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**THE ISOLATION AND DIFFERENTIATION OF PROBIOTIC STRAINS OF LACTIC ACID BACTERIA FROM NATIONAL FERMENTED MILK PRODUCTS**

Lidia Stoyanova 1 , Tatiana Sultimova 1 , Maria Napalkova 1 , Olga Ogloblina 1 , Alexander Shevtsov 2 1

Department of Microbiology, Lomonosov Moscow State University, 119991, Leninskie gory, 1–12, Moscow, Russia, + 7 (925) 8340663; stoyanovamsu@mail.ru 2 National center of Biotechnology of Kazakhstan Republic The Committee of Science of the Ministry of Education and Science of Republic of Kazakhstan, 010000, Valikhanova street 13/1, Astana, Kazakhstan, ncmrshevtsov@mail.ru

Lactic acid bacteria (LAB) have been used since time immemorial by people, in particular in some ancient communities, over time, each nation has its own national lactic acid products used not only as food products, but also as therapeutic, preventive and curative agents for the treatment of the cardiovascular system, respiratory organs, gastrointestinal infections, etc. [1, 2]. The LAB isolated from the national lactic acid products draw a special interest among the probiotic correctors of normal intestinal microbiota plays an important role in human ecology. LAB are cultures of strategic importance to the nation’s health. The basic property of LAB, by which they are combined into a separate broad group of microorganisms, is the ability to form lactic acid as the main product of fermentation. Lactic fermentation is carried out by bacterial organisms heterogeneous in morphology, physiological and biochemical properties. Conditions and habitats contribute to the variability of the morphological and cultural properties of these bacteria, which is the cause of greater difficulty in their differentiation and identification. The advantage of the method of natural selection is that nature itself tests the properties of microorganisms. Sometimes even in nature stand out the most adapted form from existing culture conditions. The basis of LAB group are the genus of Lactococcus and Lactobacillus. The genus Lactococcus of serological group was isolated from the genus Streptocoссus, which includes pathogenic forms, and under a new name Lactococcus is classified in the “GRAS” status (absolutely harmless for human health and animals) [3]. The subjects of the study were the microbiota of national home-made fermented products brought from Lebanon (Beirut) – Laban, Leben, but from Buryatia (Ulan-Ude) and Iran (Tegeran) – Kurunga and Doogh (respectively), products of mixed lactic acid and alcohol fermentation. Isolation of LAB was carried out in stages using elective media. The best environment for the cultivation of LAB is the skim milk. In test tubes, the product under study was introduced for self-fermentation at different temperatures (30°, 37°, 42°C) under steady-state conditions. Then, 112 the original test tubes were selected, in which a different density of the milk clot formed, which is characteristic of lactococci or lactobacilli. Then, they sowed on the agar medium to obtain unit colonies. The number of acid-forming LAB was recorded by adding of bromocresol magenta indicator to the medium of incubation, forming a clearing zone around the colonies on the agar medium with deep sowing of lactobacilli due to the formation of lactic acid [4]. The main requirements for probiotic cultures are their inhibitory activity against opportunistic and pathogenic bacteria and health benefits [1, 2, 4]. For this, using the replica method, colonies of isolated pure cultures were tested for antimicrobial activity on the main representatives of various taxonomic groups of bacteria that caused infections. Mesophilic lactococci were grown on agar media for 3 to 4 days, separated from other LAB by colonies on a Petri dish and by microscopy of preparations. To differentiate L. lactis subsp. lactis from L. lactis subsp. cremoris and from L. lactis subsp. lactis diacetilactis took into account the growth pattern on dense media with milk hydrolyzate: L. lactis subsp. cremoris form dark round colonies on the surface of the medium, and L. lactis subsp. lactis diacetylactis – deep colonies of irregular shape in the form of pieces of cotton wool. The cultural features were assessed using a list of cultural characteristics for the identification of bacteria [4]. But strains K-205 and IR, isolated from Kurunga and Doogh (respectively) adapted to an alcohol substratum, could ferment sugar alcohols, including mannitol, which is not characteristic for L. lactis subsp. lactis, but is a differentiating feature for L. lactis subsp. сremoris. Sixty-eight cultures of LAB were isolated: 18 and 15 acidforming lactococci clones and mesophilic lactobacilli were isolated from drinks Kurunga and Doogh, of which 3 lactococci strains with high antimicrobial activity up 2900–3100 IU / ml as compared with nisin (“Nisaplin”, Aplin and Barrett, LTD). It should be noted that lactococci had a high level of inhibitory activity, effectively inhibited the growth of both Gram-positive and Gram-negative bacteria: Proteus vulgaris, E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae also showed a fungicidal effect. Inhibitory activity against molds and yeasts is a little-known biological property of lactic acid bacteria of the genus Lactococcus. The most active colonies lactobacilli, with a wide spectrum of action, were shown on the agar medium of MRS, which is most often used for the cultivation of lactic acid bacteria. Up to 50% of colonies of lactic acid bacteria isolated from the microbiota of the Lebanese dairy products Laban and Leben, inhibiting the growth of gram-positive and gram-negative bacteria and up to 30% possessing fungicidal activity. The taxonomic position of the isolated cultures by classical microbiological methods for the identification of bacteriocin-forming strains of lactococci was confirmed by a genotypic method based on the analysis of the similarity of the nucleotide sequences of the 16S rRNA gene. The cultures of the genus Lactobacillus from Kurunga were identified as L. paracasei and L. rhamnosus related to the L. casei group and as L. brevis, L. buchneri, L. diolivorans, and L. parabuchneri (the L. buchneri group) using the classical microbiological methods and on the basis of the 16S rRNA sequence analysis. The polymorphism of the nucleotide sequences of the genes groEL, rpoB, and rplB encoding specific proteins was studied for intraspecific differentiation of the lactobacilli. To confirm the belonging of two strains 9 and 2.5 isolated from Laban and Leben to the species Lactobacillus paracsei and Lactobacillus delbrueckii ssp. bulgaricus also used a molecular-biological method of identification. The gene nucleotide sequences of all the genotyped strains of most perspective LAB were deposited in the GenBank database. 113 The purpose of further research is to study the probiotic properties of these new strains. Compared to other microorganisms used in the manufacture of fermented milk products, lactococci and lactobacilli are much more active in fermenting the main carbohydrate of milk, lactose. Reducing the concentration of milk sugar in the product is combined with the presence of a high number of living microorganisms that contain their own enzymes that digest lactose. This produces a substitution effect in the intestine for people with lactase deficiency. These strains possessed high proteolytic and antioxidant activities, are resistant to the conditions of the gastrointestinal tract. It should be noted that the new strains of LAB have replenished the collection of cultures and are of scientific and practical interest in terms of expanding biological diversity, their possible use as probiotics, as well as bacterial starter cultures, for the production of lactic acid products. **References**

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