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BIOLOGICALS TECHNOLOGY

Optimization of Cultivation Conditions for the *Lactobacillus reuteri* LR1 Strain to Improve the Biosynthesis of Bacteriocin-Like Substances

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Abstract—The article presents the results of optimization of the culturing conditions to increase the production of bacteriocin-like compounds (presumably including reuterin) by the *Lactobacillus reuteri* strain LR1. The strain *Escherichia coli* ATCC 25922, an opportunistic microorganism, the content of which is standardized in foods by the Food Safety Legislation, was selected as an indicator strain of antimicrobial activity. The nutrient medium for *L. reuteri* LR1 cultivation was optimized to increase the production of bacteriocin-like compounds, and the parameters of two-stage growth of the producer were established: (1) cultivation on the initial medium for biomass accumulation and (2) growth on a water–glycerol medium to convert glycerol to antimicrobial compounds, presumably reuterin. The first stage was carried out as follows: the *L. reuteri* LR1 culture (5%) was inoculated into a medium with the following composition: hydrolyzed milk—250 mL/L; yeast extract—10 g/L; peptone—5 g/L; glucose—20 g/L; sodium acetate—5.0 g/L; monosubstituted potassium phosphate—2.0 g/L; magnesium sulfate—0.2 g/L, and manganese sulfate—0.5 g/L, pH 6.4–6.6. The cultivation was carried out for 18 h at (37 ± 1)°C. The second stage included the incubation of the obtained biomass in 200 mM of glycerol-containing aqueous solution, pH 6.6, for 2 h at 37°C. The zone of *E. coli* inhibition caused by the produced bacteriocin-like compounds was 25 mm.

Keywords: bacteriocin-like compounds, *Lactobacillus reuteri*, antimicrobial activity, reuterin, glycerol bioconversion, optimization of the culturing conditions

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INTRODUCTION

Lactic-acid bacteria, in particular, bacteria of the *Lactobacillus* genus, play an important role in the gastrointestinal tract ecology; a number of microorganisms have a probiotic effect on the human body and farm animals. It should be noted that, in addition to their general healing effect, the metabolites of lactic acid and probiotic microorganisms in food and feed are highly active agents that inhibit pathogenic microbiota [1].

Numerous LAB species produce an antimicrobial component with a broad spectrum of action, reuterin. This compound is synthesized by some *L. reuteri* species during anaerobic glycerol fermentation. Reuterin biosynthesis is carried out via the conversion of glycerol to 3-hydroxypropionaldehyde (3-HPA) with the involvement of B12-dependent glycerol dehydratase [2]. The reuterin in the aqueous solution is a dynamic system consisting of 3-HPA, its hydrate, 1,1,3-propanetriol, its dimer, 2-(2-hydroxyethyl)-4-hydroxy-1,3-dioxane, and acrolein [2, 3]. It was recently shown that acrolein is the main component of the aforementioned dynamic system, and it is responsible for reuterin's antimicrobial activity [3]. Mutual conversions of 3-HPA and acrolein are blocked at a temperature of 4°C and pH 4.0, while the equilibrium shifts to acrolein formation at higher temperatures [3]. 3-HPA can subsequently be converted to 1,3-propanediol (1,3-PD) in the presence of glucose and with the participation of (NAD^+)-dependent oxidoreductase [4].

Reuterin production was initially observed only in *L. reuteri* species, which are a part of the endogenic bacterial microbiota in humans and animals; however, it was later shown that such species as *L. brevis*, *L. buchneri*, *L. collinoides*, and *L. coryniformis* are also capable of synthesizing this compound [5]. Apart from lactobacilli, the bacteria of the *Bacillus*, *Citrobacter*, *Clostridium*, *Enterobacter*, and *Klebsiella* genera can also produce reuterin. However, this compound, as an

Abbreviations: CFU—colony-forming unit; CL—culture liquid; LAB—lactic-acid bacteria; NAD—nicotinamide adenine dinucleotide; YE—yeast extract.

intermediate of glycerol metabolism, degrades in these bacteria to 1,3-propanediol and further to water and carbon dioxide. Only in *L. reuteri* is the activity of reuterin-degrading oxidoreductase low enough to accumulate a sufficient reuterin amount under anaerobic conditions to be toxic to other bacterial cells.

Reuterin is a water-soluble compound that is active in wide pH range and resistant to proteolytic and lipolytic enzymes. Therefore, it is well studied as a food preservative or an auxiliary therapeutic agent [6–8]. Reuterin is synthesized in vitro at pH, temperature, and aerobic conditions close to those in the gastrointestinal tract [9]. In vivo biosynthesis of the active compound can occur in the colon as a result of the intestinal microbiota (including *L. reuteri*) metabolism in the presence of sufficient amounts of glycerol, which is a product of fermentation, and the digestion of fats in the intestinal lumen [9, 10].

L. reuteri is an obligate, heteroenzymatic, lactic-acid bacillus that inhabits the gastrointestinal tract of humans and animals [11, 12]. A search for strains of this species that are capable of efficient synthesis of antimicrobial bacteriocin-like compounds (including reuterin) is of great practical interest. On the one hand, *L. reuteri* is the dominant LAB species in the intestinal tract of many mammals and birds [13, 14]; on the other hand, reuterin and other related compounds have high potential as a food preservative [1, 15, 16] and antimicrobial therapeutic agent [7, 8] due to their biochemical properties and broad spectrum of antimicrobial and fungicidal activity.

The composition and culturing conditions of the medium have a significant effect on the yield of bacteriocin-like compounds during the cultivation of a LAB strain [17, 18]. It was shown, for instance, that the production of a nisin-like bacteriocin by a *Lactococcus lactis* strain is only efficient on MRS and M17 media [19, 20].

The individual components of the media are very important for antibiotic production by microorganisms [21]. For example, salts of nitric or, less commonly, nitrous acid, ammonium salts of organic and inorganic acids or amino acids, proteins, and products of their hydrolysis (peptones, hydrolysates) usually serve as components of nutrient media. Disaccharides, polysaccharides, alcohols, and acids are used as carbon sources. A microorganism can actively develop on one carbon source and weakly, with poor bacteriocin synthesis, on another [21]. The biosynthesis and microbial growth of most microbial metabolites require the presence of phosphorus-containing compounds in the form of phosphate ions.

The active acidity (pH) of the nutrient medium and its composition significantly affect the development of microbes, their metabolism, and, consequently, the formation of bacteriocin-like compounds [22].

The goal of the present work was to optimize the nutrient medium and culturing conditions in order to improve the biosynthesis of bacteriocin-like substances, including, presumably, reuterin, by *L. reuteri* LR1.

EXPERIMENTAL

Strains and Culture Maintenance

The *L. reuteri* strain LR1 from the Microbial Collection of the All-Russia Research Institute of Dairy Industry (Moscow Russia) was used. The inoculate was obtained via strain cultivation in MRS broth (Biokompas-C, Russia) at 37 ± 1°C.

Gram-negative strains of *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, and the gram-positive strain *Staphylococcus aureus* ATCC 6538 served as test microorganisms. The strains were supplied by the Scientific Center for the Expert Evaluation of Medicinal Products (Ministry of Health of the Russian Federation) and were grown on agar slants at 37 ± 1°C.

Antagonistic Activity

The antagonistic activity was determined from a comparison of growth in mixed populations and monocultures [23]. Cocultivation was carried out in 20 mL of MRS broth at 37 ± 1°C inoculated with 1 mL of *L. reuteri* LR1 and the test strain. The number of test-strain cells grown on SPA medium (dry nutrient agar, Biokompas-C, Russia) was taken as 100%; the number of cells in all cultures was counted after 24 h and 48 h of cultivation.

Assessment of the Biosynthesis of Bacteriocin-Like Compounds

The level of the production of bacteriocin-like compounds (including, presumably, reuterin) by *L. reuteri* LR1 was determined by the change in antimicrobial activity against a selected test strain measured with the well-diffusion agar method [24]. To this end, the test strain *E. coli* ATCC 25922 was cultured at 37 ± 1°C for 24 h on agar slants, and the cell suspension (1%) was then introduced at 45 ± 2°C into regenerated SPA medium with Tween 80 (Sigma, United States). The liquid was mixed carefully and poured onto sterile Petri dishes to a thickness of the medium layer of 5 mm. A glass tube was used after agar solidification to make wells with a diameter of 5 mm; the analyzed samples were added to the wells. The dishes were kept at room temperature for 3 h and then incubated at 37 ± 1°C. The zone of inhibited test-strain growth was measured after 24 h of incubation.

Optimization of Culturing Conditions

To establish the optimal glycerol content in MRS broth, *L. reuteri* LR1 inoculate (1%) was added to the medium, and the suspension was cultured at 37 ± 1°C for 24 h. The grown culture was placed into MRS

Table 1. Central composition plan for response surface

Experiment number	Peptone	Yeast extract	Hydrolyzed milk
1	1.0	0.5	0.0
2	0.5	0.5	25.0
3	0.0	1.0	25.0
4	0.0	0.5	0.0
5	0.5	1.0	0.0
6	0.5	1.0	50.0
7	0.0	0.5	50.0
8	0.5	1.0	25.0
9	0.0	1.5	50.0
10	0.0	1.5	0.0
11	1.0	1.5	0.0
12	1.0	1.0	25.0
13	0.5	1.0	25.0
14	0.5	1.5	25.0
15	1.0	0.5	50.0
16	1.0	1.5	50.0

broth (50 mL) and cultured for another 3 h at the same temperature, after which the suspension (5%) was added to MRS broth containing various glycerol amounts (0 mM, 50, 100, 150, 200, 250, 350, or 450 mM) and was grown for 18 h at 37 ± 1°C. After cultivation, the samples were centrifuged at 14000 *g* for 10 min, and the supernatant was passed through a filter with a pore size of 0.22 (Sartorius, United States). The antimicrobial activity was then measured in the samples with the well-diffusion agar method, as described above.

To determine the optimal glycerol content in the water-glycerol medium used for glycerol bioconversion, the *L. reuteri* LR1 biomass was collected via centrifugation, washed, placed into an aqueous medium containing 150 mM, 200 mM or 250 mM glycerol, and incubated for 2 h at 37 ± 1°C. After glycerol bioconversion, the *L. reuteri* LR1 biomass was sedimented at 14000 *g* for 10 min, the obtained supernatant was passed through a filter with a pore size of 0.22 μ, and the antimicrobial activity was determined with the well-diffusion agar method, as described above.

The effect of active acidity on the production of antimicrobial bacteriocin-like compounds was studied at the following pH levels: 5.8, 6.0, 6.2, 6.4, and 6.6. *L. reuteri* LR1 was cultured in bioreactors (DAS GIP, Germany) in batch mode with constant neutralization of the nutrient medium (20% NaOH aqueous solution) for pH maintenance at a set level (see above) with constant mixing (60 rpm).

A plan for the central composition of a three-factor experiment was designed to study the effect of various nitrogen sources on biomass growth and the accumulation of antimicrobial compounds (Table 1).

Such nitrogen sources as peptone (with factor levels of 0%, 0.5%, and 1%), YE (0.5%, 1%, and 1.5%), and hydrolyzed milk (0%, 25%, and 50%) were selected as independent factors. ANOVA was used to assess the effect of each of them. The differences in the values of each factor were analyzed in paired comparison tests and recognized as statistically significant at $p \le 0.05$.

All of the experiments were carried out in three to five replications.

Statistical and regression analysis with Microsoft Office and Statistica 10 were used for mathematical processing of the data. Mathematical planning of the experiment was used for effective experiment setting and the selection of rational parameters. The reliability of the results was assessed with the Student's *t*-test, and the Fischer's criterion was used to verify the approximation of the obtained regression dependences to the experimental data.

RESULTS AND DISCUSSION

Antagonistic Activity of the Lactobacillus reuteri Strain Against Test Cultures

It was previously shown that *L. reuteri* LR1 isolated in 2014 from the feces of a healthy person has a probiotic effect in vivo [25] and a pronounced antagonistic activity against clinical *Klebsiella pneumoniae* isolates with multiple antibiotic resistance [23].

Like other probiotic microorganisms, *L. reuteri* can synthesize various antimicrobial compounds, including reuterin, which possesses a broad spectrum of antimicrobial action against bacteria, yeasts, fungi, and protozoa [26–28] and is produced during anaerobic glycerol metabolism. It is well known that the ability to synthesize antimicrobial bacteriocin-like compounds is a strain-specific characteristic. For example, various *L. reuteri* strains can produce different amounts or none of these substances.

In order to study the ability of *L. reuteri* LR1 to synthesize antimicrobial compounds, it was necessary to select an opportunistic test strain suitable for the testing of the activity of bacteriocin-like substances produced by this *L. reuteri* strain. Three opportunistic strains, *E. coli, S. typhimurium*, and *Staphylococcus aureus*, the content of which in foods is standardized by law, were selected as test microorganisms. Figure 1 presents the antimicrobial activity of *L. reuteri* against these strains.

The *L. reuteri* strain showed the highest activity against *E. coli* ATCC 25922; the number of viable test cells decreased by 2.741 lg(CFU/mL) and 5.24 lg(CFU/mL) after 24 h and 48 h of cocultivation, respectively. Indeed, it was recently shown that gram-positive

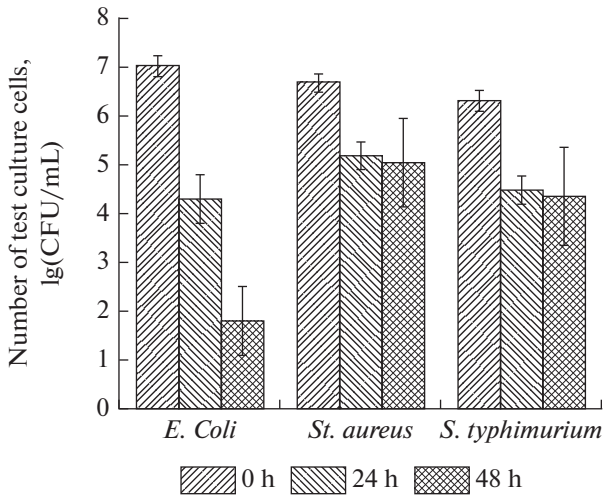

Fig. 1. Antagonistic activity of *L. reuteri* against test strains of opportunistic pathogens *E. coli*, *S. typhimurium*, and *Staphylococcus aureus.*

microorganisms, such as *Staphylodoccus aureus*, can be more resistant to antimicrobial compounds, e.g., reuterin, than gram-negative bacteria. Among the latter, *E. coli* DH5α proved to be the most sensitive [29]. Therefore, the *E. coli* strain turned out to be the best test strain, the viability of which after the cocultivation with *L. reuteri* was much lower than that in the other tested bacterial strains.

Optimization of the Composition of the Nutrient and Water-Glycerol Media

It is known that the production of the bacteriocin-like substance reuterin increases in glycerol-containing medium; therefore, the effect of various glycerol amounts on the accumulation of antimicrobial compounds by the *L. reuteri* strain was studied. Figure 2a shows that the highest *L. reuteri* LR1 activity against the *E. coli* test culture was observed at glycerol concentrations of 150 mM and 200 mM (with no significant difference between them). However, the antimicrobial activity was considerably reduced at a glycerol concentration higher than 200 mM (Figure 2a).

It was also established that *L. reuteri* cells died after some time of cultivation in MRS broth with 150 mM or 200 mM glycerol (Figure 3). It is known that reuterin inhibits the growth of gram-negative and most gram-positive bacteria, fungi, yeasts, and protozoa at concentrations from 15 mg/mL to 30 mg/mL and is toxic for LAB, including *L. reuteri*, at concentrations of 60–150 mg/mL [30].

Only live bacterial cells can synthesize antimicrobial compounds, and the isolation of these compounds requires purification from the media of complex composition; therefore, it is-appropriate to use a two-stage cultivation of the producer strain, first in a rich nutrient medium for biomass accumulation and then in a water-glycerol medium to convert glycerol into antimicrobial substances (presumably reuterin).

It was shown that the size of the zones of the inhibition of test bacteria growth was much larger when two-stage cultivation was applied (19–23 mm *versus* 12–14 mm for single stage cultivation) with a glycerol concentration of 150–250 mM (Figure 2). Since the antimicrobial activity at glycerol contents of 150 mM or 200 mM was practically the same and was higher than that at 250 mM of glycerol (Figure 2b), all subsequent experiments were carried out at a glycerol concentration of 200 mM in the water-glycerol medium.

The next stage of the work was the optimization of the medium composition at the first phase of *L. reuteri* cultivation. The effect of various nitrogen sources,

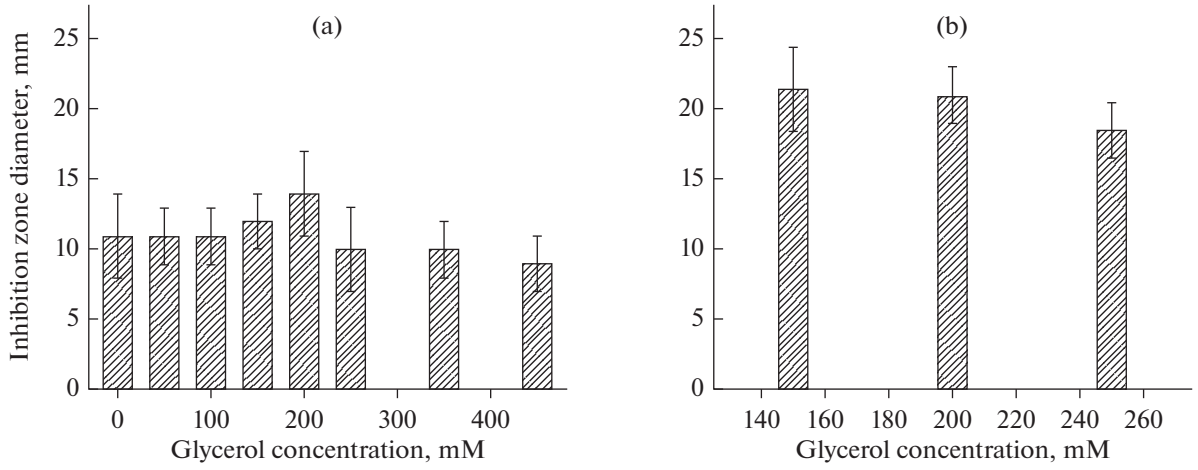

Fig. 2. Antagonistic activity of *Lactobacillus reuteri* against the *E. coli* test strain as a result of various cultivation modes: (a) single-stage cultivation on MRS medium supplemented with glycerol; (b) two-stage cultivation on MRS broth and then on water–glycerol medium.

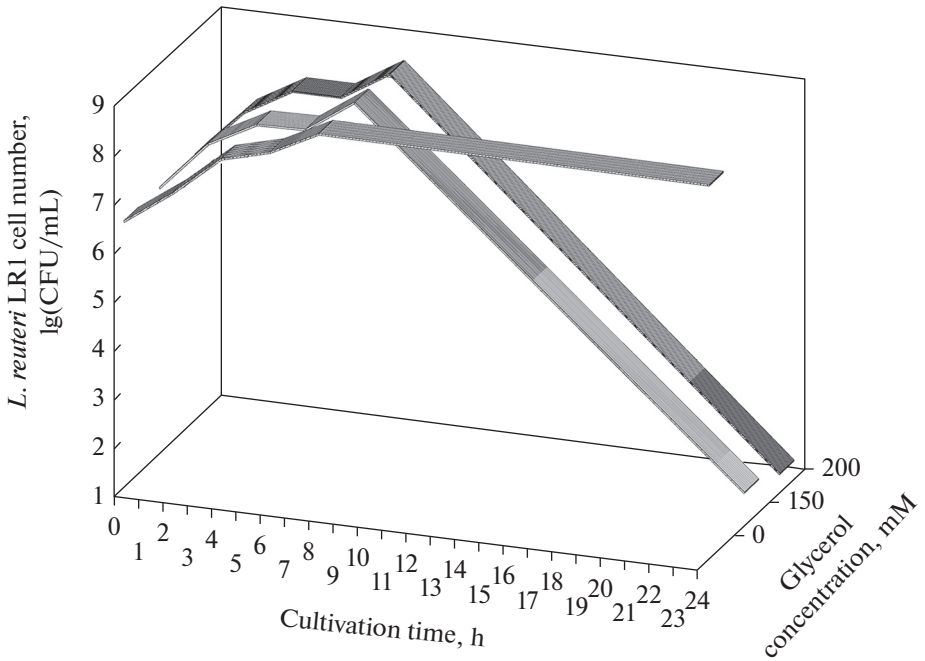

Fig. 3. *Lactobacillus reuteri* LR1 growth on MRS broth containing various glycerol concentrations.

carbohydrates, and salts on the growth and accumulation of antimicrobial compounds (presumably reuterin) was studied.

Peptone, yeast extract (YE), and hydrolyzed milk were tested as nitrogen-containing components; the cultivation was carried out in 150-mL bottles at a starting pH value of 6.2, a temperature of 37 ± 1°C, and a glucose content of 2%.

The results showed that factors such as the content of hydrolyzed milk and YE and the combination of peptone + hydrolyzed milk, were statistically significant ($p \leq 0.05$) for *L. reuteri* antimicrobial activity, while combinations of peptone + YE and YE+ hydrolyzed milk were not (Fig. 4). Based on these results, hydrolyzed milk (25%), YE (1.0%), and peptone (0.5%) were selected as nitrogen sources for further research.

Glucose, lactose, and sucrose were tested, separately and in combination, as carbon sources in a medium that was already optimized for nitrogen sources. Their effect on biomass accumulation and the biosynthesis of bacteriocin-like substances was studied. It was established that all of the aforementioned carbohydrates had no statistically significant influence on the synthesis of the antimicrobial compounds by *L. reuteri* (Table 2). Based on the obtained data, glucose (20 g/L) was selected as a carbon source for the medium containing hydrolyzed milk, YE, and peptone.

The following salts are commonly used as components of the medium for the *Lactobacillus* cultivation: potassium phosphate monosubstituted, sodium acetate, ammonium citrate bisubstituted, magnesium sulfate, and manganese sulfate [31]. The previously developed *L. reuteri* growth medium included the following salts, g/L: potassium phosphate monosubstituted—2; sodium acetate—5; magnesium sulfate—2; and manganese sulfate—0.05 [31]. A plan of the central compositional for a four-factor experiment was drawn up to study the effect of the aforementioned salts on the biosynthesis of the antimicrobial compounds by *L. reuteri* LR1. Potassium phosphate monosubstituted (with the factor level of 0 g/L, 2 g/L and 4 g/L), sodium acetate (0 g/L, 2.5 g/L and 5 g/L), magnesium sulfate (0.2 g/L, 1.1 g/L and 2.0 g/L), and manganese sulfate (0 g/L, 0.25 g/L and 0.5 g/L) were taken as independent factors (Table 3).

According to the experimental plan, 18 nutrient media were composed, and the effect of the aforementioned salts on the *L. reuteri* antimicrobial activity was studied. The greatest antimicrobial activity (the largest diameter of the *E. coli* growth-inhibition zone, 20–22 mm) was observed with nutrient media no. 8, 10, 11, 13, and 17 (Table 3). The tested salts had no statistically significant effect on the production of bacteriocin-like compounds. The largest inhibition zones (21.75 ± 1.5 mm) were observed for media containing sodium acetate—5.0 g/L; potassium phosphate monosubstituted—2.0 g/L; magnesium sulfate—0.2 g/L, and manganese sulfate—0.5 g/L.

The study made it possible to compose an optimized nutrient medium providing the highest production of antimicrobial compounds by the *L. reuteri* LR1 strain: hydrolyzed milk—250 mL/L; YE—10 g/L; peptone—5 g/L; glucose—20 g/L; sodium acetate—

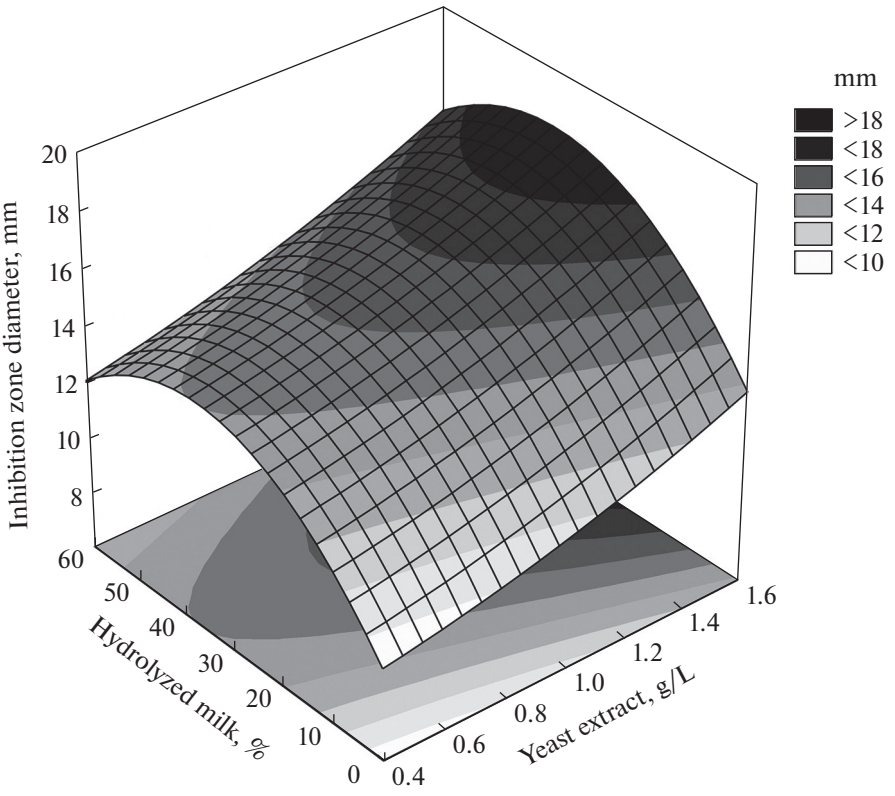

Fig. 4. Response surface for evaluation of the activity of antimicrobial compounds produced by *L. reuteri* grown in a medium with various nitrogen sources.

5.0 g/L; potassium phosphate monosubstituted—2.0 g/L; magnesium sulfate—0.2 g/L, and manganese sulfate—0.5 g/L.

Optimization of Lactobacillus reuteri Culturing Conditions

For further optimization of the production of antimicrobial compounds by *L. reuteri* LR1, the effect of the following parameters was studied: the active acidity (pH) of the nutrient medium (previously optimized in this work) during the first cultivation stage; the starting pH values of the water-glycerol medium; the amount of the introduced inoculate; and the temperature and the time of glycerol bioconversion to antimicrobial compounds.

The highest production of these compounds was observed with a nutrient medium with pH 6.2–6.4, when the zone of *E. coli* growth inhibition was 22–23 mm (Table 4).

Table 2. Effect of various carbon sources on bacteriocin-like compound production by *Lactobacillus reuteri* LR1 (assessment of the zone of inhibited growth of the *E. coli* test strain)

Variable factor, %			pH	Biomass, g	Diameter of zone of growth inhibition, mm
glucose	lactose	sucrose			
0.0	0.0	2.0	4.66	1.25	15.25 ± 0.96
2.0	0.0	2.0	4.54	1.07	17.50 ± 0.73
0.0	2.0	0.0	4.61	1.19	14.75 ± 0.26
2.0	2.0	2.0	4.46	1.10	19.75 ± 0.26
2.0	0.0	0.0	4.61	0.94	16.00 ± 0.15
0.0	2.0	2.0	4.50	1.12	17.25 ± 0.22
0.0	0.0	0.0	4.75	1.34	14.50 ± 0.73
2.0	2.0	0.0	4.50	1.00	17.25 ± 0.96

Table 3. Effect of various salts on the biosynthesis of antimicrobial bacteriocin-like compounds

Medium number	Variable factor, g/L				pH	Diameter of the zone of inhibited *E. coli* growth, mm
	sodium acetate	potassium phosphate monosubstituted	magnesium sulfate	manganese sulfate		
1	5.0	0.0	0.2	0.50	4.20	16.75 ± 0.50
2	2.5	2.0	1.1	0.00	4.03	17.50 ± 0.58
3	2.5	2.0	1.1	0.25	4.09	18.00 ± 0.82
4	2.5	2.0	1.1	0.25	4.23	16.50 ± 0.58
5	0.0	4.0	2.0	0.50	4.20	16.00 ± 0.50
6	5.0	4.0	2.0	0.00	4.20	16.00 ± 0.50
7	0.0	2.0	1.1	0.25	4.24	17.25 ± 0.50
8	5.0	2.0	0.2	0.50	4.22	21.75 ± 1.50
9	2.5	4.0	1.1	0.25	4.39	19.25 ± 0.96
10	0.0	4.0	0.2	0.50	4.16	20.00 ± 0.50
11	5.0	2.0	1.1	0.25	4.18	20.50 ± 0.58
12	5.0	0.0	2.0	0.50	4.22	19.00 ± 0.82
13	2.5	2.0	0.2	0.25	4.15	20.00 ± 0.50
14	5.0	4.0	0.0	0.00	4.31	17.50 ± 0.57
15	2.5	2.0	1.1	0.50	4.35	19.25 ± 0.50
16	5.0	0.0	0.2	0.50	4.33	18.50 ± 2.38
17	2.5	2.0	1.1	0.00	4.40	20.25 ± 0.50
18	2.5	2.0	1.1	0.25	4.23	18.00 ± 2.31

Table 4. Effect of the pH of the nutrient medium on the production of antimicrobial bacteriocin-like compounds by *Lactobacillus reuteri* LR1

Characteristic	pH				
	5,8	6,0	6,2	6,4	6,6
L. reuteri cell number, lg(CFU/mL)	8.7 ± 0.34	8.8 ± 0.21	8.9 ± 0.11	9.3 ± 0.23	8.7 ± 0.15
Diameter of the zone of inhibited *E. coli* growth, mm	17 ± 1.41	19 ± 1.41	22 ± 1.41	23 ± 1.41	21 ± 1.06

To study the effect of the amount of the added inoculate, inoculate doses of 1%, 3%, 5%, and 10% were tested. Cultivation was carried out in the optimized medium for 18 h at 37 ± 1°C with an initial pH value of 6.2. The highest accumulation of antimicrobial compounds was observed at inoculate doses of 5% and 10%; the diameter of the growth-inhibition zone was 24.5–24.8 mm in these conditions, which correlated to the *L. reuteri* biomass and cell number.

The glycerol bioconversion time varied from 1 to 4 h, and the tested temperatures were 30°C and 37°C (according to the reported data, 30°C and 37°C were the optimal temperatures for reuterin production [32]).

The biosynthesis of bacteriocin-like compounds showed a statistically significant dependence on the temperature of *L. reuteri* cultivation (at the first stage) and the temperature of the bioconversion in water-glycerol medium (at the second stage) (Fig. 5). The largest zones of *E. coli* growth inhibition (24.7 ± 0.3 mm) were obtained at 37°C at the first stage and at the same temperature at the second stage with a bioconversion time of 2 h. When the temperature was reduced to 30°C, the inhibition zones decreased; the largest growth-inhibition zones at this temperature were observed after 4 h of incubation (Fig. 5).

To study the effect of water-glycerol medium pH on the glycerol bioconversion to antimicrobial com-

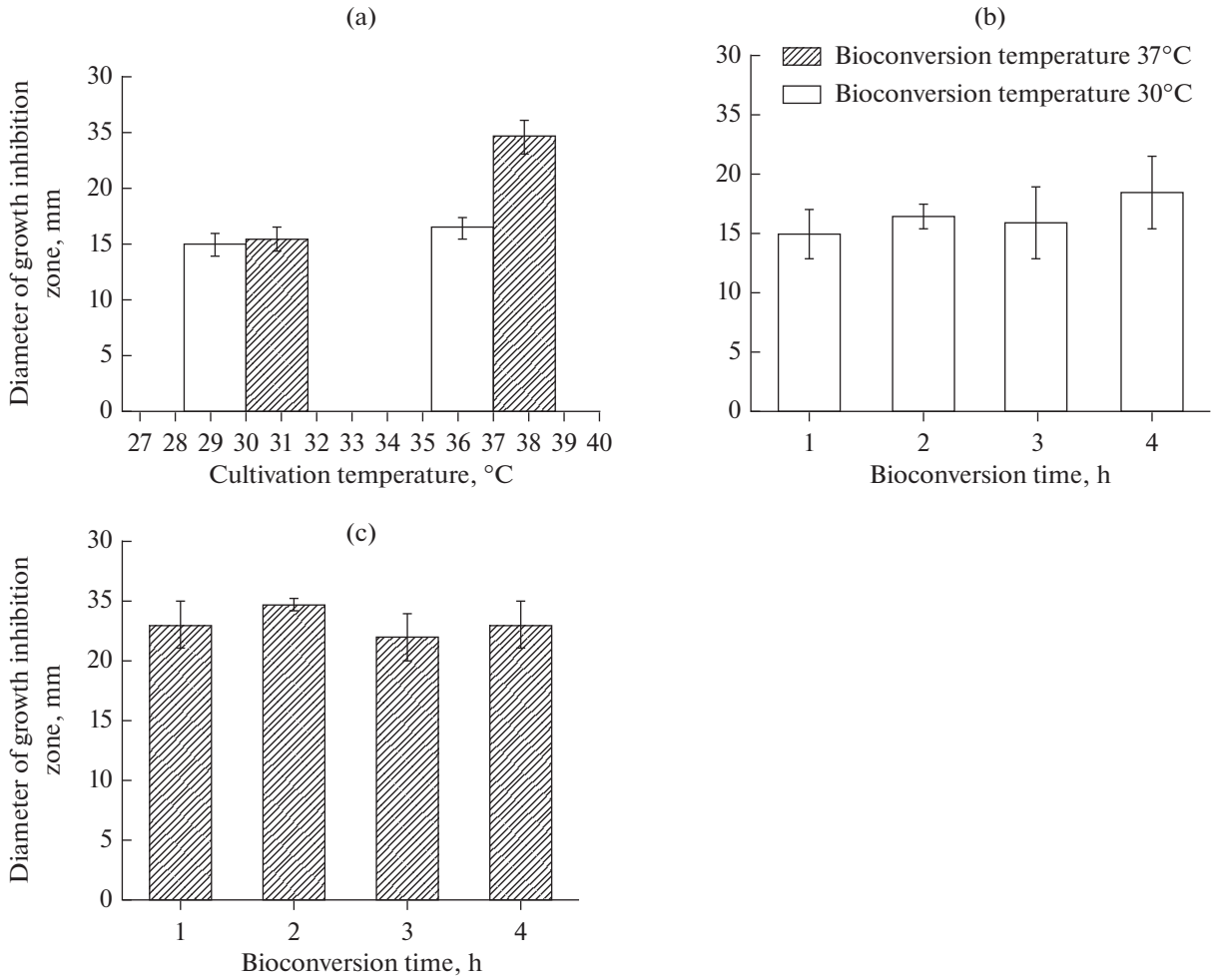

Fig. 5. Effect of cultivation temperature (first stage) and bioconversion (second stage) (a), and bioconversion time at 30°C (b) and 37°C (c) on the production of antimicrobial bacteriocin-like compounds by *L. reuteri* LR1.

pounds, the starting pH values ranged from 5.4 to 6.8 with a step of 0.2; the final pH at the end of bioconversion was 4.2 ± 0.2 in all experiments.

pH of the water-glycerin medium	Diameter of the zone of inhibited *E. coli* growth, mm
5.4	24.50 ± 0.71
5.6	24.50 ± 0.71
5.8	24.25 ± 0.35
6.0	24.50 ± 0.71
6.2	23.50 ± 2.12
6.4	24.50 ± 0.71
6.6	24.75 ± 0.35
6.8	22.50 ± 2.12

It was shown that the antimicrobial activity, which is expressed as the diameter of growth-inhibition zone, changed insignificantly, from 22.50 ± 2.12 mm to 24.75 ± 0.35 mm, at all of the tested pH values. The highest activity was characteristic of the initial water-glycerol medium pH, 6.6.

CONCLUSIONS

Thus, this study showed that the probiotic strain *L. reuteri* LR1 with antimicrobial activity against gram-positive and gram-negative bacteria synthesizes a bacteriocin-like compound, presumably reuterin, in the presence of glycerol. The optimal method for efficient biosynthesis of antimicrobial compounds is two-stage cultivation, first in a nutrient medium for cell-biomass accumulation and then in a water-glycerol medium for glycerol bioconversion to the target compounds. The optimal composition of the nutrient medium for the first stage includes hydrolyzed milk—25%; yeast extract—1.0%; peptone—0.5%; glucose—20 g/L; sodium acetate—5.0 g/L; potassium phosphate monosubstituted—2.0 g/L; magnesium sulfate—0.2 g/L; and manganese sulfate—0.5 g/L. The optimal cultivation conditions at the first stage are an inoculation dose of *L. reuteri* LR1 of 5%, a pH value of the nutrient medium of 6.4–6.6, an incubation temperature of 37°C, and a cultivation time of 18 h. The cells are then separated via centrifugation; the

biomass is washed and placed into the water-glycerol medium for glycerol bioconversion to a bacteriocin-like compound, presumably reuterin. The bioconversion parameters are as follows: the glycerol content in medium is 200 mM, the initial pH is 6.6, the temperature is 37°C, and the process time is 2 h. The diameter of the zone of inhibited growth of the *E. coli* test strain with the obtained solution is equal to 25 mm.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest.

This article does not contain any studies involving animals performed by any of the authors.

This article does not contain any studies involving human participants performed by any of the authors.

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