Bacterial Cultures Study for Creating a Nutritional Supplement With Probiotic Properties

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ABSTRACT

Food products of functional purposes, where nutritional supplements have a special position, have a positive physiological effect on human health by affecting the digestive tract. In the present research article, there are results of experiments on selection of microorganisms for making a nutritional supplement with probiotic properties. The nutritional supplement is to contain specially chosen and identified strains, including *Lactobacillus, Bifidobacterium, Streptococcus salivarius subsp. Thermophilus*. The microorganisms are identified by their physiological, biochemical and genetical properties and are deposited in Federal Institution "State Research Institute of Genetics and Selection of Industrial Microorganisms of the National Research Center" Kurchatov Institute" – GosNIIGenetika. The authors present results of experimental work on combinability of the strains with each other based on the method of coculturing of examined strains and with filter liquors of other strains. It is proved that the strains of probiotic bacteria enter into symbiotic relationship with each other as well as with the examined technologic strain of Streptococcus thermophiles. The experimental results of antagonistic activity of a supplement made on the basis of examined strains give evidence of a marked antagonism to the testing cultures of microorganisms that can cause intestinal infectious diseases. The conducted research made it possible to produce a nutritional supplement with probiotic properties that can be recommended to use in functional food product technology.

Keywords**:** *probiotic properties, nutritional supplements, Bacterial cultures*

1. INTRODUCTION

Food has always been one of the most important factors supporting the life on the Earth. The problem of intensification of activities in different countries to prevent premature mortality from noncommunicable diseases (NCDs) and to reduce risk factors for developing NCDs through preventive measures was discussed at a meeting of the World Health Organization High-Level Independent Commission on Noncommunicable Diseases [1]. Four out of the nine goals set by the WHO relate to nutrition, including correction of dietary intake and expanding the range of food containing functional components. According to the scientific literature, such components include: food fiber, vitamins, macro- and microelements, polyunsaturated fatty acids, medium-chain fatty acids, probiotics, prebiotics, synbiotics, antioxidants, flavonoids and others. While the research is deepening and expanding, the number of functional components is constantly increasing, but it is stated that they should be dosed and only those that have proven effectiveness should be used [2, 3] [4]. Chronic deficiency of these components leads to the shift of balance of the symbiotic system "the organism and its microbiome". When the severity of disorders in this system increases, the probability of pathological changes in the body grows as well. At first these changes have a functional nature, but subsequently, they manifest themselves in the form of numerous and various diseases, the spectrum of which depends on the profile and number of the affected functional biomolecules [5]. It is generally believed that many functions of the microorganism are directly or indirectly influenced by the intestinal microbiota. In view of this, timely correction and maintenance of the composition of the intestinal microbiota, that plays a key role in the pathogenesis of many diseases, is an essential prerequisite for supporting the life-sustaining activity of the body and expanding the life expectancy [6, 7]. Despite the long-term research on probiotic bacteria, the interest of the scientists remains at a high level, which is proved by numerous publications in different countries [8–10]. This is connected not only with the manifestation of functional properties by probiotic bacteria, but also with the fact that, according to their systematic position, they are internationally classified as GRAS, i.e. as microorganisms that do not cause infectious diseases of humans and animals. Some researchers believe that such effects of microbiota are based on microbial low-molecular compounds that they liberate [5, 11, 12]. Thus far we

already know the following functions of useful representatives of microbiota: morphokinetic effect; regulation of the gas composition of the cavities and water-salt metabolism; participation in the metabolism of proteins, fats and carbohydrates; immunogenic and detoxificative functions; regulation of metagenome stability, regulation of replication and phenotypic expression of genes of prokaryotic and eukaryotic cells, and some other functions [5, 11]. Probiotics and products containing them are not pharmaceuticals, as they are not used to treat functional pathologies, but to regulate the functioning of certain organs and organ systems in the human body. However, even the optimization of the body functioning allows us to talk about the successful competition of probiotics with many pharmaceuticals. The spectrum of clinical syndromes and pathological conditions, the pathogenesis of which is believed to be related to microecological imbalance in the human body, is currently quite wide and tends to expand.

One of the achievements of the beginning of the 21st century is the development and implementation of the concept "probiotics and functional nutrition". By changing the content and proportion of certain nutrients (microorganisms, minerals, vitamins, isoprenoids, unsaturated fatty acids, cholines, organic acids, and others) that enter the body with probiotics and functional food products, it is possible to regulate almost all life processes in organs and tissues through direct or indirect impact. Scientific rationale, competent selection of strains from normal microflora representatives is the main strategic direction of the development of probiotics and functional food products industry that determines their use by individuals depending on their gender, age, race and nationality, working and living conditions, physiological and health conditions. Important criteria for development of nutritional supplements from probiotic bacteria of different taxonomic groups include the relationship between different strains and their ability to inhibit the development of pathogenic and potentially pathogenic microorganisms. The nature of their relationship depends on the taxonomic group of bacteria, cultivation conditions and culture medium, the time of adaptation, the growth activity of microorganisms, as well as the ability of each individual strain to produce various metabolism products [13–15]. It is to be noted that not all probiotic bacteria actively reproduce themselves in unpasteurized milk, so *Streptococcus thermophilus* is used to speed up the growth of these bacteria and to obtain products with the required characteristics. The analysis of scientific and technical literature allows us to conclude the relevance of research in the field of increasing the number of probiotic strains with proven properties for creating nutritional supplements and prophylactic products.

2. THE AIM OF THE RESEARCH

The aim of the research was to study the compatibility of probiotic strains with each other and with *Streptococcus thermophilus*, as well as to analyze the antagonistic activity of the developed nutritional supplement with probiotic bacteria against some representatives of pathogenic and potentially pathogenic microorganisms that can cause intestinal infectious diseases and, as a result, other diseases.

3. OBJECTS OF STUDY

The objects of study have been strains of probiotic bacteria *Lactobacillus acidophilus*: AD-3 (ВКПМ B-8152), AE-5 (ВКПМ B-8153), AST-44 (ВКПМ B-9647), AB-259 (ВКПМ-B-6543); *Bifidobacterium bifidum* GSB-15 (ВКПМ S-1539), *Bifidobacterium adolescentis* B-1 (ВКПМ Ac-1243), *Bifidobacterium longum* VGB-21 (ВКПМ S-1540); *Streptococcus salivarius* subsp. *Thermophilus* strain ST-95 (ВКПМ V-7985), which has technological properties. All strains were deposited in the All-Russian Collection of Industrial Microorganisms (ВКПМ) of NRC "Kurchatov Institute" – GosNIIGenetika. A nutritional supplement has been developed on the basis of the studied strains.

4. MATERIALS AND METHODS

4.1. Biochemical research methods

The antagonistic activity of the created nutritional supplement *in vitro* was measured by the method of developing mixed populations in comparison with the growth of test cultures in monoculture. The developed nutritional supplement was cultured on nutrient medium MRS-broth (Merck KgaA, Germany). Fresh samples of the test microorganisms of the second generation were washed off from meat and peptone agar slant (Federal Budget Institution of Science «State Research Center for Applied Microbiology & Biotechnology» (FBIS SRCAMB) Obolensk, Russia) with physiological saline. The inoculation dose of microorganisms, determined using McFarland standard, for all strains was 10^8 CFU/cc. 1 cc of suspension of each test microorganism was added into test tubes with 9 cc of liquid nutrient medium. To control the growth of test microorganisms in monocultures, the control tubes were incubated for as long as 48 hours at the temperature of (37 ± 1) ºC. In the experimental tubes, seeded with test microorganisms in the same way, the test nutritional supplement was added in the amount of 10^6 CFU/cc. Thus, the prepared mixed populations of microorganisms (experiment) were incubated at the temperature of (37 ± 1) ºC for as long as 48 hours. At the end of the incubation process, a series of ten-fold serial dilutions in physiological saline were prepared from experimental and control tubes. Culture media were taken from these dilutions to register the correlative test-microbe: *E.coli* for Endo agar (FBIS SRCAMB) Obolensk, Russia); *Staph. Aureus* for Baird-Parker selective agar base (HiMedia Laboratories, India); *Proteus vulgaris* and *Salmonella Dublin* for meat-and-peptone agar (FBIS

SRCAMB, Obolensk, Russia). Inoculations of media in Petri dishes were thermostated at (37±1) ºC during 24–48 hours and the number of CFU in both control and test samples was counted. *E. coli* B-125, *Staph. Aureus* 209-P, *Proteus vulgaris* F-30, *Salmonella Dublin* D-11 received from the Scientific Centre for Expert Evaluation of Medicinal Products were used as a test sample culture. Check studies were conducted in a special accredited and certified testing laboratory. Antagonistic activity in research works is expressed by the microbistatic quotient Pi (%) that is calculated by the following formulae:

$$P_i = \frac{C_м - C_c}{C_м} \times 100\%,$$

where $C_м$ is the number of test-culture cells, developed in monoculture; C_c is the number of test-culture cells, developed in a mixed population with the examined probiotic bacterial strain.

4.2. Evaluation of active acidity

Evaluation of active acidity was carried out by the potentiometric method with the help of a potentiometric analyzer with the variation range of 1–14 pH to a precision of ±0.02 pH.

4.3. Evaluation of combinability of strains

100cc boiled and cooled up to (37±1)ºC milk was added with 5% of examined culture combination. The inoculated milk was thermostated at (37±1) ºC until bunching. The time of bunching process was registered and its active acidity was measured. The combinability of strains was evaluated by the active acidity of clots and the time of milk ripening with the help of strains mixture in comparison with the stated indices for singular strains.

To carry out the analysis only fresh 16–18 hours Bifidus bacteria developed from Blaurock medium (FBIS SRCAMB) Obolensk, Russia) and 4–6 hours media of *Str. Thermophilus* и *Lb. acidophilus* developed from MRS-agar (Merk KgaA, Germany) were used. Germ-free filter liquor was prepared from the 16–18 hours bifidus bacteria medium and 4–6 hours media of Lactobacillus in aseptic conditions. Then 5 % inoculum and 5 % filter liquor of examined mixture of strains were filled in the test tubes (experiment). Filter liquors of examined strains were the control samples in the experiment. Later they were thermostated during 16–18 hours at (37 ± 1) ºC. Compatibility of the examined strains of Bifidus bacteria, Lactobacillus and Streptococcus thermophiles was evaluated by the number of CFU in 1 cc of the medium and by the active acidity of the medium before and after thermostation.

5. DISCUSSION OF RESULTS

The interactions of the examined microorganisms for the production of nutritional supplement were under examination during the experiment on the active ability to reproduction while a separate species of strain influenced the development of the strain of the other species. The indices of active acidity of the medium and the cells number were registered twice: in the beginning of the experiment and after 18 hours of co-culturing. The experiment results are showed in Tables 1 and 2.

The submitted data represent the highest activity of development of the strain *B. adolescentis* B-1 in presence of filter liquors of all strains of Bifidus bacteria and the strain of *Str. Thermophilus* CT-95. The number of cells increased hundred and fiftyfold from 6.6 Lg CFU/cc to 9.2 Lg CFU/cc. The lowest cell growth was registered in combination of *B. adolescentis* B-1 and filter liquor of *B. longum* ВГБ-21 – from 7.3 Lg CFU/cc to 9.2 Lg CFU/cc.

B. bifidum ГСБ-15 strain was highly active while developing with the filter liquor of the strain *Lb. acidophilus* АЕ-5. At the same time the number of cells increased from 7.3 Lg CFU/cc to 9.2 Lg CFU/cc. The lowest cell growth was registered in the control sample (pure *B.bifidum* ГСБ-15 without any additional microorganisms).

Table 1 The compatibility of the strain *B. adolescentis* B-1

Filter liquor	Strain *B. adolescentis* B-1			
	Active acidity, pH		Cells number, CFU, Lg/cc	
	0 hours	18 hours	0 hours	18 hours
B. adolescentis B-1 (control)	6.5±0.01	3.80±0.01	7.45±0.1	8.20±0.1
B. bifidum ГСБ-15	6.5±0.01	4.25±0.01	7.1±0.1	8.30±0.1
B. longum ВГБ-21	6.5±0.01	4.27±0.01	7.1±0.1	7.47±0.1
Str. thermophilus CT-95	6.5±0.01	4.08±0.01	6.0±0.1	8.20±0.1
Lb. acidophilus АЕ-5	6.5±0.01	3.90±0.01	6.7±0.1	8.50±0.1
Filter liquors of all strains	6.5±0.01	4.00±0.01	6.6±0.1	9.20±0.1

Table 2 The compatibility of the strain B. bifidum ГСБ-15

Filter liquor	Strain *B. bifidum* ГСБ-15			
	Active acidity, pH		Cells number, CFU, Lg/cc	
	0 hours	18 hours	0 hours	18 hours
B. bifidum ГСБ-15 *(control)*	6.5±0.01	4.20±0.01	7.7±0,1	8.30±0.1
B. adolescentis B-1	6.5±0.01	4.10±0.01	7.6±0.1	8.50±0.1
B. longum ВГБ-21	6.5±0.01	4.45±0.01	7.6±0.1	8.95±0.1
Str. Thermophilus CT-95	6.5±0.01	4.62±0.01	7.5±0.1	8.95±0.1
Lb. acidophilus AE-5	6.5±0.01	4.43±0.01	7.3±0.1	9.20±0.1
Filter liquors of all strains	6.5±0.01	4.07±0.01	7.3±0.1	9.00±0.1

The results of examination of compatibility of the strain B. longum ВГБ-21 showed its highest activity while developing together with *B. adolescentis* B-1, as well as with *Lb. acidophilus* AE-5. The number of cells increased from 7.2 Lg CFU/cc to 9.2 Lg CFU/cc and relatively from 7.0 Lg CFU/cc to 8.7 Lg CFU/cc. The lowest cell growth was registered in the control sample and in combination of *B. longum* ВГБ-21 with filter liquors of all strains.

The results of examination of compatibility of the strain *Str. thermophilus* CT-95 showed its highest activity while developing together with filter liquors of all strains. At the same time the number of cells increased from 6.8 Lg CFU/cc to 8.4 Lg CFU/cc. The lowest growth was observed in combinations of *Str. thermophilus* CT-95 with *B. adolescentis* B-1 and *Str. thermophilus* CT-95 with *Lb. acidophilus* AE-5. The number of cells increased in this case from 7.8 Lg CFU/cc to 8.4 Lg CFU/cc.

The received data of the compatibility of *Lb. acidophilus* AE-5 represent the highest activity of its development together with the filter liquor of *B. bifidum* ГСБ-15 and B. *longum* ВГБ-21. They gave a cell boost of *Lb. acidophilus* AE-5 from 5.8 Lg CFU/cc to 9.0 Lg CFU/cc. The lowest cell growth was observed in the control sample. In case of adding filter liquors of all strains the number of *Lb. acidophilus* AE-5 cells extensively increased that was an important factor in food supplement production.

The results of examination of antagonistic activity of the nutritional supplement with probiotic bacteria in comparison with testing cultures that can trigger GIT diseases are shown in Figure 1.

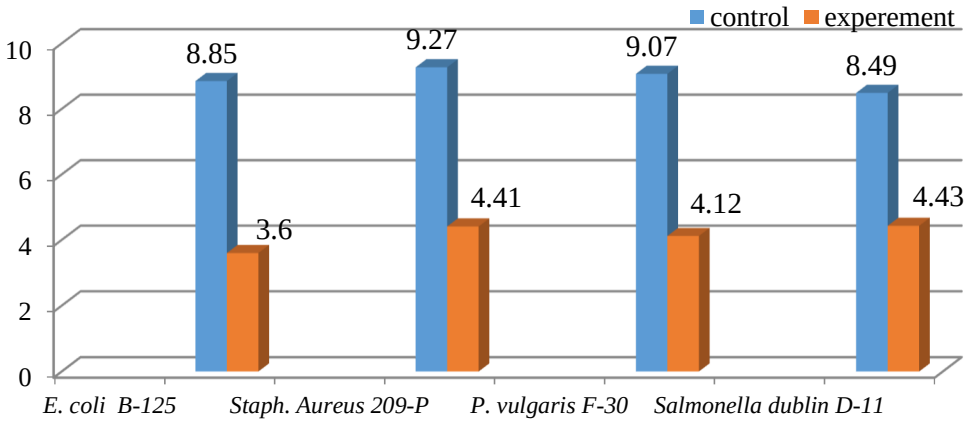

Figure 1 Antagonistic activity of a nutrient supplement with probiotic properties

The submitted data show an evident antagonistic activity of a nutritional supplement with probiotic properties against the testing cultures of microorganisms (Figure 1). As provided in the experiment all strains of

probiotic cultures have the antagonistic activity against pathogenic and potentially pathogenic microorganisms. The inhibition rate of *E. coli* B-125 is 59 %; *Staph. aureus* 209-P – 52 %; *Proteus vulgaris* F-30 – 54 %; *Salmonella dublin* D-11 – 47 %.

6. CONCLUSIONS

The examined data revealed the fact that the studied strains of probiotic microorganisms potentiate the growth of each other in comparison with the control sample, but they do not have an antagonistic activity against each other. The research shows that the examined strains of probiotic bacteria enter into symbiotic relationship with each other as well as with the examined technologic strain of Streptococcus thermophilus. The obtained data made it possible to create a nutritional supplement with probiotic properties. The research of antagonistic activity of the nutritional supplement on the basis of the examined strains show that there is an evident antagonistic activity against the testing cultures of microorganisms: *E. coli* B-125, *Staph. aureus* 209-P, *Proteus vulgaris* F-30, *Salmonella dublin* D-11. Therefore, the nutritional supplement with probiotic properties can be recommended to use in functional food product technology.

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