ABSTRACT
of the Thesis for the Philosophy Doctor (PhD) degree
Specialty "8D05101-Biology"
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Application ways of adsorptive and spatial immobilization of probiotics for their protection and delivery to the gastrointestinal tract

General characteristics of the dissertation work. Comprehensive studies have been carried out aimed at creating a new polysaccharide prebiotic matrix with a probiotic biofilm immobilized in it. As a result, synbiotic microgranules based on bacterial cellulose (BC), prebiotic – pullulan (PUL), and *Lactobacillus rhamnosus* GG biofilm were obtained and characterized.

Relevance of the topic. Probiotics are live microorganisms that, when consumed in adequate quantities, benefit the human body. The idea is that taking probiotics can help restore and strengthen the local gut microbiota. To do this, exogenous probiotics must successfully, without significant losses, reach the large intestine and at least transiently colonize its mucosa in order to "work" in it. During delivery to the target site, probiotics are exposed to the negative effects of stomach hydrochloric acid, bile, and digestive enzymes. Therefore, creating a method for protecting probiotic cells from stress during transportation is an urgent task, which was implemented in the dissertation research.

In recent years, there has been increasing interest in obtaining microencapsulated forms of probiotics. However, the inadequacy of the existing hardware design limits the industrial use of the microencapsulation method. This served as a prerequisite for this work, the main goal of which is to develop another, simpler and cheaper technological method for immobilizing probiotics through the inclusion of cells in the spatial structure of the polymer, known as "membrane capture" or the adhesive method. It is based on the fact that many microorganisms in their natural habitat, after initially attaching to a substrate, form colonies and then biofilms, which are defined as populations of microorganisms enclosed in a matrix.

Biofilm provides cells with many beneficial properties, including reduced cellular attrition, increased biochemical stability, and stimulation of cell-cell communication by including quorum stimulators, and improved substrate distribution. From a microecological perspective, living within a gut biofilm is a selective advantage that allows microorganisms to exist in a protected niche that allows them to interact directly with the host. Since not all probiotic microorganisms can form biofilms, artificially "force" inclusion of them in a special delivery vehicle could facilitate the colonization and retention of beneficial strains in the gastrointestinal tract (GIT), thereby enhancing their probiotic properties.

The use of BC as a supporting matrix seems promising. Along with high porosity and strength, BC has a high adsorption capacity and is completely biocompatible with both microorganisms and the human body.

Although biofilm immobilization provides a physical protective barrier against external stresses, an additional technique can be used to increase cell stability and viability. It involves the inclusion in the matrix of a special polysaccharide component

with prebiotic activity. It is expected that the presence of a prebiotic in the matrix will stimulate cell proliferation, thereby promoting the formation of a probiotic biofilm within it, providing additional protection. It seems that a working model probiotic strain should, firstly, have a beneficial effect on human health and, secondly, be able to form biofilms on abiotic surfaces. *Lactobacillus rhamnosus* GG has these properties.

The development and production of probiotics immobilized in biopolymer membranes requires a reasonable choice of additional ingredients for the matrix, a method for immobilizing the strain, and obtaining a stable commercial form of the biologically active component based on the biofilm of the probiotic culture. It is assumed that this synbiotic system (neutral carrier + prebiotic + probiotic) can be used to deliver the "working strain" to the target niche – the large intestine.

The purpose of the work: study the possibility of using a complex polysaccharide matrix with probiotic biofilms (PMPB) as a transport system for their delivery to the host body.

Objectives of the work:

- screen microbial polysaccharides for their prebiotic activity;
- choose an optimal method for including a prebiotic component in the BC matrix;
- determine the influence of the composition of the culture medium on the formation of *L. rhamnosus* GG biofilm;
- obtain PMPB microgranules by immobilizing *L. rhamnosus* GG into the bacterial cellulose/pullulan (BC/PUL) matrix complex;
- determine the viability of the probiotic during transportation through the GIT and its controlled release in the large intestine under *in vitro* and *in vivo* conditions;
- determine the composition and diversity of the microbiome of volunteers after taking an experimental sample of the "Lactocell" dietary supplement.

Research objects: polysaccharide matrix complex BC/PUL; microgranules of a polysaccharide matrix with probiotic biofilms.

Research methods: Prebiotic activity – hydrolysis degree, serial dilutions followed by inoculation on MRS medium. Preparation of BC/PUL films – adding a prebiotic polysaccharide to the fermentation medium with a BC producer, co-cultivating the BC and PUL producers. Study of the structure of films and microgranules – scanning electron microscopy. Film strength – Instron tensile testing machine. IR spectrum – Fourier spectrophotometer Jasco FT/IR6200. Biofilm formation – microplate method. Immobilization of probiotics – incubation in a biofilm-inducing medium. Fluorescence microscopy – Biozero fluorescence microscope. Study of tolerance to gastric acid and bile salts, release of probiotics – an artificial model of the GIT. *In vivo* study on rats – microscopy, fluorescent *in situ* hybridization method, viability assessment. Metagenomic analysis – volunteers who took "Lactocell" dietary supplement.

Scientific novelty of the research results.

The nutrient medium has been optimized, cultivation on which induces biofilm formation of *Lactobacillus rhamnosus* GG.

A nutrient medium was constructed, and conditions were selected for the cocultivation of exopolysaccharide producers *Komagataeibacter xylinus* C-3 and *Aureobasidium pullulans* C-7 to obtain the BC/PUL biocomposite.

For the first time, it was shown that a cell-free fecal extract, "fecalase," can be used to model the enzymatic activity of the intestinal microbiome.

For the first time, synbiotic microgranules with a biofilm of a probiotic culture have been obtained.

A new probiotic dietary supplement "Lactocell" has been developed, intended for the correction and restoration of the intestinal microbiome.

Theoretical significance of the work. The dissertation work is of an applied nature and contains new knowledge about the process of immobilization of a probiotic culture into microgranules of biopolymers of the neutral carrier–prebiotic system. This study examines the possibility of immobilizing probiotic biofilms rather than planktonic cells. The choice of the composition of the composite material with prebiotic action is theoretically and experimentally substantiated. The ways of an effective and affordable method for producing BC composites with a biologically active component are outlined. This study confirmed the advantage of cocultivation of producers as a more efficient approach that increases the cost-effectiveness of the method for obtaining a complex polysaccharide matrix. The information obtained about changes in the composition of the intestinal microbiota after taking "Lactocell" dietary supplement may be useful in the further development of the scientific basis for the use of the microbiome in disease prevention.

Practical value of the study.

The nutrient medium has been created for an artificial symbiotic microbial system: BC producer + PUL producer.

The developed new microbial metabiotic colon model may become an important predictive tool to determine the effectiveness of probiotic delivery systems to their ecological niche.

The new complex immobilized probiotic dietary supplement "Lactocell" can be used to prevent microecological changes in the GIT and correct its microflora.

Microgranules of a polysaccharide matrix with an immobilized probiotic culture can find application in functional foods.

Basic statements for the defense:

- the choice of optimal carbon sources and mineral components of the nutrient medium contributes to the induction and stimulation of biofilm formation by *Lactobacillus rhamnosus* GG;
- joint cultivation of biopolymer producers simplifies and reduces the cost of obtaining a composite from microbial polysaccharides;
- the synbiotic system (neutral carrier + prebiotic + probiotic) protects stressful conditions of the GIT and preservation of the volume of viable cells of the probiotic culture, allowing for the effective delivery of probiotics to the large intestine.

The main research results and conclusions:

1. Using an artificial stomach juice, the resistance to hydrolysis of three microbial polysaccharides was determined, which was in the following order: PUL (97%) > XG (93%) > GG (90%). It was found that PUL better stimulates the growth

of *Lactobacillus rhamnosus* GG probiotic strain compared to xanthan and gellan. The prebiotic score of PUL was 0.33, and the xanthan and gellan scores were 0.11 and 0.23, respectively.

2. The strategy of cocultivation of producers is preferable for obtaining the BC/PUL biocomposite than the *in situ* method. The weight of the film when adding a pre-synthesized prebiotic to the fermentation medium with the BC producer was 11.9 g/l, and for cocultivation, it was 16.8 g/l. Using IR spectroscopy, it was established that the inclusion of PUL in the BC film occurs through chemical interaction. The BC/PUL composite forms a network of fibers (average size – 80±4 nm), providing high mechanical properties (tensile strength – 27±0.7 MPa; elongation at break – 14±0.5%).

3. The optimal modification of the PYG_m medium composition has been selected when grown on which the formation of *Lactobacillus rhamnosus* GG ATCC 53103 biofilm is induced and stimulated. The proposed composition of the modified PYG_m medium (g/l): peptone – 15, casein hydrolysate – 5, glucose – 5, yeast extract – 10, $MgSO_4$ – 0.1, inulin – 1, bile – 0.5.

4. A scheme has been developed for the production of PMPB microgranules: cocultivation of polysaccharide producers to form a BC/PUL composite → grinding and granulation of the matrix → immobilization of the probiotic in a medium that induces biofilm formation → lyophilization. The ready-to-use synbiotic microgranules contained 9.82 log CFU/g.

5. The survival rate of immobilized LGG in PMPB microgranules after incubation in the stomach + duodenum model system is 6 log higher than that of free cells. The LGG titer in colonic juice increased by 1.5 log (11.4 log CFU/g) compared to the initial value (9.75 log CFU/g). Oral administration of PMPB microgranules to rats demonstrated that probiotics were successfully released from the matrix in the colonic environment. The highest concentration of viable immobilized LGG in fecal samples was found between 3 and 7 days after the start of consumption (7.38-7.63 log CFU/g), with 5.12 log CFU/g found one week after the last intake.

6. The use of "Lactocell" dietary supplement contributed to an increase in the diversity of the microbiome of volunteers. Before taking probiotic microgranules, *Firmicutes* bacteria predominated, but after the probiotic course, an increase in the level of *Bacteroidetes* was observed. After consuming "Lactocell", a significant presence of bacteria with probiotic properties was detected, including *Lactobacillus rhamnosus* species. An increase in the metabolic activity of amino acids, fatty acids, vitamin B1, polyamines, and pyruvate was established.

Connection with the plan of basic scientific works. The dissertation work was carried out within the framework of the project AP09259491 "Biotechnology application in the production of combined dairy products using polysaccharide matrix with probiotic biofilms" (2021-2023).

Publications. The main content of the dissertation is reflected in 9 published works, including 3 articles in journals indexed in the Web of Science and Scopus databases, 2 articles in republican scientific journals included in the list of CQAFSHE MSHE RK, 4 abstracts in materials of international conferences.

The author's personal contribution lies in carrying out the main volume of theoretical and experimental research, analysis, interpretation, presentation of the results obtained, and preparation of manuscripts for publications.

The volume and structure of the dissertation. The dissertation is presented on 107 pages. It contains 4 tables, 28 figures, 1 appendix, and references used from 260 sources.