The development of a biologically active additive to reduce the blood cholesterol level

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Abstract. The relationship between the content of polyunsaturated fatty acids (PUFAs) of flaxseed oil and the cholesterol-metabolizing activity of various strains of bifidobacteria was studied. The optimum dose of linseed oil in a nutrient medium for the cultivation of bifidobacteria was selected to provide high cholesterol destruction compared to the control. Of all the studied strains of bifidobacteria, the most pronounced destructive activity against cholesterol is displayed by the strain Bifidobacterium longum DK-100, which, with the biomass growth in a nutrient medium of linseed oil destroys 74% of the total cholesterol. When studying the fatty acid composition of the biomass of bifidobacteria, the oleic acid was found to predominate among monounsaturated fatty acids, and the α-linolenic acid to prevail among polyunsaturated fatty acids, that amounted to 44-45%. A decrease in the content of linolenic acid during the cultivation of bifidobacteria was noted, which is probably due to their participation of bifidobacteria in the metabolism. As a result of the studies, the optimum conditions for the cultivation of bifidobacteria were selected and the technological parameters of producing dietary supplements were justified.

1 Introduction

The prospects of using probiotic microorganisms in biotechnology are due to their ability not only to synthesize various useful products, but also to decompose or degrade a wide range of toxic compounds.

In recent years, a significant amount of data has been accumulated that the host and transient microflora of the host while synthesizing, transforming or destroying exogenous and endogenous sterols, are actively involved in cholesterol metabolism. This allows to consider the microflora of the host as the most important metabolic and regulatory organ involved in cooperation with the host cells in maintaining cholesterol homeostasis [1, 2, 3].

An analysis of published data on biologically active compounds produced by probiotic microorganisms showed that the biotechnological potential of anaerobic microorganisms of bifidobacteria, propionic acid and lactobacilli has not been used so far. Lactobacilli have long attracted the attention of biotechnologists because of their potential significance for the purposes of maintaining health, preventing and treating many diseases. The number of publications on the ability of certain strains of lactobacilli to exhibit a hypocholesterolemic effect, i.e. reduce blood cholesterol is increasing [4, 5, 6].

Hypocholesterolemia is an increase in blood cholesterol that can lead to the development of such serious diseases as atherosclerosis, arterothrombosis, coronary heart disease, diabetes, obesity, hypertension, and others. The reason for the increase in blood cholesterol is its excessive intake with food and insufficient decomposition of cholesterol in the body. Cholesterol is deposited at the sites of damage in the vascular wall and subsequently causes the formation of atheroma that leads to the development of vascular diseases [7, 8].

There is a known feedback between the content of polyunsaturated fatty acids (PUFAs) in the human diet and the concentration of cholesterol and triglycerides in the blood. A study of the lifestyle, health and nutritional characteristics of Mediterranean people or people who regularly consume fish showed that low levels of cardiovascular disease among population groups are due to high levels of polyunsaturated fatty acids (mainly omega-3).

The biological effects of omega-3 PUFAs are primarily associated with their effect on cell membrane functions. Being an essential component of phospholipids of all cell membranes, omega-3 PUFAs determine their fluidity, thereby they can affect the properties of membrane-bound receptors, signal transduction, and the activity of a large number of membrane-bound enzymes [9, 10].

It should be noted that the role of PUFAs in the processes associated with hypocholesterolemia is still not well understood.

The relevance of research in the field of microbial ecology, the study of cholesterol metabolism by probiotic microorganisms, is determined by the need to create bio-products of mass consumption to maintain the

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Table 1. Effect of various doses of linseed oil on cholesterol-metabolizing activity of bifidobacteria.

Strain of microorganisms	Added components	Added component dose, %	Content of cholesterol in nutrient solution, mmol/l							The level of cholesterol decompo-sition, %
			The duration of cultivation, h							
			0	4	8	12	16	20	24	
B. bifidum 8_3	Control		4.92	4.92	4.9	4.76	4.42	3.72	3.12	36.59±0.02
	linseed oil	0.5	4.92	4.92	4.81	4.46	3.89	3.04	2.24	54.47±0.03
		1	4.92	4.91	4.76	4.27	3.76	2.97	1.69	65.65±0.04
		1.5	4.92	4.9	4.71	4.19	3.57	2.75	1.61	67.28±0.02
B. longum DK-100	Control		4.92	4.92	4.87	4.61	4.31	3.63	2.95	40.04±0.02
	linseed oil	0.5	4.92	4.91	4.75	4.38	3.76	2.58	2.19	55.49±0.02
		1	4.92	4.9	4.69	4.21	3.57	2.23	1.54	68.70±0.04
		1.5	4.92	4.88	4.61	4.03	3.28	2.04	1.26	74.39±0.03
B. longum B379M	Control		4.92	4.92	4.91	4.81	4.54	3.84	3.21	34.76±0.02
	linseed oil	0.5	4.92	4.92	4.85	4.53	4.14	3.41	2.47	49.80±0.02
		1	4.92	4.91	4.79	4.41	3.95	3.27	2.02	58.94±0.03
		1.5	4.92	4.91	4.74	4.31	3.86	3.18	1.83	62.80±0.03

health of the population, which will compete with medicines.

It should be noted that humanity is experiencing a large deficit in physiologically important long-chain omega-3 PUFAs, which contribute to an extremely high mortality from circulatory diseases.

The level of PUFAs in a human body directly depends on the food consumed. In this regard, the creation of biologically active additives combining probiotic microorganisms and polyunsaturated fatty acids with high therapeutic and prophylactic properties is of great interest.

Purpose of work.

The paper presents a study into the effect of flaxseed oil PUFA on the cholesterol-degrading activity of probiotic dietary supplements.

2 Objects and Research Methods

The objects of research were strains of bifidobacteria Bifidobacterium longum DK-100, Bifidobacterium longum B379M, Bifidobacterium bifidum 83, obtained from the All-Russian collection of industrial microorganisms of Federal State Unitary Enterprise GosNII Genetika, activated by the biotechnological method developed at East Siberia State University of Technology and Management.

For the cultivation of probiotic microorganisms, a clarified serum-based nutrient medium with growth components was added, developed at the East Siberia State University of Technology and Management [11].

Cold-pressed linseed oil (TU 9141-001-45437467-09) was used as a functional ingredient. An activated culture of bifidobacteria of a stationary growth phase in the amount of 5% of the volume of the nutrient medium was used as an inoculum. Cultivation was carried out in flasks at a temperature of (37-1) °C.

The viability of bifidobacteria was judged by the number of colony forming units (CFU) when plating cell suspensions from the appropriate dilutions on MMC medium.

The concentration of cholesterol in the nutrient medium was determined by the enzymatic method [12].

The fatty acid composition was determined by the content of fatty acid methyl esters according to All-Union State Standard (GOST) R 51483-99 on a Crystal 2000M gas chromatograph with a PID flame detector, using HP-FFFAP capillary column (USA) - 50 m, 0.32 mm 0.52 μm and the nitrogen as a carrier gas. Determination conditions are programming mode at a speed of 4 ° C / min, column temperature ranging between 180 to 220 ° C, evaporator temperature being 250 ° C, detector temperature being 250 ° C [13].

All experiments were performed in 3-5 replicates. Statistically significant differences are discussed at p> 0,05.

3 Results and Discussions

At the first stage of the research, the effect of various doses of flaxseed oil on the cholesterol-metabolizing activity of bifidobacteria was studied. The research results are presented in Table 1.

From table 1 it is seen that with an increased dose of introduced oil from 0.5% to 1.5%, a more active cholesterol degradation occurs during the cultivation of all strains of probiotic microorganisms as compared to the control dose. The strain B. longum DK-100 is characterized by the most pronounced destructive activity against cholesterol when 1.5% linseed oil is added to the nutrient medium at the end of cultivation destroying 74.39% of cholesterol.

Thus, on the basis of the studies, the introduction of linseed oil in a nutrient medium was found to increase the cholesterol-metabolizing ability of bifidobacteria by 1.5-1.8 times.

When evaluating fats, it is necessary to take into account the balance of PUFAs of the omega-6 and omega-3 families, which should be 10: 1 in the diet of a healthy person, and from 3: 1 to 5: 1 for medical nutrition according to the recommendations of the Nutrition Institute of the Russian Academy of Medical Sciences [14].

Table 2. Fatty-acid composition of the bifidobacteria biomass in a nutrient solution with linseed oil.

Name of the indicator		The name of the studied sample			
		linseed oil	**B. longum DK-100 with linseed oil**	**B. longum B379M with linseed oil**	**B. bifidum 8₃ with linseed oil**
The content of fatty acids, %	Rich:	9.95	10.71	9.83	10.83
	With$_{10:0}$ capric	-	-	-	0.07
	With$_{12:0}$ lauric	-	-	-	0.09
	With$_{14:0}$ myristic	-	1.94	0.09	0.31
	With$_{16:0}$ palmitic	5.8	6.61	5.8	6.28
	With$_{18:0}$ stearic	3.91	2.16	3.82	3.96
	With$_{20:0}$ arachidic	0.2	-	0.12	0.12
	With$_{22:0}$ begenova	0.04	-	-	-
	Monounsaturated:	17.68	18.51	16.53	16.42
	With$_{16:1}$ palmitoleic	0.09	-	0.11	0.13
	With$_{18:1}$ oleic	17.31	18.51	16.29	16.19
	With$_{20:1}$ andonova	0.14	-	0.13	0.1
	With$_{22:1}$ erucic	0.14	-	-	-
	Polyunsaturated:	72.23	70.76	72.23	71.9
	With$_{18:2}$ linoleic	16.8	26.59	27.21	26.24
	With$_{18:3}$ linolenic	55.37	44.17	44.9	45.54
	With$_{20:2}$eykozadienovaya	0.06	-	0.12	0.12
	Total:	99.86	99.98	98.59	99.15
The content of polyunsaturated fatty acids, %	ω-6	16.80	26.59	27.12	26.24
	ω-3	55.37	44.17	44.90	45.54

However, the problem is that in most industrialized countries, including Russia, the ratio of omega-6: omega-3 in food currently ranges from 15: 1 to 25: 1. A characteristic feature of modern human nutrition is an increase in omega-6 PUFAs in food, which leads to a relative deficiency of omega-3 [15].

In this regard, the fatty acid composition of the biomass of bifidobacteria was studied. The research results are presented in Table 2.

In the studied fatty acid composition of biomass of bifidobacteria cultivated in a nutrient medium with linseed oil, among monounsaturated fatty acids oleic acid predominates, among polyunsaturated fatty acids linolenic acid prevails, which is in average 44-45% (Table 2). Table 2 demonstrates an increase in the amount of linoleic acid omega-6 and a decrease in linolenic omega-3 and oleic omega-9 acid when cultivating bifidobacteria in a nutrient medium with linseed oil, which is probably associated with the participation of unsaturated fatty acids in the metabolism of bifidobacteria.

According to modern concepts, ALA in the human body is a biochemical precursor of physiologically significant long-chain PUFAs with 20-22 carbon atoms - eicosopentaenoic (EPA) and docosahexaenoic (DHA), which belong to the omega-3 family, and LK - the arachidonic (ARA) of the omega family -6 [16].

Thus, as a result of the studies, it was found that PUFAs of linseed oil not only stimulate the growth of bifidobacteria, but also increase their cholesterol-metabolizing activity.

The obtained experimental data made it possible to develop a technology for the production of probiotic dietary supplements enriched in PUFAs.

A peculiar feature of the proposed technology is the introduction of linseed oil in an amount of 1.5% in a cooled nutrient medium, due to the instability of fatty acids during sterilization. A strain of Bifidobacterium longum DK-100 is used as an inoculum. Qualitative characteristics of dietary supplements are presented in Table 3.

The analysis of the data in Table 3 shows that the dietary supplement has a high cholesterol metabolizing activity and can be recommended for the prevention of diseases caused by high cholesterol in the blood.

Optimization of the nutrient medium with linseed oil increases the number of viable bifidobacteria cells that carry out intensive cholesterol metabolism.

The mechanism of interaction of unsaturated fatty acids with bifidobacteria cells can be explained by their adsorption and incorporation into the outer membrane, changes in the physicochemical properties of the cell surface, as well as the properties of the lipid bilayer [17]. In the context under consideration, it is important to note that they, at certain concentrations, are growth factors for bifidobacteria and can be attributed to prebiotics, and the developed dietary supplements to synbiotic products.

Based on the research results, an innovative product category of probiotic biologically active additives with high consumer properties was developed. Their main difference from pharmaceuticals is their physiological nature, absence or low risk of side effects [18]. A quality indicator has been introduced for cholesterol-metabolizing activity, which determines the functional properties and innovative character of products.

Table 3. Qualitative characteristics of probiotic dietary supplements, enriched in polyunsaturated fatty acids.

Indicator name	Indicator value
	BAD linseed oil
Consistency and external appearance	Homogeneous. Allowed the separation of serum
Color	From white to light yellow
Taste and smell	Clean fermented, with a touch of linseed oil
Limit value of pH, units	5,3-7,5
Cholesterol-metabolizing activity, %	74,39
Temperature at release from the factory, at most, °C	4-6
The amount of bifidobacteria, CFU./cm^3, not less than	$1*10^{12}$
The volume of the product (cm^3), which should not content:	
Coliform bacteria (coliforms)	10
S. aureus	10
Pathogenic microorganisms (including Salmonella)	50
Yeast and mold, CFU./cm3, at most	10

4 Conclusion

As a result of the studies, a biologically active supplement was developed to lower blood cholesterol. It has been established that polyunsaturated fatty acids of linseed oil increase the cholesterol-metabolizing activity of bifidobacteria.

The most pronounced hypocholesterolemic effect is characterized by a bifidobacteria strain Bifidobacterium longum DK-100, which destroys 74% of cholesterol in a nutrient medium with linseed oil.

The results obtained open up broad prospects for the creation of functional nutrition products for the prevention of diseases caused by high cholesterol in the blood.

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