acidic conditions, which releases hydroxide ions that neutralize hydrogen ions, thereby maintaining a constant neutral pH (until completely dissolve). It has been experimentally proven that $CaCO_3$ is a more effective antacid for protecting probiotics compared to $Mg(OH)_2$ [56].

It has also been proven that doping microgels with cellulose nanoparticles significantly improves the resistance of probiotic microorganisms in the GIT by filling the pores and thereby reducing the ingress of gastric juice [57]. pH-sensitive carrier particles have been developed, consisting of a mixture of calcium, alginate, and EDTA. This system is jelly-like under acidic conditions but decomposes at neutral pH values, so it can be used for the programmed release of probiotics in the small intestine.

Microgels with added nutrients. Another approach to increase the survival of encapsulated probiotics during storage and in the GIT is to provide them with a sufficient quantity of beneficial nutrients — prebiotics. Encapsulation of prebiotics in the core of microgels is known to improve the viability of probiotic microorganisms [59]. For example, the addition of oligosaccharides (β-glucan, plant extracts) improves probiotic delivery [65]. Oligosaccharides are not normally degraded by GI enzymes but can be used by some bacteria, especially *Lactobacillus* species in the colon. Co-microencapsulation of *Lactobacillus fermentum* with oligosaccharides protects the probiotic from exposure to low temperatures [59]. The inclusion of sea buckthorn extract in microgels also contributes to the protection of *L. casei* during heat treatment and passage through the GIT, which is explained by its antioxidant activity [73, 74].

Selection of biomaterial for microencapsulation. Biomaterials used to encapsulate probiotics include natural and synthetic polymers. These biomaterials are in direct contact with liv-

ing cells. For this reason, the following criteria have been developed for their selection:

- physical and chemical properties (chemical composition, morphology, mechanical strength, stability in the GIT, and intestinal fluid;
- toxicological analysis;
- production and sterilization processes [75—79].

Biomaterials are inorganic or organic macromolecules consisting of a repeating chain of monomers linked by covalent bonds. Their chemical structure and conformation of monomeric chains provide them with specific functionality — the ability to form gels. The most common biomaterial used to encapsulate probiotics is alginate. Other supporting biomaterials include carrageenan, gelatin, chitosan, whey proteins, cellulose acetate phthalate, locust bean gum, and starches [62, 76—79].

Alginate is a linear polymer of a heterogeneous structure, consisting of two monosaccharide units: α-L-guluronic (G) and β-D-mannuronic (M), connected by β (1—4) glycosidic bonds. Alginate is soluble in water over a temperature range of 60 °C to 80 °C. It is known that alginate gels do not dissolve in an acidic environment. The success of using alginate in microencapsulation of probiotics is due to the protection of cells from acidic conditions. It is an ideal bacterial encapsulation material for delivery to the intestines. Alginate is well known for its biocompatibility, environmental friendliness, low cost, and ease of use. The carboxylic groups on the alginate chain can be crosslinked with polyvalent ions, such as calcium ions. Cross-linked alginate materials are stable in the low-pH environment of the stomach, and the crosslinks are reversed in high-pH environments such as intestines. The controllable and reversible nature of these cross-links makes alginate a promising encapsulating polymer for the targeted delivery of probiotics to the gut.

The selection of a suitable biomaterial is a preliminary study requiring careful methodological research. The search for new materials for encapsulation is of paramount importance. These materials must meet the requirements of non-toxicity, resistance to gastric acidity, and compatibility with cells of probiotic microorganisms [79—82].

Modern methods for evaluating the effectiveness of probiotic microorganisms' delivery systems. To evaluate the effectiveness of a probiotic delivery system, it is very important to characterize its structural organization, physicochemical properties, functional characteristics, and viability of probiotic microorganisms. It is advisable to carry out such assessment with a combination of *in vitro* and *in vivo* models. *In vitro* models are useful for scanning many different compounds quickly and cheaply, but they often do not accurately model the complex processes that take place inside the human gut. *In vivo* models are more expensive and time-consuming, but they allow a more accurate assessment of the potential effectiveness of the delivery system in real applications.

***In vitro* methods for evaluating the effectiveness of delivery systems**:

1. Characterization of the structure and physicochemical properties

The structural organization of microgels is usually characterized by light or electron microscopy [37, 43, 52]. Atomic force microscopy can also be used to study the surface morphology of microgels. Fluorescence microscopy can be used to visualize selectively labeled microgel biopolymers [52]. Fluorescent staining is also used to measure the internal pH of microgels and determine the location and viability of probiotic microorganisms in microgels [3, 52]. The ratio of living-to-dead cells is detected by confocal microscopy or flow cytometry [16]. It is also possible to detect probiotics by incorporating a luminescence emitting plasmid (pGENluxCDABE), which allows the detection of live cells of probiotic microorganisms using an *in vivo* imaging system (IVIS) [30]. The advantage of this bioluminescent plasmid is that it can be used to

track probiotics as they pass through the GIT, as well as to monitor the proliferation of probiotics in the gut, such as *E. coli* DH-5α. One of the main disadvantages of this method is that it is currently not suitable for the detection of gram-positive bacteria, which include most probiotics [30].

2. Viability and functionality of probiotics

The viability of probiotics is determined using plate counting or flow cytometry methods as described by the International Organization for Standardization [83]. There are static and dynamic models of digestion *in vitro*.

The static model assumes a constant ratio of food to digestive fluid, as well as a constant pH throughout each stage of digestion, making the method easy to use. Digestive juices include fluids mimicking saliva, simulated gastric fluids (consisting of HCl, salts, and pepsin; pH 1—2) and intestinal fluids (consisting of bile salts and pancreatin; pH 5—7) [65].

The dynamic model involves pH regulation, food flow control, and real-time delivery of digestive enzymes to different parts of the GIT, which better mimics the digestive process itself [65]. For example, the SHIME system, which consists of five vessels containing various simulated gastrointestinal fluids, simulates gastric and lower intestinal activity. A computer-controlled *in vitro* gastrointestinal model called the Dynamic Gastrointestinal Simulator (SIMGI) has been developed. This simulator allows one to simulate the physiological processes in the stomach, small intestine, as well as the ascending, transverse, and descending regions of the large intestine. In addition, this system also promotes the multiplication of the colonic microbiota [65].

The TNO models for the upper GIT (stomach and small intestine — TIM-1) and the large intestine (TIM-2) are widely used to model the human intestine [84]. This model is also applicable to studying the survival of probiotic microorganisms. An *in vitro* model of the GIT called the «Thiny Intestine» (TSI) has been developed to simulate the human small intestine, which maintains pH, temperature, bile salt levels, microbiota, and enzyme composition at a physiologically significant level [85].

***In vivo* methods for evaluating the delivery systems' effectiveness.** While *in vitro* models are more practical for rapidly screening many different formulations, they do not accurately mimic the human intestine. Therefore, promising candidates for probiotic delivery systems should be tested in more accurate *in vivo* models. To determine the viability of microencapsulated probiotic microorganisms after oral administration, real-time PCR and fluorescence *in situ* hybridization (FISH) are used [3]. Sometimes, there is a poor correlation between *in vitro* and *in vivo* studies.

The effect of encapsulation on the functional characteristics of probiotics is also being evaluated using animal models. For example, mice with DSS-induced colitis were fed with free and microencapsulated LGG [68]. The results showed that encapsulated LGGs were more effective in preventing intestinal inflammation than free LGGs.

Although rodents, especially rats and mice, are commonly used to test probiotics, it should be noted that their GIT is not very similar to a human. Conversely, dogs and humans have fairly similar gastric morphology and gastric emptying characteristics, while pigs and humans have fairly similar colon morphology [86]. Therefore, it is more appropriate to use the above-mentioned animal species to obtain more convincing data, but this is associated with higher costs and ethical issues.

Conclusions. Due to the importance of the intestinal microbiota for human health, as well as the increasing number of negative factors affecting the microbiota of the host organism, the development of systems for the oral delivery of microencapsulated active viable probiotic microorganisms to the large intestine is one of the important tasks of modern biotechnology.

Microencapsulation of probiotics into polymer microcapsules successfully protects them from aggressive and changing conditions of the GIT, and also allows the delivery of living cells of probiotic microorganisms without loss of their functional activity to the target biotope of the host organism. Microcapsules also protect probiotic cells during storage over a wide range of temperatures and can significantly extend the shelf life of the final product. Joint microencapsulation of prebiotics with probiotic microorganisms can further increase the survival of the latter during storage and passage through the GIT.

It has been shown that alginate is an ideal biopolymer material for microencapsulation of probiotic microorganisms for targeted delivery of them to the intestine. Alginate is biocompatible, environmentally friendly, has a low cost, and, most importantly, is characterized by ease of use.

Thus, the development of functional foods enriched with microencapsulated probiotic microorganisms as effective means of maintaining and restoring the intestinal microbiota is one of the urgent and important tasks of modern science.

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СУЧАСНІ АСПЕКТИ МІКРОКАПСУЛЮВАННЯ ПРОБІОТИЧНИХ МІКРООРГАНІЗМІВ

Численні дослідження останніх років показали, що кишковий мікробіом відіграє важливу роль у підтримці різних фізіологічних процесів в організмі, включаючи травлення, метаболізм, роботу імунної системи, захист від патогенів, біосинтез унікальних метаболітів, виведення токсинів і регулювання функції вісі кишечник-мозок. Мікробіота кишечника формується під впливом багатьох факторів: способу народження і вигодовування немовляти, генетичного фону та способу життя, включаючи дієту, фізичні навантаження, прийом лікарських препаратів, стрес та загальний стан здоров›я господаря. Популяції кишкових мікроорганізмів можуть істотно відрізнятися в різних людей, зокрема і здорових. Несприятливі зміни в мікробному складі та в його функціях є характеристикою дисбіозу та свідчать про патологічні порушення в організмі [1].

Введення в організм про-, пре-, синбіотиків та інших похідних, а також трансплантація фекальної мікробіоти здатні відновлювати порушену мікробіоту шлунково-кишкового тракту. В даний час зростає інтерес до функціональних інноваційних продуктів харчування як ідеальних носіїв для пробіотиків. Однак, багато комерційних пробіотичних продуктів неефективні, оскільки корисні бактерії, що входять до їх складу, не виживають при процесингу харчових продуктів, зберіганні та проходженні через верхні відділи шлунково кишкового тракту. Отже, необхідні сучасні ефективні стратегії підвищення стабільності пробіотичних мікроорганізмів. Однією з таких стратегій є сучасний метод мікрокапсулювання. Застосування такої технології при виготовленні функціональних продуктів харчування дозволяє підтримувати стабільність пробіотичних мікроорганізмів при зберіганні, захищає їх від агресивних умов шлунково-кишкового тракту та сприяє їх колонізації на слизовій оболонці товстого кишечника. Для досягнення кращого захисту та контрольованого вивільнення пробіотиків найбільш широко використовують альгінатні мікрогелі як матеріал для оболонок мікрокапсул.

Ключові слова: *пробіотичні мікроорганізми, мікробіота кишечника, мікробіом, інкапсуляція, функціональні продукти харчування.*