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LACTIC ACID BACTERIA: MICROBIAL AND FUNCTIONAL ASPECTS TO SEARCH BIOACTIV STRAINS

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Modern methodological approaches were applied and strains of lactic acid bacteria Lactobacillus casei, Lactobacillus acidophilus and Streptococcus thermophilus with high biological activity level, perspective in functional dairy products manufacturing, were selected. The high biological potential of lactic acid bacteria selected cultures was determined, which is capable of ensuring the stability of technological process flow and bacterial agents' essential characteristics and their fermented products. Experiments in vitro showed that selected strains were characterized by valuable industrial properties, namely the ability to reduce the level of cholesterol and lactose during development in milk, the resistance to virulent bacteriophages and gastrointestinal tract aggressive compounds, the high antagonistic and adhesive activity. On the basis of studies technological passports on strains were made, the deposit of strains was held in in National collection of industrial microorganisms of Institute of Microbiology and Virology of Zabolotny of Ukrainian National Academy of Sciences.

Keywords: *lactic acid bacteria, selection, adhesive activity, cholesterol, virulent bacteriophage*

МОЛОЧНОКИСЛІ БАКТЕРІЇ: МІКРОБІОЛОГІЧНІ ТА ФУНКЦІОНАЛЬНІ АСПЕКТИ ПОШУКУ БІОЛОГІЧНОАКТИВНИХ ШТАМІВ

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Застосовано сучасні методологічні підходи та відібрано штами молочнокислих бактерій Lactobacillus casei, Lactobacillus acidophilus і Streptococcus thermophilus з високим рівнем біологічної активності, перспективні у виробництві функціональних молочних продуктів. Визначено високий біологічний потенціал обраних культур молочнокислих бактерій, який здатний забезпечити стабільність технологічних процесів і основних характеристик бактеріальних агентів і їх ферментованих продуктів. У дослідах in vitro показано, що селекціоновані штами характеризувались цінними промисловими властивостями, а саме: здатністю знижувати рівень холестерину та лактози під час розвитку в молоці, були стійкими до вірулентних бактеріофагів і до агресивних сполук шлункового тракту, проявляли високу антагоністичну та адгезивну активність. На підставі досліджень було складено технологічні паспорти штамів, проведено депонування штамів в Національній колекції промислових мікроорганізмів Інституту мікробіології та вірусології ім. Заболотного Національної академії наук України.

Ключові слова: *лактобактерії, селекція, адгезивна активність, холестерин, вірулентний бактеріофаг*

In recent years, functional dairy products popularity steadily grows, because a significant role they play in diet and clinical nutrition became known. In many ways the functional product biological value is determined by properties of starter cultures that make up their composition [1]. That is why it is important to extend the probiotic microorganisms range and to involve them in the diet. Search and directed selection of probiotic cultures is an important task that requires study of these microorganisms [2]. For industrial use probiotic strains are selected for a number of biological properties and expression of their functional activity, particularly in *in vitro* experiments.

Purpose of the work was to search bioactive strains of lactic acid bacteria, perspective in functional products production.

Materials and methods. The object of the study – newly selected cultures of lactic acid bacteria. Strains identification and selection research were performed according to Bergey's Manual of Systematic Bacteriology (2009). In experiments 17-h pure cultures grown twice in a suitable liquid medium: MRS with 1% glucose and 1% lactose for bacteria Lactobacillus, hydrolyzed broth (HB) – for Streptococcus thermophilus were used. Milk coagulation activity, acid formation limit and other technological parameters were determined according to [3]. Pure cultures growth laws were studied using periodic cultivation in 10% sterile skim milk. The number of microorganisms was determined by CFU number on agar nutrient medium, using the method of critical tenfold dilutions. Calculation of cultures growth parameters was performed on the basis of microorganisms growth curves in coordinates *lg CFU* – cultivation duration. Cultures relation to bacteriophages was determined by method of double agar supplemented with 10 mM $CaCl_2$ [4]. Llytically active species-specific bacteriophages isolated in Ukrainian enterprises, from dairy phages collection of the Institute of Food Resources, were used in experiments. Antagonistic activity of lactic acid bacteria as to test cultures of pathogenic and conditionally pathogenic microorganisms was determined by the method of common cultivation during 24 h, while calculating antagonistic activity titre as the highest dilution of the bacterial mixture from which a test culture was planted. Cells hydrophobicity was determined by method that is based on the bacteria ability to be distributed in the biphasic system "suspension of bacteria:n-hexadecane". Hydrophobicity was expressed as a percentage and determined by the ratio of bacterial suspension optical density after interaction with n-hexadecane to its output value. Electrokinetic study of cells surface structures was performed at the facility for microelectrophoresis. After increasing in MRS and HB medium for 17 h the cells were precipitated by centrifugation at 3000 rev/min for 15 minutes and washed twice with sterile distilled water (pH 6.6). Electrophoretic mobility of 30 cells in an electric field (10 V/cm, 6.7 mA) was measured. Lactobacilli ability to adhere was investigated in a model system with glass beads. For this purpose glass columns with a diameter of 15 mm were filled with chemically pure glass beads with a diameter of about 1 mm (5 g) and there was added 5 cm3 of bacterial suspension, optical density of which was 0.8 units (wavelength λ = 540 nm). A column with bacteria suspension without glass granules was used as a control for counting cells that were fixed at its walls. After 30 min the suspension was poured into measuring vessel, the column was washed twice with saline solution for removal of loose cells. The control and experimental volumes of suspensions were adjusted to 10 cm^3 with saline solution and measured the suspension optical density. Bacteria adhesive ability was determined by the proportion of fixed cells from output amount. Also bacteria adhesion was studied as to human laryngeal adenocarcinoma cell line HEp-2, which was kindly provided by Doctor of Medical Sciences V.I.Zadorozhna (L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases of Ukrainian National Academy of Medical Sciences). Microorganisms were prepared as described above and suspension density was adjusted by optical turbidity standard with a rate of 109 cells per 1 cm3. In a sterile Petri dish a covering glass with washed cell culture was brought (grown on glass slides in Petri dishes in an incubator with 5% CO_2) and bacterial suspension (10 cm3). It was incubated for 1 h at room temperature. Then glass slides were washed 8 times with saline solution to remove loose bacteria, dried, fixed and dyed with methylene blue. In the optical microscope, a number of lactobacilli that are fixed on the culture cells HEp-2 was counted. For results accuracy at least 100 cells HEp-2 were viewed. Adhesiveness degree was expressed by adhesiveness index (AI) by average number of bacteria that were fixed on 1 HEp-2 cell. Lactobacilli acid resistance was evaluated by viable cells number by exposure to medium acidified with hydrochloric acid to pH 2.0; 2.5; 3.5. Resistance to bile («Oxgall», Sigma, USA) was evaluated by the difference in time at which the culture optical density (at λ = 540 nm) in medium with 0.3 % bile (experiment) and without (control) reached a value of 0.3. Bacteria cholesterol activity was investigated in milk with a fat content of 3.2% and in MRS or HB

mediums (depending on bacteria type) in which cholesterol sources are, respectively, milk fat and water soluble mixture of lipids enriched in cholesterol (Aldrich Co. LTD, Sigma). Cultures cholesterol activity was assessed by the difference between the values of residual cholesterol in the culture fluid and sterile environment. Micropreparations analysis was carried out using a microscope Motic (Fischer Bioblock) with integrated video camera TopView with an increase in 1000 times. All experiments were conducted in three replications. For experimental data processing the software package STATISTICA©5.XX for Windows (StatSoft Inc., USA) was used with the advice given in S. N. Lapach's (with co-authors) manual [5].

Results and discussion of research. Search and selection of active strains of lactic acid bacteria was performed from natural sources of distribution: from stool samples obtained from healthy people and self-fermented dairy products. The selected material was planted in liquid mediums – MRS with 1% glucose and 1% lactose, pH 5.9, and recovered skim milk and were cultured at a temperature of 37°C. From accumulative cultures, using traditional techniques of microorganisms selection in pure culture, there were got some colonies. Colonies with characteristic lactic acid bacteria morphology were selected and transferred to liquid mediums and cultured at a temperature of 37°C. Grown cultures purity degree was evaluated on the basis of morphological homogeneity at microscopy. This procedure was performed for each accumulative culture for 2-3 times. As a result of this selection work 322 isolates in pure culture were removed. The isolates obtained conventionally were divided into 4 groups according to the morphological structure of cells and selection source (Table 1).

Table 1

Selected cultures characteristics

Group	Cells morphology	Cells dimension, mcm	Selection source	Number of isolates
I	Thick rods of various lengths, separate or in chains	0.6-0.9 – 1.4-6.0	Human intestines	73
II	Thick rods of various lengths, separate or in chains	0.7-0.9 – 1.5-7.0	Dairy products	115
III	Thin rods, sometimes in chains	0.5-0.6 – 1.0-4.4	Human intestines	52
IV	Cocci, diplococci, chains	0.6-0.8	Dairy products	82

According to the data presented in this table 125 isolates were isolated from the intestine and from dairy products – 197. Most of them had a rod shape of different sizes – 74.53% of the total. Number of rods isolated from intestine and dairy products was almost on the same level – 38.82% and 35.71%, respectively. Coccal microorganisms were isolated only from dairy products.

The initial screening of isolates was performed by indices which characterize the cultures technological suitability, namely milk coagulation activity (MCA) and acid formation limit (AFL). In addition, such important cultures properties as resistance to bile (RB) and acid resistance (AR) were studied. The use of these indices is due to the direction of selection – selection of biologically active microorganisms. In Table 2 it is shown the distribution of selected isolates by selected criteria for each group.

Cultures that were isolated from dairy products, so-called "milk" strains of 2nd and 4th groups fermented milk more actively, MCA index rate fluctuated within 5-10 hours. Such cultures proportion was 46.9% of the total number of selected isolates. Among the "intestinal" origin cultures of the 1st and 3rd groups, only 21 isolates of the 1st group were characterized by high milk coagulation activity.

Table 2

Distribution of selected isolates by selective criteria

Index	Range	Group							
		I		II		III		IV	
		Number of isolates							
		pcs	%	pcs	%	pcs	%	pcs	%
MCA, h	5-10	21	6.5	84	26.1	0	0	67	20.8
	10-20	38	11.8	29	9.0	7	2.1	15	4.7
	20-30	14	4.3	2	0.6	45	13.9	0	0
AFL, °T	80-150	0	0	0	0	37	11.5	78	24.2
	150-250	12	3.7	39	12.1	15	4.7	4	1.2
	250-350	61	18.9	76	23.6	0	0	0	0
RB, Δ h	1-2	16	4.9	0	0	11	3.4	0	0
	2-4	31	9.6	22	6.8	39	12.1	15	4,7
	More than 4	26	8.1	93	28.9	2	0.6	67	20.8
AR,%	10-25	18	5.6	73	22.7	7	2.8	51	15.8
	25-50	43	13.4	36	11.2	29	9.0	27	8.4
	50-90	12	3.7	6	1.7	16	4.9	4	1.2

Note: the total number of selected isolates - 322 units – was taken 100%.

It is known that lactic acid rods are capable to superproduction of lactic acid, and this often leads to defects of fermented dairy products. It is therefore advisable to search strains that have moderate acid formation ability. Based on this, share of rods of "milk" origin (isolates of the 2nd group) with AFL values not more than 250°T was 12.1%. Share of "intestinal" isolates (1st and 3rd groups) for which AFL ≤ 250 was higher by 1.6 times and was 64 isolates.

Most bile resistant isolates also had the "intestinal" origin. Also noteworthy is the fact that 8.4% of isolates of the 1st and 3rd groups were characterized by high resistance to bile (stunting not more than 2 h) and 21.7% - average (stunting to 4 hours). Bile affected the "milk" isolates of the 4th group most destructively and among them there were no cultures with high resistance to bile.

For bioactive strains of lactic acid bacteria acid resistancy is very important, first, for successful transit through the alimentary canal, especially through the stomach with its high concentration of HCl, and secondly – to ensure their guaranteed number in ready functional foods and during storage. Analysis of selected isolates by acid resistance index showed that resistant strains of "intestinal" origin were 2.8 times more than in "dairy". At the same time, the number of isolates that over exposure for 2 hours in an environment of active acidity with pH value of 2.5 to retain till 50% of living cells (average) was about the same regardless of selection source (see Table 2).

Therefore, the selected isolates in a given degree are characterized by important biological properties. However, the "intestinal" strains were characterized by greater resistance to bile and acid, and lower indices of milk coagulation activity and acid formation limit compared to "milk" strains. This is primarily due to greater adaptability of dairy cultures to development in milk - a natural environment for them [6].

As a result of screening by 4 indices 8 strains were selected as promising, five of which were "intestinal" origin and three – "milk". Featured bacteria pure cultures were analyzed and identified to species. Further investigation of isolated cultures properties performed by *in vitro* criteria, that allow examining thoroughly the strains functional potential, namely, antagonistic and adhesive activity, the ability to reduce cholesterol content.

Antagonistic activity of isolated cultures was investigated by the method of joint cultivation with test cultures of pathogenic and conditionally pathogenic microorganisms. It was

found that in pure culture the number of test cultures was 10^8 CFU/cm3, and during the common growth with lactobacteria the number of test cultures cells reduced by thousands and millions times (Table 3).

Study of isolated cultures antagonistic activity showed that lactobacilli actively suppressed the development of test cultures compared to thermophilic streptococci. Proteus and intestinal rods were the most sensitive to their action – antagonistic activity titer ranged from 1 to 2 lg CFU/cm^3. The strain *L. casei* 302 had the highest antagonistic activity titer – ≥ 1 lg CFU/cm^3. Strains *S. thermophilus* 21 and 37 were characterized by slightly lower antimicrobial activity, as evidenced by lower values of the antagonist titre – from 3 to 5 lg CFU/cm^3 (Table 3).

Table 3

Titre of different strains antagonistic activity, in CFU/cm^3 *(common cultivation)*

Lactobacteria		Test cultures*			
Species	strain	*E. coli*	*St. aureus*	*Pr. vulgaris*	*Bac. subtilis*
S. thermophilus	21	4	4	3	4
S. thermophilus	37	5	5	3	5
L. acidophilus	35	2	1	less than 1	1
L. acidophilus	310	2	3	2	3
L. casei	205	2	2	less than 1	3
L. casei	218	2	3	1	3
L. casei	302	less than 1	1	less than 1	1
L. casei	330	1	2	less than 1	3
L. casei Shirota	«Yacult»	2	4	3	3
Control №1, pH 3,8		5	6	6	6
Control №2, test cultures		8	8	8	8

Note: (Arithmetic mean error – 3-5%, $p < 0.05$)

One of the mechanisms of lactic acid bacteria antimicrobial action is their ability to accumulate during their life a large number of organic acids, primarily lactic acid. Therefore, in the experiments, as a control №1, appropriate sterile environment with proven acidity to 3.8 pH were used. In addition, expression of antagonistic activity by studied Lactobacteria was compared with the effect of strain *L. casei* Shirota (recognized probiotic), which we have isolated from "Yacult" drink (Japan). As can be seen from Table 3, lactobacilli kept greater inhibitory effect on all test cultures compared with control (pH 3.8). Thus, it is possible to suggest that their inhibitory effect is associated not only with the production of acid, but also with some antimicrobial agents.

Action spectrum of used as an additional control strain *L. casei* Shirota was not significantly different from the studied cultures. Lactic acid bacteria *L. casei,* isolated by us, did not yield to this probiotic culture by antagonistic activity level, or even exceed it.

Adhesion of lactic acid bacteria was studied in model systems *in vitro,* which imitated nonspecific interaction with hydrophobic (n-hexadecane) and hydrophilic (glass beads) surfaces and specific – with cell culture HEp-2.

Analysis of cell surface hydrophobicity of lactic acid bacteria showed that this property is dependent on the conditions of cultivation and is strain specific feature (Table 4).

The "intestinal" strain of *L. casei* 302 had the highest value of this index – 14.8%, whereas the hydrophobicity degree of "milk" strain *L. acidophilus* 310 among MRS medium was lower on average by 2.5 times.

During the development in MRS medium cell surface was characterized by smaller by (1.1-1.9) times degree of hydrophobicity compared with growth in HB. Obviously, the presence

of additional carbohydrate in MRS medium stimulated hydrophilic compounds formation on lactic acid bacteria cell surface, whereas in HB it was not observed.

Table 4

Hydrophobic and electrokinetic properties lactic acid bacteria cell surfaces

Lactobacteria		Hydrophobicity, %		ζ- potential, - (mV)
Species	strain	MPC	HB	
S. thermophilus	21	9.17±0.31	12.29±0.57	5.52±0.23
S. thermophilus	37	8.64±0.79	9.28±0.44	2.53±0.12
L. acidophilus	35	7.04±0.23	13.17±0.29	7.21±0.15
L. acidophilus	310	5.96±0.54	9.26±0.26	6.26±0.13
L. casei	205	9.51±0.35	-*	5.12±0.14
L. casei	218	12.21±0.64	-	7.35±0.21
L. casei	302	14.75±0.61	-	11.76±0.30
L. casei	330	9.74±0.29	-	5.09±0.24

Note: *- not determined

Research of cells electrophoretic mobility in an electric field showed that the total potential of lactic acid bacteria cell population had a negative charge. ζ-potential value of "intestinal" strains was by (2.0-2.1) times lower than that of "milk" origin cultures. The smallest ζ-potential was determined for *L. casei* 302 strain – (-11.8) mV (see Table 4). Some authors connect this feature with high adhesive properties of bacteria [7].

Changes in the surface properties of lactic acid bacteria during development in different environments affected on their ability to be fixed to the glass granules (Table 5).

Table 5

Lactic acid bacteria adhesion in different model systems

Lactobacteria		Glass beads, %		HEp-2, AI
Species	strain	MPC	HB	
S. thermophilus	21	11.13±0.45	50.49±2.21	0.99±0.14
S. thermophilus	37	8.63±0.26	22.65±0.48	0.34±0.11
L. acidophilus	35	37.01±1.57	45.84±1.71	0.36±0.18
L. acidophilus	310	16.89±1.64	21.05±1.81	0.21±0.10
L. casei	205	16.63±1.56	-*	0.22±0.10
L. casei	218	19.17±0.41	-	0.20±0.13
L. casei	302	33.90±0.88	-	1.43±0.24
L. casei	330	30.49±1.26	-	0.43±0.20

*Note: *- not determined*

As can be seen from Table 5 the number of fixed bacteria grown in HB was (1.2-4.5) times greater than that of MRS. Thus, the adhesiveness of strain *S. thermophilus* 21 accumulated in HB was 4.5 times higher than that of MRS. The influence of the growth environment on the adhesive properties of acidophilic bacillus strains was minimal. Total number of adherent cells to glass granules depended on the strain and ranged from 8.6 to 50.5%.

In the model of cell line HEp-2 it is shown that the strain of *L. casei* 302 had the best adhesive ability, adhesiveness index (AI) was much higher compared to other strains. On fig. 1 it is shown microphotogram illustrating adhesion of *L. casei* 302 to the cell line HEp-2.

Thus, the isolated strains showed a high capacity for adhesion *in vitro*, and *L. casei* 302 strain – even more than the cultures, which were effective in treatment of respiratory tract in children [8].

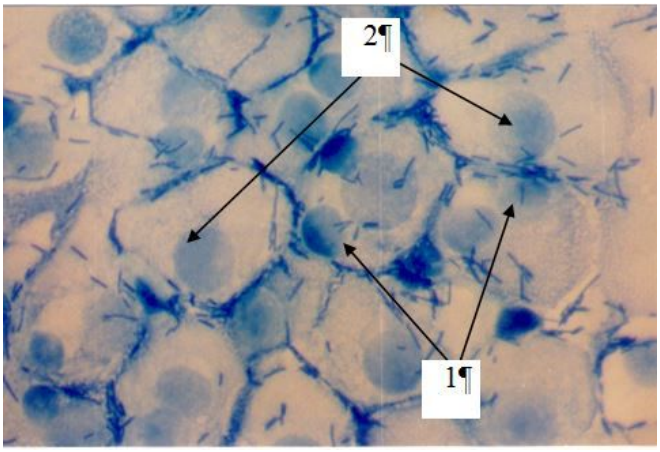

Fig. 1. Adhesion of lactic acid bacteria to human laryngeal adenocarcinoma cell line HEp-2:
1 – *L. casei* 302; 2 – HEp-2 (*light microscope, magnification 10×100*)

Research of cholesterol activity showed that the strains differed significantly by this property and linked from 7,7% to 64,0% of environment cholesterol (Table 6).

Table 6

Reduction of cholesterol content by lactobacteria *in vitro*
(Arithmetic mean error – 3-5%, $p < 0,05$)

Lactobacteria		HB, MPC*		Milk**	
Species	strain	*mkg/cm³*	*%*	*mkg/cm³*	*%*
S. thermophilus	21	63.3	12.7	60.1	35.4
S. thermophilus	37	60.1	12.0	35.1	20.6
L. acidophilus	35	47.1	9.4	43.9	25.8
L. acidophilus	310	38.4	7.7	42.8	25.2
L. casei	205	101.5	20.3	79.4	46.7
L. casei	218	104.4	20.9	86.5	50.9
L. casei	302	120.6	24.1	108.7	64.0
L. casei	330	105.9	21.2	99.8	58.7
L. casei Shirota	(Yakult)	77.0	15.4	65.4	38.5

*Note: * $Control_{HB, MPC}$ – 170 mkg/cm³, ** $Control_{Milk}$ – 500 mkg/cm³*

The strains belonging to *L. casei* reduced cholesterol content the most actively. After 20 h of growth in milk cholesterol amount decreased by 79,4 ÷ 108,7 g/cm^3. The strain *S.thermophilus* 21 affected the content of cholesterol almost at the same level – the number of extracted cholesterol in milk was 60.1 mg/cm ^{3}. The effect acidophilus bacteria during cultivation in milk was much weaker – cholesterol level decreased by 42.8 ÷ 43.9 g/cm^3.

It should also be emphasized that the strain *L. casei* Shirota, used to compare the properties of the probiotic cultures with known probiotics had no significant advantages under the conditions of described experiment.

The data obtained as for high cholesterol activity of *L. sasei* are consistent with literary. In M. Brashears's experiments [9] the strains *L. casei* isolated 60 mkg of cholesterol from 1 cm^3 of MPC broth. The selected strains showed cholesterol activity at the same high level, and *L.sasei* 302 isolated from the environment even in 2.01 times more cholesterol.

Lactic acid bacteria strains, involved in starter cultures composition must together with functional activity have a good progress in milk, during fermentation to form milk coagulate of smooth, dense texture, with pleasant taste and aroma.

It was found that thermophilic streptococci and acidophilic bacteria strains developed in milk more actively – cells number increased by 55 ÷ 110 times from the original amount. The intensity of *L. sasei* cells accumulation was significantly lower – they increased the number only by 5 ÷ 20 times, depending on the strain (Table 7).

Differences between strains within species concerning growth nature in milk were found based on growth parameters analysis, namely, specific growth rate (μ, h^{-1}), lag phase duration (T_l), separation speed constant (number of cell separation for 1 h) (ν, h^{-1}) and regeneration term, which characterizes time, necessary for one cycle of cell separation (g, h) [10].

Among the streptococci the strain *S. thermophilus* 21 was selected and from *L.acidophilus* – pcs. 35 , as the most active. These cultures began to grow after a short phase of growth retardation – an indicator T_l is the smallest among all the studied cultures for *S.thermophilus* 21 – 0.22 h and for *L. acidophilus* 35 – 1.76 h. Active growth in periodical culture continued for 6 h for *S. thermophilus* 21 and 8 h – *L. acidophilus* 35 with specific speed in log-phase, respectively, 0.97 and 0.76 h^{-1}. Thermophilic streptococci strain was reproduced faster compared to acidophilic rod, regeneration time was shorter by 2.1 times. The number in stationary phase for both strains was similar and it was – (8,50 ± 0,5) lg CFU/cm^3.

Table 7

Indicators of lactic acid bacteria growth in milk

Strain	MCA, h	The number of bacteria, lg CFU/cm^3	μ_{max}, h^{-1}	T_l, h	ν, h^{-1}	g, h
S. thermophilus						
21	5.0	8.50	0.97	0.22	1.08	0.94
37	6.0	8.31	0.91	0.30	0.85	1.18
L. acidophilus						
35	5.0	8.47	0.76	1.76	0.51	2.00
310	5.5	8.20	0.90	1.95	0.56	1.80
L. casei						
205	21.0	7.90	0.26	3.08	0.15	6.67
218	24.0	7.00	0.11	3.20	0.10	11.11
302	16.5	8.25	0.42	2.94	0.20	5.00
330	19.5	8.00	0.37	3.00	0.17	5.88

Note: cultivation duration – 12 h for S. thermophilus and L. acidophilus; 24 h – for L. casei

L. casei cultures, because of their "intestinal" origin needed more adaptation period before active growth – from 2.94 to 3.20 h (see Table 7). After growth retardation phase they grew at a moderate rate μ_{max} – (0,11-0,42) h^{-1} for a long time – from 7 to 10 h depending on the strain. The strain *L. sasei* 302 distinguished by the shortest period of cell regeneration – in 1,2-2,2 times lower compared with other strains of this species and the highest performance – (8,25 ± 0,5) lg CFU / cm^3. The milk coagulation activity of this strain was also 1,2-1,5 times higher in comparison to them. Thus, the growth rate of this strain in milk indicates the effectiveness of the selection in the direction of adaptation to milk.

These data are important in the development of nutrient medium composition for biomass accumulation of experimental cultures in bacterial agents production.

It is known that starter cultures sensitivity to phage infection creates a number of difficulties in the production of various dairy products and cheeses, leading to significant economic losses [9]. So important to dairy industry property of starter cultures as the ability to withstand the devastating impact of phages was also investigated. It was found that the selected strains were phage-resistant as for industrial species-specific virulent bacteriophages from the collection of biotechnology department of the Institute of Food Resources.

The cultures had homogeneous cells population with stable characteristics (variation coefficients less than 10%) and for multiple biological properties they were recognized as promising targets in future biotechnological developments.

Conclusion

1. As a result of directed selection by the criteria that characterize the expression of functional activity *in vivo*, industrially valuable strains were isolated from natural sources: *L.casei*, *L. acidophilus* and *S. thermophilus*. High biological potential of lactic acid bacteria selected cultures was determined, capable of ensuring the stability of production process flow and essential characteristics of bacterial agents and their fermented products.

2. Experiments *in vitro* showed that the selected strains are characterized by the ability to reduce the level of cholesterol and lactose during milk fermentation and they are phage-resistant. In addition, they are resistant to bile and acid, antagonistically active against pathogenic and conditionally pathogenic bacteria and have good adhesive properties.

3. Based on the research results technological passports for strains are done, the deposit of strains was held in National collection of industrial microorganisms of Institute of Microbiology and Virology (Ukraine).

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