Brief information about the project

Name of the project	AP09259491 “Biotechnology application in production of combined dairy products using polysaccharide matrix with probiotic biofilms” (0121PK00253)
Relevance	The fundamental idea of the project is to protect probiotics from stress factors to ensure their effective delivery to the human gastrointestinal tract by immobilization in a hydrogel polysaccharide matrix. The inclusion of a matrix with probiotic biofilms in fermented dairy products will ensure a high degree of cell viability of probiotic microorganisms during the production, storage and consumption of such functional food products. The assortment of probiotic foodstuffs available on world and Kazakhstani market is represented mainly by fermented products made from cow's milk. There is practically no use of such a food matrix as mare's and goat's milk, each of which has a lot of useful properties. In this regard, it is relevant to create combined products having all useful properties of mare and goat milk, as well as maximally level the negative aspects of each of them separately. The introduction of a specially selected strain of probiotic bacteria with proven therapeutic efficacy into their formulation will allow such combined products to be classified as functional, since they will have not only high nutritional, but also medicinal properties.
Purpose	Creation of technology for obtaining combined products from mare and goat milk with probiotics immobilized in a symbiotic polysaccharide matrix based on bacterial cellulose.
Objectives	1. Selection of the composition and method of obtaining a polysaccharide matrix with probiotic biofilms (PMPB). 1.1 Screening of polysaccharides: pectin, xanthan, pullulan by their prebiotic activity; 1.2 Selection of the method for including the prebiotic component into the BC-matrix and determination of its physicochemical and organoleptic parameters; 1.3 Immobilization of a probiotic into a polysaccharide matrix, lyophilization, preparation of PMPB microgranules. The implementation of this objective involves the screening of polysaccharides by their prebiotic activity, followed by the selection of a method for including the most active biopolymer-prebiotic into the matrix base - bacterial cellulose (BC) hydrogel. The production of the polysaccharide matrix complex will be carried out by including the selected polysaccharide into the finished gel film or BC globules, or in the process of its biosynthesis. This will be followed by an assessment of the effectiveness of the prebiotic component inclusion in the BC-matrix (analysis of IR spectrum and electron microscopy) and the determination of the physicochemical and organoleptic parameters of the polysaccharide matrix. Then, it will be followed by the selection of a method for immobilizing the culture of the probiotic strain into this matrix. The choice of method will be carried out by determining the number of immobilized cells (seeding on agar medium and electron microscopy). The resulting PMPB will be lyophilized and ground up.

	As a result, an additional prebiotic ingredient will be selected for the matrix, in which the probiotic culture will be immobilized in an optimal way to create a biofilm. Physicochemical properties (viscosity, water activity, porosity, surface morphology) of the polysaccharide hydrogel and the titer of bacteria in it will be determined. The parameters of the technology for PMPB obtaining will be worked out. 2. Investigation of the protective effect of probiotics immobilization in a polysaccharide gel against aggressive conditions of the technological cycle and the gastrointestinal tract (GIT). 2.1 Determination of the survival rate of probiotics in PMPB during freeze drying and on a model of an artificial gastrointestinal tract; 2.2 Selection of milk base and starter cultures for fermentation of milk mixture from mare's and goat's milk; 2.3 Determination of a complex of physicochemical, microbiological, organoleptic indicators of fermented dairy products. The objective is realized by determining the level of viability of immobilized bacteria (the method of limiting dilutions using the MRS medium) during freeze-drying and on experimental models *in vitro* (artificial gastrointestinal tract). Evaluation of the cryoprotective effect of the polysaccharide matrix components on the viability of the lyophilized probiotic is necessary to determine the shelf life. Determination of the cell titer in PMPB in the model stomach and intestine system is required to assess the degree of validity of their use as a means of delivering a live probiotic to the target niche - the large intestine. As a result, the level of survival of probiotic microorganisms in PMPB will be monitored under freeze drying and simulated gastrointestinal conditions. 3. Development of technology of combined products from mare and goat milk with PMPB microgranules. 3.1 The choice of the method and stage for the inclusion of PMPB in products; 3.2 Determination of food products storage; 3.3 Development of technical and regulatory documentation. Production approbation of the process of obtaining enriched PMPB products. The way to accomplish this objective is to select a method for including PMPB in the developed products, which ensures the preservation of sensory properties, nutritional value and an increase in their shelf life. The objective involves the development of product recipes and technologies, followed by the development of their fermentation modes in production conditions. As a result, 3 types of products with PMPB will be developed: yogurt, fermented milk drink, curd paste. Their nutritional value, organoleptic properties, physicochemical and rheological characteristics will be determined, the dose, method and technological stage of PMPB introduction will be established, fermentation modes and shelf life of new functional products will

	be worked out. Industrial testing of the technology for obtaining products will be carried out, technical and regulatory documentation for new types of combined probiotic products based on mare and goat milk will be developed.
Achieved results	A cost-effective method for the production of the polysaccharide matrix BC/PUL has been established – cocultivation of producers. Symbiotic microgranules were obtained: BC/PUL with LGG biofilm. The physicochemical characteristics of the microgranules and the titer of bacteria in them were determined. Ready-to-use lyophilized granules contained 10^9 CFU/g viable cells. It has been established that immobilization of biofilms provides protection from stress and preservation of viable cells of the probiotic culture. A technology has been developed for producing the dietary supplement "Lactocell" and new probiotic combined products of the "Argymak" series: yogurt, fermented milk drink, curd paste. The viability of the probiotic in products and organoleptic indicators were determined. Storage conditions and periods have been established.
Research team members with their identifiers (Scopus Author ID, Researcher ID, ORCID, if available) and links to relevant profiles	1. Savitskaya Irina Stanislavovna – Doctor of Biological Sciences, Professor. h-index 10; CiteInd.-353, Scopus author ID 36705310600; https://orcid.org/0000-0003-2417-8463; Researcher ID F-5542-2013. 2. Kistaubayeva Aida Serikovna – candidate of biological sciences, associate professor. h-index 8; Scopus author ID 57197801138; http://orcid.org/0000-0002-9385-7155; ResearcherID F-5542-2013. 3. Yuriy Sinyavskiy – Doctor of Biological Sciences, Professor. h-index 2; Scopus Author ID 57212555889. 4. Ignatova Ludmila Viktorovna – candidate of biological sciences, associate professor. h-index 6; Scopus Author ID 57216761870. 5. Talipova Aizhan – Master of Technical Sciences. h-index 3; Scopus author ID 57211535311; https://orcid.org/0000-0001-6874-5760. 6. Abdulzhanova Malika – Master of Technical Sciences. h-index 2; Scopus author ID 57211532475. 7. Zhantlessova Sirina – Master of Technical Sciences. h-index 2; Scopus Author ID 57869399100; https://orcid.org/0000-0001-6604-8056; ResearcherID: ADA-3537-2022.
List of publications with links to them	2022: - in peer-reviewed foreign scientific publications indexed in the Web of Science or Scopus databases, with a non-zero impact factor: 1. Zhantlessova S., Savitskaya I., Kistaubayeva A., Ignatova L., Talipova A., Pogrebnjak A., Digel I. Advanced "Green" Prebiotic Composite of Bacterial Cellulose/Pullulan Based on Synthetic Biology-Powered Microbial Coculture Strategy / / Polymers – 2022. – Vol. 14, No. 15. P. 3224. doi:10.3390/polym14153224 (Scopus Percentile – 76; Q1) 2. Vassilyeva N., Savitskaya I.S., Zhantlessova S.D., Mansurov Z.A., Smagulova G.T. Morphological and Physicochemical Properties of Nanostructured Cellulose Obtained through

	Chemical and Biological Methods // Vestnik Tomskogo gosudarstvennogo universiteta. Biologiya – 2022. – No. 58. P. 55–70. doi:10.17223/19988591/58/3 (Scopus Percentile – 35; Q4) - in publications recommended by CQAFSHE MSHE RK: Zhantlessova S.D., Khamitkyzy Zh., Talipova A.B., Savitskaya I.S., Kistaubaeyva A.S. Selection and optimization of cultivation conditions for bacterial cellulose producer // International Journal of Biology and Chemistry – 2022. – Vol. 15, No. 1. P. 55–63. doi:10.26577/ijbch.2022.v15.i1.06 2023: - in peer-reviewed foreign scientific publications indexed in the Web of Science or Scopus databases, with a non-zero impact factor: 1. Kistaubayeva A., Abdulzhanova M., Zhantlessova S., Savitskaya I., Karpenyuk T., Goncharova A., Sinyavskiy Y. The Effect of Encapsulating a Prebiotic-Based Biopolymer Delivery System for Enhanced Probiotic Survival // Polymers – 2023. – Vol. 15, No. 7. P. 1752. doi:10.3390/polym15071752 (Scopus Percentile – 76; Q1) 2. Talipova A.B., Buranych V.V., Savitskaya I.S., Bondar O.V., Turlybekuly A., Pogrebnjak A.D. Synthesis, Properties, and Applications of Nanocomposite Materials Based on Bacterial Cellulose and MXene // Polymers – 2023. – Vol. 15, No. 20. P. 4067. doi:10.3390/polym15204067 (Scopus Percentile – 76; Q1) - in publications recommended by CQAFSHE MSHE RK: Abdulzhanova M., Kistaubaeva A., Ignatova L., Zhantlesova S., Kabykenova A., Sobhi el-Sohaimi. Preparation of yogurt based on mare's milk powder, enriched with probiotic microcapsules // Microbiology and Virology – 2023. – No. 2(41). – P. 96–123. doi:10.53729/MV-AS.2023.02.06
Patents	1 Utility model patent No. 35575 dated March 18, 2022 *Komagataeibacter xylinus* C-3 bacterial strain – producer of bacterial cellulose. Authors: Savitskaya I.S., Kistaubaeva A.S., Shokataeva D.Kh., Abdulzhanova M.A. 2 Utility model patent No. 7876 dated March 10, 2023 Method for producing yogurt with functional and probiotic properties. Authors: Abdulzhanova M.A., Kistaubaeva A.S., Savitskaya I.S., Sinyavskiy Yu.A., Zhantlesova S.D. 3 Utility model patent No. 8172 dated June 16, 2023 Method for producing curd paste with functional and probiotic properties. Authors: Abdulzhanova M.A., Kistaubaeva A.S., Savitskaya I.S., Talipova A.B.

Йогурт

Кисломолочный напиток

Творожная паста