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Biological activity of consortia based on lactobacillus strains extracted from traditional lactic acid starter cultures

Currently, one of the most promising and demanded areas of microbiology is search for new strains of lactic acid bacteria to create probiotics and functional foods. Therefore, this article considers the search, selection, study and comparison of biological properties of *Lactobacillus* spp. cultures of lactic ferments from starter cultures (ayran) fermented in the countryside of the Karaganda region.

Key words: probiotics, microorganisms, lactobacilli, strain, biological properties.

The origins of many branches of the practical use of lactic acid bacteria arose in ancient times, when people began to spontaneously apply them in their daily lives.

Long since has been noted as well the curative value of a number of products made using lactic acid bacteria for the treatment of several diseases. Widely used them doctors of antiquity. Indications of these we meet from the XII century physician Abu Ali Ibn Sina in his «Canon of Medicine». I.I.Mechnikov in search of ways to combat premature aging indicated lactic acid bacteria as antagonists of harmful microbes that live in the human intestinal tract. These studies resulted in a boost to the study of their antibiotic properties. Steady interest in the study of lactic acid bacteria is caused not only by the fact that they play a large and diverse role in the economy of man. These microorganisms have been extremely convenient object for the study of a number of issues related to the metabolic processes of living cells. The human body is a balanced ecosystem, populated by countless variety of microorganisms. A collection of microorganisms of skin, oral cavity, colon and other organs and cavities is called microbiocenosis. Each beneficial microorganism is assigned on its own specific function. Equilibrium state of microbiocenosis of the body characterize overall health. Currently, there is a steady increase in diseases, related to the violation of the natural balance. This is primarily due to the continued deterioration of the ecology [1–2].

Lactic acid bacteria are the most important representatives of symbiotic microflora of intestinal tract of humans, which are indispensable for normal existence of organism. The oppression of their livelihoods and reducing biological activity cause intestinal colonization by opportunistic and pathogenic microorganisms, also there is dysbacterioses, plaguing currently to 90–95 % of the population. It is shown that disbiotic violations are both a consequence and a cause of many pathological processes in the human body, such as chronic inflammatory and infectious diseases of gastrointestinal tract, broncho-pulmonary, cardiovascular system and other. Curing properties of lactic acid bacteria have long attracted the attention, and research in obtaining new therapeutic and prophylactic products of directed action, such as probiotics, are now one of the priorities of biotech science. Identified and used for practical use as probiotics lactic acid bacteria, actively suppressing the growth of pathogenic bacteria and possessing antimutagenic and probiotic properties, enhancing immune response. At the same time, they are not always successfully adapted in the human body, and to stimulate their growth and metabolic activity the so-called prebiotics are used. Since the human body is characterized by its own genetically determined lactic organisms there is a particular interest as the use of a wide range of prebiotics of biologically active substances of natural origin that have a positive impact on them [3–4].

The modern definition of probiotics was given by WHO in 2002, «Probiotics are live microorganisms which, when applied in adequate amounts cause improving the health of the host organism» [5]. According to the requirements of the Food and Agriculture Organization of the UN (FAO) and WHO (2002) microorganisms that are part of a probiotic must have the following characteristics: be non-pathogenic and non-toxic; survive in the intestine; maintain the stability of content and livelihoods for the duration of storage; composed of living cells, which have highly adhesive and antagonistic ability to pathogenic and conditionally pathogenic microorganisms; should not inhibit the normal intestinal microflora; have a genetic passport and proof of genetic stability (to be sensitive or have a natural resistance to antibiotics).

A significant achievement of modern medicine and biology is the progress in the study of human symbiotic microflora and its role in maintaining health. Currently, one of the most promising and popular areas of microbiology is to find new strains of lactic acid bacteria to create probiotics and functional foods. One of the main components of starter cultures for such products and preparations are likely to be bacteria of the genus *Lactobacillus* spp. In this regard, the study of the biological properties of new strains of these microorganisms is relevant, timely objective and are the focus of foreign and Kazakhstan specialists.

For Kazakhstan it is actual to create new highly consortia, based on *Lactobacillus* strains isolated mainly from local sources, promising for applications in medicine, veterinary medicine, ecology, food and other industries. It is known that lactobacilli are used as a component of the leaven for numerous medical and dietary dairy products. Currently lactobacilli are administered either in monoculture or in conjunction with various types of bifidobacteria in the biologically active formulations, dietary supplements and dairy products.

The aim of our work is the selection, study and comparison of biological properties of lactic acid cultures of *Lactobacillus* spp. from starter cultures (ayran) fermented in the countryside of our region (Karkaraly district, village Koktas, Aktogai district, village Aktogai) Karaganda region (research *in vitro*).

Studies on culture-morphological characteristics indicate their belonging to the genus *Lactobacillus*. When the microscoping still sticks, arranged singly or gathered in the chain. Using digital eyepiece USB camera 14 megapixels, the high quality microphotographs were reproduced to create photo of atlas of selected probiotic cultures in the Karaganda region. Cells size of 0.5–1.2 microns. Did not form spores. Optimum growth temperature 37 °C. Well grow on MRS medium. Gram positive. Facultative anaerobes, microaerophiles. On agar media of a company «Laktobakagar» TU 9398-104-78095326-2012 colonies were grown 1–2 mm in diameter, convex edge with solid, opaque and not pigmented. Culture collection of genus *Lactobacillus* spp. was identified by morphological, tinctorial, cultural, biochemical properties. To isolate were used conventional methods. Antagonistic activity was determined by agar layers (A.Y.Likhachev) 20 strains of *Lactobacillus*, to 6-strain test: fungi of *C.albicans*, *C.tropicalis*, *S.marcescens*, *B.subtilis*, *S.aureus*, *E.coli*, *P.mirabilis* (Museum of strains of the Department of Microbiology KSMU), the result was taken into account by the appearance of plaques around the zone of lysis, the lack of growth around colonies of tested strain of lactobacilli.

Results

Thus, the antagonistic activity of *Lactobacillus* investigated against the test strains of *S.aureus* was the highest of 49.7 %, with low inhibitory activity against *S.aureus* found in three crops, in 76.5 % lactobacilli average antagonism to staphylococcus aureus was detected, *E.coli* — 37,1 %, regarding *S.marcescens* — 33,1 %, against *P.mirabilis* — 9,7 % of the cultures showed a low degree of antagonistic activity to fungi of the genus *Candida*, marked by weak antagonism it is associated with high production of *Candida* species lysozyme, which acts as a protective factor. Probiotic potential of isolated bacteria of the genus *Lactobacillus* spp. authors studied in 48 isolated from local milk products of the genus *Lactobacillus* bacteria spp., to this end, experimental studies of bacterial survival kind *Lactobacillus* spp. in simulated gastrointestinal conditions of tract *in vitro*. Survival of probiotic strains depends on their own resistance and microorganism factors. These factors should first include lysozyme, gastric juice, bile, the enzyme component (trypsin). In our studies of 48 crops studied only 2 samples showed high resistance to crystalline lysozyme (Bryntsalov Russia, lyophilized powder 0.05 g), 4 strains to medical bile, 2 strains to hydrochloric acid gastric juice, 4 strains of the enzyme trypsin. Gastric acid is one of the main factors preventing the penetration of the body's defense microorganisms, in such an environment only microorganisms having acid resistance survive. In the small intestine the probiotics are exposed to bile acids and pancreatic enzymes. Consequently, many microbes almost completely die. This may be due to increased permeability of the cell membrane of bacteria, which occurs in response to bile acids and pancreatic enzymes, so that the detection ability at 4 *Lactobacillus* strains in the literature is considered a good predictor of outcome [5].

When resistance to bile study found 10 % of the studied cultures are resistant to 40 % concentration of bile medical.

Spectrum of antimicrobial activity was studied in relation to such representatives of pathogenic microflora as *S.aureus*, *B.subtilis*, *S.marcescens*, *E.coli*, *P.mirabilis*, and fungi *C. albicans*, *C.tropicalis* by method of deferred antagonism. It was established that the average values of stunting indicator crops range from 10 mm to 28 mm ($p \leq 0,001$). It should be noted that the highest sensitivity to bacterial activity expressed *E.coli*, *B.subtilis*. Thus, the study of antagonistic activity of bacteria of the genus *Lactobacillus* spp. regarding opportunistic and pathogenic microflora showed that the vast majority are antagonists with high

activity from 10 mm to 28 mm ($p \leq 0,001$), low levels of antagonistic activity showed 22.0 % isolates with a zone of stunting indicator cultures less than 7 mm. Basic mechanisms of antagonistic activity are the biological effects of various metabolites produced by bacteria of the genus *Lactobacillus spp.* Among these metabolites include lysozyme, lactic acid, hydrogen peroxide, gastric juice, bile, the enzyme component (trypsin), bacteriocins. At this stage the authors investigated the ability of strains to withstand high rates of resistance to natural factors of the body protection, exploration of the spectrum of antimicrobial activity to the test strains was done, the results are characteristic for *Lactobacillus* strains with high biological activity [5–12].

The use of modern microbiological techniques allow to conduct screening of isolated cultures and make a selection of the most biologically active associated strains, which will form the basis of the consortium of microorganisms cultivated in our region. Urgency to develop new highly active consortia based on *Lactobacillus* strains extracted mainly from local sources dictates the continuation of this study.

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Дәстүрлі сүт қышқыл өнімнің ұйытқысынан бөлініп алынған лактобактериялар штаммы негізіндегі консорциумның биологиялық белсенділігі

Қазіргі заманда микробиологияның тиімді бағытының бірі — пробиотикалық препараттарды және функционалды тамақтану өнімдерін дайындау үшін сүт қышқыл бактерияларының жаңа штаммын іздеу болып отыр. Осыған байланысты мақалада Қарағанды облысының ауылдық жерлерінің айран ұйытқысынан бөлініп алынған сүт қышқыл дақылдарының бірі *Lactobacillus spp.* тұқымдасының биологиялық қасиеттерін зерттеу және салыстыру көрсетілген.

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Биологическая активность консорциумов на основе штаммов лактобактерий, выделенных из традиционных молочнокислых заквасок

В статье отмечено, что в настоящее время одним из перспективных и востребованных направлений микробиологии является поиск новых штаммов молочнокислых бактерий для создания пробиотических препаратов и продуктов функционального питания. В связи с этим авторами рассмотрены поиск, выделение, изучение и сравнение биологических свойств молочнокислых культур рода *Lactobacillus spp.* из заквасок (айран), заквашиваемых в сельской местности Карагандинской области.

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