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## Functional Properties of Lactic Acid Bacteria and Bifidobacteria

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### Abstract

A comprehensive research was carried out to substantiate theoretically and experimentally the application of probiotic polyspecific concentrates Bifilakt A and Bifilakt D as highly effective symbiotic additives. The physiological and biochemical properties of the following bifidobacteria strains were studied: *B. bifidum* 791, *B. longum* B339M, and the Bifilakt A and Bifilakt D concentrates. At that, it was revealed that polyspecific bacterial concentrates Bifilakt A and Bifilakt D have high biochemical and colonizing potential as well as antagonistic and antimutagenic activity, and the prospects of their application as probiotic cultures for the production of symbiotic products were substantiated.

### Keywords

Lactic acid bacteria; Bifidobacteria; Probiotics; Symbiosis

### Introduction

Assessment of existing or newly developed potential products of functional nutrition requires taking into account whether they are capable of improving the composition of normal microflora or not [1,2].

A prospective method of developing symbiotic functional products is the search and implementation into production of the substances of natural origin, which have both technological and physiological functionality simultaneously [3].

So, we selected various probiotics as the study objects – strains and consortia of bifidobacteria and lactic acid bacteria that were the most typical representatives of normal human microflora.

### Methods

Identification of bifidobacteria was carried out in compliance with Methodical Instructions 4.2.999, and identification of bifidobacteria in cultures mixed with lactic-acid bacteria – in compliance with Methodical Instructions 4.2.577.

Probiotics viscosity was determined by rheological methods using an Ostwald viscometer at 20°C; specific proliferation rate of microorganisms was assessed by an increase in biomass; concentration of exopolysaccharides was assessed by the anthrone method; quantitative count of microorganisms was performed by limiting dilution in the hydrolysate lactose medium or corn-lactose medium according to Specification 10-02-02-789-192-95; pH was assessed by the potentiometric method according to GOST 3624-87; and antimutagenic activity was assessed by the Ames test.

### Results and Discussion

Application of bifidobacteria in probiotics is particularly timely, as these microorganisms have been found to be among the most important representatives of human microbiocenosis.

Analysis of the works of Pool-Zobel and Sauer; Yazawa and Tamura [4,5] on the study of biological properties of different strains of bifidobacteria and lactobacilli showed that bifidobacteria are of particular importance among a wide range of microorganisms inhabiting the human intestine. Dominating in the biocenosis of the intestine of children and adults, these bacteria fulfill important physiological functions while enhancing immunological resistance. Producing a large amount of acetic acid and lactic acid, bifido- and

lactobacteria create an acidic medium in the intestine and actively prevent proliferation of pathogenic and putrefactive microflora. These microorganisms participate in enzymatic processes and help to normalize bowel movements; reduce flatulence; promote absorption of calcium, iron and vitamin D; and synthesize vitamins B and K. They are also used in the synthesis of vitamins B<sub>2</sub>, B<sub>6</sub> and K in considerably larger amounts than other bacteria of the gut microflora, such as *Escherichia coli* and *Lactobacillus acidophilus*. There is evidence that the cultures of these microorganisms supply us with a number of essential amino acids, including tryptophan.

When probiotics are decided to be used, they must be isolated from humans; useful for the host organism, which needs laboratory research and clinical test; safe when taken for a long time; have strong colonizing potential; have stable properties in clinical and technological terms; show minimum ability for translocation into the internal environment of the human organism when large quantity of them is taken in; and show fast rate of proliferation [6].

Based on the above factors and taking into account the unique capabilities of bifidobacteria and their influence on various functions of human organs, we can recommend probiotics or master seed strains of starter cultures for making special dietary sorts of therapeutic flour confectionery goods. However, it is obviously impossible to select a certain one of them, which would be able to inhibit proliferation of pathogens naturally, assimilate cholesterol and oxalates, hydrolyze lactose, and demonstrate antitumor and antiallergic activity. Besides, strains that are meant to be used must be resistant to low pH and bile acids, compatible with other microorganisms, and resistant to antimicrobial agents; and show high rate of proliferation.

Industrial production of starter cultures of bifidobacteria and their application in production processes have become possible due to the intensive selection of bifidobacteria and study of their biochemical and cultural properties, as one of the important criteria in developing

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products with probiotic cultures is the selection of microorganisms with a stable, given battery of properties. Based on the available data on the composition of the microflora of the human gastrointestinal tract, as well as the experience in using pure cultures in special products to create symbiotic systems, the following strains were selected: *B. bifidum* 791, *B. longum* B339M, and the polyspecific concentrates Bifilakt A and Bifilakt D.

Selection of strains was performed taking into account their impact on other microorganisms in a mixed medium. An important concern is that some strains of bifidobacteria, such as *B. bifidum* 791 and *B. longum* B339M, have adhesive properties. Specific activity of the combined bacterial preparation is caused by a number of viable cells of *B. bifidum* and *B. longum* strains in a single dose of preparation. It also depends on the acid-forming and antagonistic ability.

It was revealed that both strains of bifidobacteria such as *B. bifidum* 791 and *B. longum* B339M, and Bifilakt A and Bifilakt D concentrates didn't produce catalase and hydrogen sulfide; didn't reduce nitrate to nitrite; and were not able to liquefy gelatin.

After studying the colonizing potential, we revealed that the strains of the *B. bifidum* 791 and *B. longum* B339M bifidobacteria as well as the Bifilakt A and Bifilakt D concentrates were resistant to high concentration of bile and to phenol solution and common salt, and developed in the environment with low and high pH values, which can be seen in Tables 1 and 2 provided below.

The most important characteristic of probiotic preparations applied in production of functional products [7,8] is their antagonistic impact on pathogenic and opportunistic microorganisms. In this view, we studied *in vitro* the antagonistic activity of *B. bifidum* 791, *B. longum* B339M, Bifilakt A, and Bifilakt D.

The results of the conducted studies indicated that all strains showed pronounced antagonistic activity. The index of proliferation inhibition of pathogenic and opportunistic microflora mostly depends on the type of test culture, rather than on the strain or the concentrate.

The results of the proliferation inhibition index of pathogenic and opportunistic microflora are shown in Table 3.

To use starter cultures for production of flour confectionery goods, it is necessary to take into account their biochemical activity, resistance to sucrose, and the acid-forming property, which is important for regulation of the pH environment.

In view of this, we studied the biotechnological potential of probiotic microorganisms. The results of the study are provided in Table 4.

Analysis of the obtained results shows that all represented microorganisms have intense biochemical activity, which is evidenced by the number of viable cells –  $10^{10}$ - $10^{11}$  CFU/cm<sup>3</sup>.

As evidenced by the data, Bifilakt A synthesizes less exopolysaccharides and has lower viscosity when compared with other

Probiotic culture	Proliferation of microorganisms					
	NaCl, %			Phenol, %	Bile, %	
	2	4	6.5	0.4	20	40
<i>B. bifidum</i> 791	Active proliferation	Moderate proliferation			Active proliferation, grading, sterile area 3 mm	Moderate proliferation in the lower part of the environment
<i>B. longum</i> B339M						
Compound starter Bifilakt D	Active proliferation, sterile area 10 mm		Moderate proliferation in the upper part of the environment			
Compound starter Bifilakt A						

Table 1: Comparative analysis of the colonizing potential of probiotic microorganisms

Probiotic culture	pH value			
	3.5	4.5	8.3	9.2
<i>B. bifidum</i> 791	No proliferation	Active proliferation, sterile area 13 mm	Active proliferation	Active proliferation, sterile area 2 mm
<i>B. longum</i> B339M				
Compound starter Bifilakt D	Moderate proliferation			
Compound starter Bifilakt A				

Table 2: Influence of the pH environment on the proliferation of probiotic microorganisms

Probiotic culture	Degree of proliferation suppression, %							
	<i>E. coli</i> 259-22	<i>Staph. aureus</i> 6538-P	<i>Pr. mirabilis</i> 46	<i>Kleb. pneumoniae</i> 5057	<i>Shig. sonnei</i> 5063	<i>Pr. vulgaris</i> F-30	<i>Citr. freundii</i> 101/57	<i>Bac. subtilis</i> 6633
<i>B. bifidum</i> 791	6.4	22.9	25.7	17.7	65.5	26.5	54	51
<i>B. longum</i> B339M	6.8	0.2	23.5	20.2	69	27	54	77.2
Compound starter Bifilakt D	10.4	32.5	49.3	24.8	76.2	34.4	73.5	60.4
Compound starter Bifilakt A	10.4	30.4	49.3	48.7	76.2	29.2	65.5	77.2

Table 3: Index of proliferation inhibition of pathogenic and opportunistic microflora

Strain	Indicators			
	Viscosity, cSt	Specific proliferation rate, h <sup>-1</sup>	Concentration of exopolysaccharides, mg/ml	Number of viable cells, CFU/cm <sup>3</sup>
B. bifidum 791	5.81	0.65	6.80	3 × 10 <sup>11</sup>
B. longum B339M	7.38	0.77	10.50	5 × 10 <sup>10</sup>
Compound starter Bifilakt D	4.65	0.68	2.30	7 × 10 <sup>11</sup>
Compound starter Bifilakt A	2.15	0.58	1.08	5 × 10 <sup>11</sup>

Table 4: Comparative analysis of the biotechnological potential of probiotic microorganisms

probiotics. This property is important for reducing the water-binding power of foods.

Thus, taking into account the biotechnological potential of probiotic microorganisms, we find it possible to select the most prospective strains of mono- and polycultures that meet the requirements of technological peculiarities of confectionery goods production.

Various additives used in the food industry [9] and chemicals used in the agricultural industry contain certain quantity of carcinogens and mutagens that adversely affect human health. Lactic acid bacteria and bifidobacteria are known to be the representatives of the gastrointestinal tract microflora and have antimutagenic properties, which depend on the species strain affiliation of microorganisms and cultivation conditions.

In this view, further research targeted studying antimutagenic activity of probiotic microorganisms. As evidenced by the data, the most pronounced antimutagenic effect is achieved by the cells of the Bifilakt A compound starter.

It is to be noted that the cultural fluid of the studied strains also shows antimutagenic activity, yet it is slightly less than in the microorganisms' cells.

According to Y.K. Alekperov and L.I. Vorobyova, antimutagens of the cultural fluid are certain amino acids – arginine, histidine, methionine, cysteine, and glutamic acid – which produce lactic acid bacteria and bifidobacteria. Apparently, the effect of cultural fluid's antimutagenic effect is conditioned by this fact.

The most illustrative owners of the antimutagenic effect were the cells of compound starters and lactic acid bacteria and bifidobacteria. The antimutagenic nature of cells of the Bifilakt D polyspecific concentrate was rather high and equaled to 72%.

For selecting the starter cultures for production of flour confectionery goods, their resistance to sucrose is to be taken into account, as concentration of sucrose in these products is considerably above the margin of the microorganisms' sensibility. The lactic acid bacteria and bifidobacteria are known to be resistant to moderate concentrations of sucrose; however, in literature, there are no evidences of displays of this property by the studied strains and polyspecific concentrates. Therefore, selection of the most prospective probiotic preparations resistant to sucrose for production of flour confectionery goods is of great interest. In this view, we studied the resistance of probiotic microorganisms to various sugar concentrations. It is to be noted that the most adequate indicators of the state of stress are decrease of the rate of proliferation and viability of probiotic microorganisms' cells.

The results of the study of the sucrose concentration's influence on the viability of probiotic preparations' cells evidence that as the

sugar concentration reaches 60%, the number of viable cells remains at a sufficiently high level. Further increase of sugar concentration worsens the viability of probiotic microorganisms' cells. The highest resistance to extreme sweetness is demonstrated by the B. bifidum 791 strains. With 70% concentration of sugar, the number of viable cells in these cultures equals to 10<sup>10</sup> CFU/cm<sup>3</sup>, and as the sugar concentration increases further, the number of microorganisms decreases.

As sucrose is not a specific hydrocarbon for the proliferation of probiotic microorganisms [10], but is able to promote the proliferation of *E. coli*, bacteroids, and clostridia, there was interest in studying the influence of the sucrose concentration on the probiotic microorganisms' proliferation rate.

The results of determination of the dependence of the probiotic preparations' cells on the sucrose concentration showed that as the extent of sweetness of the environment increases, the proliferation rate of the studied probiotic cultures slows down, and the minimum proliferation rate was noticed for the B. longum B339M strain after increasing the sucrose concentration in the environment up to 60%.

## Conclusion

The obtained results evidence the high adaptive abilities of bifidobacteria strains, which are further used in the mixed bioconcentrates of Bifilakt A and Bifilakt D.

Thus, the data, which we have obtained, on the high physiological and biochemical properties of bifidobacteria strains and complex bacterial concentrates as well as the ability of probiotic microorganisms to synthesize antimutagenic substances, create true opportunities for development of new products with antimutagenic properties. The results of the research evidence the high survivability of probiotic cultures, the metabolism of which ensures microbiological safety in production of flour confectionery goods.

Taking into account that the complex bacterial concentrates include the renowned strains of bifidobacteria as well as lactic acid bacteria, we selected the polyspecific bacterial concentrates Bifilakt A and Bifilakt D as the probiotic additives.

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