ANNOTATION

to the doctoral thesis for Doctor of Philosophy (PhD) in the Educational Program « 8D05105-Biotechnology »

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«Development of technology of a domestic original drug against human intestinal infections»

General characteristics of the work. In the course of the work, the technology for the production of the active pharmaceutical substance of the probiotic drug was developed and the technology for the production of the finished form of the probiotic drug was implemented. In addition, preclinical studies were conducted and phase 1 and 2 clinical trials were successfully completed.

The relevance of research. Acute intestinal infections (AII) are still at the forefront of the structure of infectious diseases worldwide, including in Kazakhstan. According to WHO experts, 600 million people, that is, one in ten people in the world, get sick every year after eating contaminated food, and 420,000 die as a result of this disease. A special feature of OCI is its high rate of transmission, and both adults and children are more likely to suffer from this disease.

The incidence of intestinal infections is also increasing in Kazakhstan. NCW epidemiologists have recorded an increase in the number of patients with intestinal infections. In the first five months of 2024, 4,986 cases of acute intestinal infections were registered in the republic (4,764 cases in 2023), 316 cases of salmonellosis (405 in 2023), 79 cases of dysentery (84 in 2023), and 765 cases of rotavirus enteritis (623 in 2023).The sanitary and epidemiological system carried out in the country.

The widespread occurrence of intestinal infections, the frequent occurrence of moderate and severe forms, as well as the presence of complications -all this requires finding ways to optimize treatment tactics for this group of diseases.

Currently, the modern strategy of treating AII gives priority to therapeutic measures aimed at correcting intestinal microbiocenosis in order to eliminate the infectious focus located in the intestine.

In this regard, probiotics based on symbiont microorganisms of the gastrointestinal tract have been increasingly used in the world in place of antibiotics in the treatment of intestinal and genitourinary tract infections. The most common are lactic acid and bifidobacteria, the pathogenic potential of which, according to experts, is low enough for oral administration in conventional doses. These drugs quickly suppress pathogenic microflora and boost the body's immune system.

These probiotic drugs actively suppress pathogenic microflora and improve the immune system of the human body. The use of probiotics in effective doses can have a positive effect on the human body.

Probiotics are used in dysbiosis to regulate and maintain intestinal microflora, as well as to prevent its disorders.

Currently, there are a large number of probiotic drugs based on living microorganisms on the market. However, some probiotic drugs for intestinal infections are not always effective. The reason for this is the insufficient spectrum of antimicrobial action; due to the fact that antagonistic strains of specific pathogens are not selected.

In addition, the analysis of probiotics presented on the pharmaceutical market shows that the data indicated on their packaging does not always correspond to reality.

Domestic probiotic drugs are not produced in Kazakhstan, mainly imported products are used. In this regard, there is very little domestic data on their production.

The production of probiotic drugs is a laborious, complex process consisting of several stages. Before probiotic microorganisms can be included in a medicinal product or dietary supplement, they must be checked for compliance with a number of requirements.

The selection of microorganisms with all of the above properties, along with their effectiveness against pathogens, is a lengthy and complex process.

The industrial production of drugs containing lactic acid bacteria is a complex process. One of the main problems in the production of probiotic drugs is the accumulation of biomass while maintaining the initial properties of the selected strain or community of strains. Since biomass scaling is usually carried out by fermentation, this process needs to be carefully planned and controlled.

The production of domestic technology for drugs against intestinal infections is of high scientific and practical importance, as it requires an approach that includes pharmaceutical development, microbiological research, preclinical and clinical assessment of efficacy and safety, contributes to improving the healthcare system of Kazakhstan, strengthening pharmaceutical independence and improving public health.

The purpose of the research is to develop a technology for a medicinal probiotic drug against human intestinal infections.

Research Objectives:

1. To study and identify the morphological, cultural, and biochemical properties of lactobacilli.
2. To develop the production technology of the active pharmaceutical substance (API) and the finished dosage form of the probiotic drug.
3. To conduct preclinical trials of the drug on experimental animal models.
4. To conduct clinical trials (Phases 1 and 2) of the drug in humans.

Objects of research:

To develop the active pharmaceutical ingredient (API), lactic acid bacteria with high antagonistic activity against intestinal pathogens - *Lactobacillus fermentum* 30 and *Lactobacillus cellobiosus* 36- were used.
The probiotic drug «AC-Probionorm» was developed based on *Lactobacillus fermentum* 30 and *Lactobacillus cellobiosus* 36.

The antagonistic activity of the *L. fermentum* 30 + *L. cellobiosus* 36 culture association was tested against the following test cultures:

Escherichia coli ATCC 8739™, *Escherichia coli* ATCC 11229™, *Salmonella enterica* subsp. *enterica* serovar typhimurium ATCC 14028™, *Salmonella enterica* subsp. *enterica* serovar typhimurium ATCC 29630™, *Staphylococcus aureus* ATCC 6538-p, *Klebsiella pneumoniae* subsp. *pneumoniae* ATCC 10031™, *Pseudomonas aeruginosa* ATCC 9027™.

Preclinical trials of the «AC-Probionorm» probiotic drug were conducted on laboratory white mice weighing 30–32 g (*Mus musculus*) and rats weighing 180–200g (*Rattus*). Phases 1 and 2 clinical trials of the «AC-Probionorm» probiotic drug involved human subjects.

Research Methods:

During the dissertation work, experiments were conducted under laboratory conditions using microbiological methods. Antagonistic activity was determined using antagonism methods such as the well diffusion method**,** cross-streak method**,** and the double-layer agar method.

The industrial strains of lactobacilli were characterized by their morphological**,** cultural**,** and biochemical properties, and their molecular-genetic identification was performed using the Sanger sequencing method based on the 16S rRNA gene sequence**.**

Microorganisms were cultivated and biomass was obtained using fermentation with nutrient media. Quantitative analysis of DNA was performed using a NanoDrop ND 2000 spectrophotometer at a wavelength of 260 nm. In addition, the qualitative assessment of DNA was carried out using electrophoresis in 1% agarose gel**.** For clinical trial data analysis, the following statistical methods were used: Student's t-test for parametric data, Mann–Whitney U test for non-parametric comparisons, Kruskal–Wallis H test for group comparisons and the Chi-squared test for categorical data.

Scientific Novelty and Theoretical Significance of the Research:

In the fight against intestinal infections (IIs), studies aimed at improving the effectiveness of probiotic drugs by expanding their spectrum of action—through the optimal selection of microbial composition and development of efficient production technologies—remain highly relevant.

During the implementation of this dissertation work:

1. The morphological, cultural, and biochemical characteristics of lactobacilli were studied and identified.
2. For the first time, the production technology of the active pharmaceutical ingredient (API) and the finished dosage form of the probiotic drug «AC-Probionorm**»** was developed.
3. For the first time, preclinical studies of the «AC-Probionorm» probiotic drug were conducted on experimental animals.
4. For the first time, clinical trials (Phases 1 and 2) of «AC-Probionorm» were conducted in human subjects.

To enhance the effectiveness of treating intestinal infections, a domestic technology for the production of a probiotic drug has been developed.
The developed production technology, together with the conducted preclinical and clinical trials and organized manufacturing, contributes to the development of

Kazakhstan's domestic pharmaceutical market.
The production of this new domestic probiotic drug opens opportunities for local distribution and export to other countries.

Scientific and Practical Significance of the Work:

To improve the effectiveness of treating human intestinal infections, a **new** domestic production technology for the «AC-Probionorm» drug has been developed.

The technology, developed through preclinical and clinical studies, along with the organization of its production, will contribute to the development of Kazakhstan's domestic pharmaceutical market**.**

The production of this drug can be organized both for domestic use in Kazakhstan and for export to other countries.

Main Principles Proposed for Defense:

1. The production technology of the active pharmaceutical ingredient (API) and the finished dosage form of the probiotic drug ensures the manufacture of a high-quality and effective probiotic preparation for the treatment of human intestinal infections.
2. Clinical trials of the probiotic drug allow for the confirmation of its biological activity, safety, and efficacy in experimental animals.
3. Clinical trials (Phases 1 and 2) in humans demonstrate the drug's tolerability, safety, and effectiveness.

Main Results and Conclusions:

1. The taxonomic identity of the probiotic lactic acid bacteria was confirmed. The biological activity of the drug was validated according to production-quality indicators (the inhibition zones against intestinal pathogens reached up to 24 mm, and the viable cell count was not less than 2.0 × 10^9 CFU/g). The mono strains *Lactobacillus fermentum* 30 and *Lactobacillus cellobiosus* 36 exhibited high antagonistic activity. The association of *L. fermentum* 30 and *L. cellobiosus* 36 microorganisms, which constitute the active pharmaceutical ingredient, demonstrated higher antagonistic activity compared to the individual mono strains.

2. A production technology was developed for the active pharmaceutical ingredient (API) consisting of microbial biomass of probiotic bacteria *Lactobacillus fermentum* 30 and *Lactobacillus cellobiosus* 36 with lyophilized drying medium components including 7% sucrose, 1.5% gelatin, and 7% carboxymethyl starch (CMS). Additionally, a production technology for the finished dosage form of the drug, based on the API with added magnesium stearate, was developed for the probiotic drug «AC-Probionorm». During the development of the API production technology, the optimal nutrient medium (**MRS medium**) and the freeze-drying mode No. 1 were selected. A schematic diagram of the API production and the finished dosage form of the drug were developed. Five experimental batches of the **«AC-Probionorm»** probiotic drug were produced at the manufacturer's production site. The quality of the drug batches was evaluated based on their antagonistic activity against pathogenic microorganisms such as *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538-P, and *Salmonella enterica* ATCC 14028. No coliform bacteria, staphylococci, yeasts, or phages were detected in the drug composition.

3. The biological activity, safety, and efficacy of the probiotic drug were demonstrated in preclinical stages using in vivo experimental animal models, showing comparable results to the commercial drugs Fertal® and Lacidofil-WM. The drug was active, non-toxic, and stable in acidic conditions (pH 3) and in bile.

4. Clinical Trials Conducted (Phases 1 and 2):

In the first phase of the clinical trial, data obtained from 20 healthy volunteers demonstrated the good tolerability and safety of the «AC-Probionorm» probiotic drug.

In the second phase, which included 210 patients with dysbiosis, the efficacy of the drug was confirmed. A significant decrease in the growth of enterococci (by 25%) and *Candida* fungi (by 27%) was observed, along with a notable increase in lactobacilli (up to 80%) and bifidobacteria (up to 76%**)**, indicating the drug's potential to improve the state of the microbiota. The drug's effect remained stable throughout the entire study period (up to 42 days)**.**

The connection with the plan of the main scientific works. The dissertation work was carried out within the framework of the AR14870162 project «Clinical trials of the domestic probiotic drug «AS-Probionorm» with a wide spectrum of action against human intestinal infections» (2022-2024).

Approbation of the work. The materials of the dissertation have been reported and discussed at the following conferences:

- X International Farabi Readings. Proceedings of the international scientific conference of students and young scientists «Farabi Alemi» (April 6-8, 2023, Almaty).

- The International Scientific Conference of Young Scientists dedicated to the 40th anniversary of the founding of the M.A. Aitkhozhin Institute of Molecular Biology and Biochemistry. Almaty 2023.

Publications. The main content of the dissertation is reflected in 12 publications, including 4 articles in journals indexed in the Web of Science and *Scopus* databases (Percentile 56, Q2), 6 articles in republican scientific journals included in the list of the National Research Council of the Republic of Kazakhstan, 2 abstracts in the materials of international conferences.

The author's personal contribution consists in carrying out the bulk of theoretical and experimental research, analyzing, interpreting and formatting the results obtained, and preparing publication manuscripts.

The volume and structure of the dissertation. The thesis is presented on

100 pages of computer text and consists of the following sections: abbreviations, introduction, literature review, research materials, results and discussion, conclusion, list of sources used from 203 titles. The work includes 17 tables, 13 figures and 5 appendices.