

ABSTRACT

dissertation for the degree of Doctor of Philosophy (PhD.)
the specialty " 6D070100-Biotechnology»

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on the topic «Development of new whey - based functional beverages using consortia of lactic acid bacteria and lactose-fermenting yeasts».

General description of work: In the course of the work, antagonistically active strains of microorganisms were isolated and selected, consortia were created, their antagonism, survival in the gastrointestinal tract and normoflora-stimulating activity were increased, and the effectiveness of the selected consortia in the culture of Caco-2 intestinal cells was confirmed. Whey-based beverages have been developed using selected microorganisms. The introduction of one of the developed beverages into production has begun.

Relevance of the research topic: Being a secondary product of the dairy industry, whey is mainly disposed of in waste, despite the fact that it contains up to 50% of milk solids, up to 250 different compounds (nitrogenous, micro-and macro-compounds, milk fat, mineral salts, lactose, vitamins, enzymes, organic acids), and its products have dietary and therapeutic value along with nutritional value. The nutritional value of secondary dairy raw materials, as well as milk, is very high, it is characterized by good digestibility, an optimal ratio of nutrients, biological and physiological compatibility.

Candida yeast is a commensal microorganism and the most common human fungal pathogen worldwide. Candidiasis has become a serious clinical problem due to the growing number of people with weakened immune systems who are susceptible to infection. The mortality rate from invasive candidiasis is 46-75%.

One of the most environmentally sound ways to control pathogens is the use of their natural antagonists. The most important antagonists of fungal microorganisms are lactic acid bacteria, whose healing effect allows normalizing the microflora of the human gastrointestinal tract with simultaneous positive effects at its immunity.

The ambiguity of data on the relationship between yeast of the genus *Candida* and lactic acid bacteria requires detailed research and development of products that suppress fungal, namely yeast, microorganisms along with bacterial ones.

The ambiguity of the available data requires the study of *Candida* - lactic acid bacteria interactions and screening new producers of antifungal metabolites, as well as investigation of conditions for the maximum manifestation of their activity.

The relevance of the topic is confirmed by an avalanche of scientific publications in the field of research of antifungal metabolites of lactic acid bacteria, but the full nature of the interactions of lactic acid bacteria and yeast of the genus *Candida* has not yet been established. Moreover, the inhibition of the growth of yeast microorganisms in the presence of their cellular contact with lactic acid bacteria is not shown.

Despite the close attention of researchers to the problem under discussion, there are no practical results in the form of functional products based on lactic acid

microorganisms that contribute to the prevention and treatment of candidomycosis. Meanwhile, the urgent need to develop natural therapeutic and preventive products that contribute to the normalization of the microflora of the human gastrointestinal tract by eliminating the pathogens of candidomycosis from it is already overdue.

At the Institute of Microbiology and Virology of the KN NAS RK, it was previously shown that only drinks based on mare's and camel's milk, based on a certain combination of lactic acid bacteria and lactose-fermenting yeast, active against *Candida* yeast in a joint culture, exhibit pronounced antagonistic activity against *Candida* yeast. However, the global industry is focused on the use of cow's milk, so it is necessary to develop functional fermented milk products that suppress the growth of opportunistic yeast on its basis or whey, which is often not recycled.

Purpose of the research: development of new whey-based functional beverages using consortia of lactic acid bacteria and lactose-fermenting yeast.

Research objectives:

- Isolation and selection of new strains of lactic acid microorganisms antagonistically active against opportunistic yeast of the genus *Candida*, compilation of consortia of lactic acid bacteria and yeast with high antifungal and antibacterial activity.

- Molecular identification of selected antagonistically active strains.

- Identification of antifungal compounds of selected microorganisms.

- Increasing the survival rate of microorganisms of an antagonistically active consortium in the conditions of the gastrointestinal tract and stimulating the indigenous microflora of exposure.

- Increasing the antifungal activity of the obtained consortia by introducing herbal supplements.

- Development of technology for a new functional fermented milk drink based on whey with an antifungal effect.

Objects of research: lactic acid, acetic acid bacteria and lactose-fermenting yeast and their consortia, antagonistically active against yeast of the genus *Candida*, mold fungi and intestinal opportunistic bacteria.

Subject of the research: The subject of the study is the biotechnologically valuable indicators of new strains of microorganisms isolated from Kazakh national beverages, their antagonistic activity, the possibility of increasing antifungal activity by enriching the medium with plant-based additives and developing functional table and dietary-preventive drinks based on whey.

Research methods: Antagonistic activity was determined by the methods of delayed antagonism. Identification was performed by Sanger sequencing of the 16S rRNA genes of bacteria and the ITS regions of yeast. Volatile metabolites of active associations were analysed by gas chromatography-mass spectrometry. Physical immobilization on wheat bran was used to increase survival in the gastrointestinal tract. The protective effect of selected associations against damaging effects of *Candida albicans* was studied in the culture of Caco-2 cell line of human intestinal epithelium.

Scientific novelty of the research: New strains of microorganisms were isolated from compiled starter associations with antifungal and antibacterial activity,

including lactic acid, acetic acid bacteria lactose-fermenting yeast. For the first time, the correlation between the abundance of acetic acid bacteria in kumis samples and their anti-*Candida* activity has been shown. The data will expand the modern understanding of the metabolism of mono- and mixed cultures of lactic acid bacteria, acetic acid bacteria and lactose-fermenting yeast in terms of their antagonistic activity. The effectiveness of the developed associations in the culture of Caco-2 cells of the intestinal epithelium was confirmed. Also, milk whey drinks with antifungal effect have been developed.

Theoretical significance of the research: The data obtained as a result of the work expand the current understanding of the metabolism of mono-and mixed cultures of lactic acid bacteria and yeast in terms of their antagonistic activity. For the first time, the relationship between the antifungal anti-*Candida* activity of koumiss and the presence of significant amounts of acetic acid bacteria was revealed, and the significance of their inclusion in starter cultures for the production of fermented milk products was justified.

The practical value of the work: The use of whey prevents it from being dumped into wastewater, increase the profitability of milk processing plants and get additional profit from the sale of the drink. Drinking the drink reduces the incidence of intestinal infections, improve public health and reduce the cost of medicines, including imported ones.

Provisions for Defense:

1. The most promising source for the isolation of antagonistically active and biotechnologically valuable strains of lactic acid and acetic acid bacteria is the Kazakh national drink kumis.

2. In suppressing the growth of opportunistic yeast by samples of kumis and associations of lactic acid microorganisms, the presence of acetic acid bacteria in them is of great importance.

3. Increasing the survival rate of starter microorganisms can be achieved by physical immobilization on wheat bran.

4. By introducing acetic acid bacteria and lactose-fermenting yeast into the starter culture and introducing wheat bran into the cultivation medium, the effect of probiotic lactic acid bacteria, which suppresses the indigenous microflora, can be reduced.

5. The introduction of various herbal supplements can increase anti-*Candida* antagonism of ferment associations.

6. Development of a technological scheme for a functional table drink based on whey.

Key research findings and conclusions: Lactic acid bacteria, acetic acid bacteria and lactose-fermenting yeast were isolated from the milk of various animal species and kumis. Suppression of growth of conditionally pathogenic yeast of the genus *Candida* by a number of samples of Kazakh national drinks kumis and shubat was shown.

According to antagonistic activity 12 isolates of lactic acid bacteria were selected. Their molecular genetic identification was carried out and they were assigned to the species *Lactobacillus paracasei*, *L. fermentum*, *L. rhamnosus*, *L.*

diolivorans. The component composition was determined and the identification of microorganisms of the collection association KG, antagonistically active against opportunistic yeast of the genus *Candida*, was carried out. The bacterial components of the association were identified as *L. delbrueckii* 5, *L. gallinarum* 1, *L. parabuchneri* 3, *L. paracasei* 33-4, *A. syzigii* 2. A metagenomic study of kumis samples in the context of their anti-*Candida* activity showed its dependence on the amount of acetic acid bacteria, as well as a negative effect on the antagonism of the abundance of lactic acid cocci (*Lactococcus*, *Streptococcus*).

Compiled associations of lactic acid bacteria, acetic acid bacteria and lactose-fermenting yeast, inhibiting the growth of *Candida albicans*. The best associations in terms of antagonism, organoleptic characteristics and acid formation in whey were selected: A6, including *L. fermentum* A15, *L. paracasei* 4m-2b, *A. fabarum* 4-4M, *K. marxianus* 4MA; KG-3V, consisting of *L. delbrueckii* 5, *L. gallinarum* 1, *L. parabuchneri* 3, *L. paracasei* 33-4, *A. syzigii* 2 and *K. marxianus* 19.

Antifungal compounds with the most antagonistically active KG-3V association have been identified. Acetic acid in the amount of $16,6 \pm 0,64$ mg/ml was shown in the culture liquid after 24 h of cultivation. The relationship of part of the anti-*Candida* activity of the KG-3V association with the cellular fraction has been shown.

The absence of toxicity of two associations, including lactic acid bacteria, acetic acid bacteria and lactose-fermenting yeast, in the culture of intestinal epithelium Caco-2 cells was shown, their anti-*Candida* efficacy in the culture of Caco-2 cells was confirmed.

The effect of introducing lactose-fermenting yeast and acetic acid bacteria into the starter culture, along with lactic acid bacteria, and a prebiotic addition of wheat bran to the cultivation medium, was revealed to protect the indigenous microflora. The protective effect of physical immobilization of the fermenting association on wheat bran on the survival of the constituent microorganisms under acid and bile stress has been demonstrated.

The possibility of increasing the antagonistic activity of starter associations by introducing herbal additives into the cultivation medium has been shown.

Formulations of synbiotic drinks based on milk whey have been developed to suppress the growth of opportunistic yeast *Candida albicans*, mold fungi from the genera *Fusarium*, *Penicillium*, *Aspergillus*, as well as opportunistic bacteria from the genera *Escherichia*, *Salmonella*, *Staphylococcus*, *Sarcina*, *Mycobacterium*, based on the A6 association, including *L. paracasei* 4m-2b, *L. fermentum* A15, *K. marxianus* 4MA, and *A. fabarium* 4-4M.

Connection with the plan of the main scientific works. The dissertation work was carried out within the framework of the project № AP05132352 "Development of new symbiotic functional drinks with antifungal and antibacterial effects based on milk whey" 2018-2021.

Approbation of the work. The results obtained in the dissertation work were reported and discussed at: VI International Scientific Conference of young scientists and students. Prospects for the development of biology, medicine and pharmacy. «New consortia of lactic acid bacteria and lactose-fermenting yeast active against conditionally pathogenic yeast of the *Candida* genus» South Kazakhstan Medical

Academy. Shymkent, 2018; VI International Scientific Conference of students and young scientists «World Farabi». «Isolation of koumiss microorganisms with antifungal activity against opportunistic yeast of the genus *Candida*» Almaty, 2019; Conference «Kazakh National Academy of Sciences», International conference. «Alternative Approaches to Combatting Anti-Microbial Resistance» (AMR). America. 2019; «World Farabi» «Antagonistic activity of lactic acid bacteria associations against opportunistic yeast of the genus *Candida*» Almaty, 2020; Vestnik KazNU, the series is biological «Isolation of microorganisms from Kazakh dairy products with antagonistic activity against yeast species from the *Candida* genus» Almaty, 2019; Vestnik KazNU, the series is biological «Selection of antagonistically active strains of lactic acid bacteria from milk of various animal species» Almaty, 2020; Vestnik KazNU, the series is biological «Development of new functional synbiotic dairy beverage based on the base of whey» Almaty, 2021; Monograph. «Research and Production Center of Microbiology and Virology", Functional drinks based on whey». Almaty, 2020; AIMS Agriculture and Food. «Impact of probiotics and their metabolites in enhancement the functional properties of whey-based beverages» America, 2020; Applied Food Biotechnology. «Immobilization of dairy starter on wheat bran enhance viability under acid and bile stress» Iran, 2020; World Journal of Microbiology and Biotechnology. «Dairy associations for the targeted control of opportunistic *Candida*» Italy, 2021.

Publications and personal contributions of the author. The main content of the dissertation is reflected in 15 printed works, including 3 articles in international journals cited in Scopus, 3 articles in national scientific journals from the list of vestnik, 6 theses in the materials of the conference of the national and international level.

The structure of the dissertation. The dissertation consists of an introduction, a review of the literature, materials and methods, results and discussion, a conclusion and a list of sources used from 119 titles, contains 11 tables and 28 figures.