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MICROBIAL REGULATION OF THE ANTAGONISTIC ACTIVITY OF LACTIC ACID BACTERIA STRAINS UNDER THE INFLUENCE OF TRANSIENT BACILLI OF THE *BACILLUS* GENUS

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Aim. To study the effect of metabolites and components of aerobic spore-forming bacilli cells (MCASB) of the *Bacillus* genus on the antagonistic activity of lactic acid bacteria. **Methods**. Lactic acid bacteria, sampled from rectal and oral swabs from calves, were used in the study. Strains of conditionally pathogenic *Escherichia coli*, 055k59 No. 3912, *Staphylococcus aureus* No. 906, *Proteus mirabilis*, No. 3177, obtained from the collection of the city sanitary-epidemiological inspection service, were used as test cultures. MCASB were prepared using autoclaved microbial mass of *Bacillus subtilis* production strain 44-p via treatment with lysozyme and ultrasound. The antagonistic activity of lactic acid bacteria was determined by agar blocks method. **Results.** It was demonstrated that the addition of MCASB to the culture medium stimulates the antagonistic activity of lactic acid bacteria to pathogenic and conditionally pathogenic microorganisms of intestinal bacteria. The highest average stimulation index (2.56) of the antagonistic activity of lactic acid bacteria was found for *E. coli* 055k59 No. 3912. **Conclusions**. The results obtained may be used to improve the microbiological foundations of elaborating novel probiotic preparations.

Key words: antagonism, lactic acid bacteria, pathogenic and conditionally pathogenic bacteria, stimulation, metabolites, microbial cell components.

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INTRODUCTION

Sustaining natural microbiocenosis of intestines is one of the conditions of normal activity of both humans and animals. One of the mechanisms of the formation and functioning of microbiocenoses is presented by antagonistic properties of the dominant normal flora of intestines. In the majority of cases the antagonistic properties of microorganisms are connected to the synthesis of specific products of their metabolism: organic acids, antibiotic-like substances, lytic enzymes, bacteriocins *etc*., which are susceptible to microbial regulation [1].

Some researchers proved the possibility of microbial stimulation of the antagonistic activity of lactic acid bacteria both at the influence of microbial components (for instance, peptidoglycans of cellular walls of conditionally pathogenic, associative microorganisms) and their metabolites [2].

The application of probiotics – living microorganisms and the products of their fermentation, capable of optimizing the microbiocenosis of intestines due to the inhibition of the growth of pathogenic and conditionally pathogenic microflora (with the purpose of treating dysbacteriosis), motivates the search for novel efficient mechanisms of regulating the antagonistic activity of probiont strains [3–6].

The aim of the study is to investigate the microbial regulation of the antagonistic activity of lactic acid bacteria strains under the influence of transient bacilli of the *Bacillus* genus.

MATERIALS AND METHODS

Bacterial cultures. The objects of research were nine strains of lactic acid bacteria (LAB), isolated from the

intestines of up to three-month-old calves and naturally preserved herbage. The strains of conditionally pathogenic microorganisms *Escherichia coli,* 055k59 3912, *Staphylococcus aureus* 906 and *Proteus mirabilis* 3177 were used as test cultures.

The scheme of isolating lactic acid bacteria. LAB were obtained by the following scheme: three-fold passaging of rectal and oral sample swabs from up to three-month-old calves in the medium of sterile milk; subculturing from the sterile milk to cabbage agar with chalk; cultivation of LAB-notable colonies in the standard medium of MRS [7].

The affiliation of bacteria to the *Lactobacillus* genus was determined by their responding to the Gram staining, the mobility capability, the presence of spores, capsules, *etc*., the presence of catalase enzyme, the capability of fermenting some carbohydrates, and the activity of acid-formation.

Culture media. Semisolid culture media of the following composition were used: medium No. 1 (%) − lactoserum – 20; hydrolyzed milk – 15; sodium acetate – 2; corn extract – 1.5; manganese sulphate – 0.02; agar − 0.8;

medium No. 2 (%) − metabolites and components of aerobic spore-forming bacilli cells – 10; lactoserum – 20; hydrolyzed milk – 15; sodium acetate – 2; corn extract – 1.5; manganese sulphate – 0.02; agar − 0.8.

The metabolites and components of aerobic spore-forming bacilli cells (MCASB) were prepared using autoclaved microbial mass of *Bacillus subtilis* production strain 44-p via treatment with lysozyme and ultrasound [8].

The control (standard medium MRS, %): yeast extract – 0.5; meat extract – 1.0; soluble starch – 0.5; peptone – 1.0; glucose – 2.0; citric ammonium – 0.2; sodium acetate – 0.5; twin-80 – 0.1; K_2HPO_4 – 0.2; $MgSO_4 \times 4H_2O$ – 0.005; agar − 0.8.

The conditions of cultivating LAB strains on the investigated media. LAB strains were cultivated in the thermostat for five days at the temperature of 37 ± ± 0.5 °C. The dose of inoculate introduction was 1.0 ± 0.1 %. The results were estimated by the optical method.

The criterion of estimating the influence of MCASB on the growth and antagonistic activity (AA) of LAB was the data of the accumulation of the number of bacterial cells of lactic acid bacteria and their AA in the media with MCASB and without them. The stimulation index for the growth activity of bacterial cells was calculated using the following formula:

Stimulation index = (*titer of microbial cells with MCASB*)/(*titer of microbial cells without MCASB*).

The determination of the antagonistic activity of lactic acid bacteria strains. AA of LAB was determined using the agar blocks method. The suspensions of test cultures of *E. coli* 055k59 3912, *S. aureus* 906, *P. mirabilis* 3177 were cultivated on Petri dishes with the meat infusion agar (MIA) by the depth point method in the concentration of 10^7 of colony-forming units (CFU) in 1 cm^3. After agar gelation the holes of up to 7 mm diameter were cut on its surface and the blocks of media with deeply cultivated probiotic bacteria were introduced. Petri dishes were kept in the refrigerator, then in the thermostat at the temperature of optimal growth of test-strains. The determination of the diameters of delay zones for test culture growth was conducted 24–48 h later.

The criteria of estimating the influence of metabolites of aerobic bacilli on AA of LAB were the indices of the stimulation index: The index of stimulation for AA of LAB was determined by the following formula:

Stimulation index = (*diameter of growth delay zone with MCASB*)/(*diameter of growth delay zone without MCASB*).

The experiment was performed in triplicate.

RESULTS AND DISCUSSION

It is known that exometabolites and components of bacterial cells may serve as an additional source of organic substances (aminoacids, vitamins) for the growth and synthesis of biologically active substances of LAB.

There have been experiments on the influence of metabolites and components of aerobic spore-forming bacilli cells on the growth and AA of LAB. The strains of bacteria were isolated from the normobiocenosis of intestines of up to three-month-old calves and naturally preserved herbage.

It was determined that the investigated variants of media Nos. 1 and 2 enhance LAB growth compared to the control medium MRS. The maximal indices of bacterial mass accumulation on these media are observed for LAB strains Lkr-7, Lch-11, Lpl-18, which is confirmed by the titer value of viable cells of these cultures (in medium No. 1 − 10^{10} CFU/ml, in medium

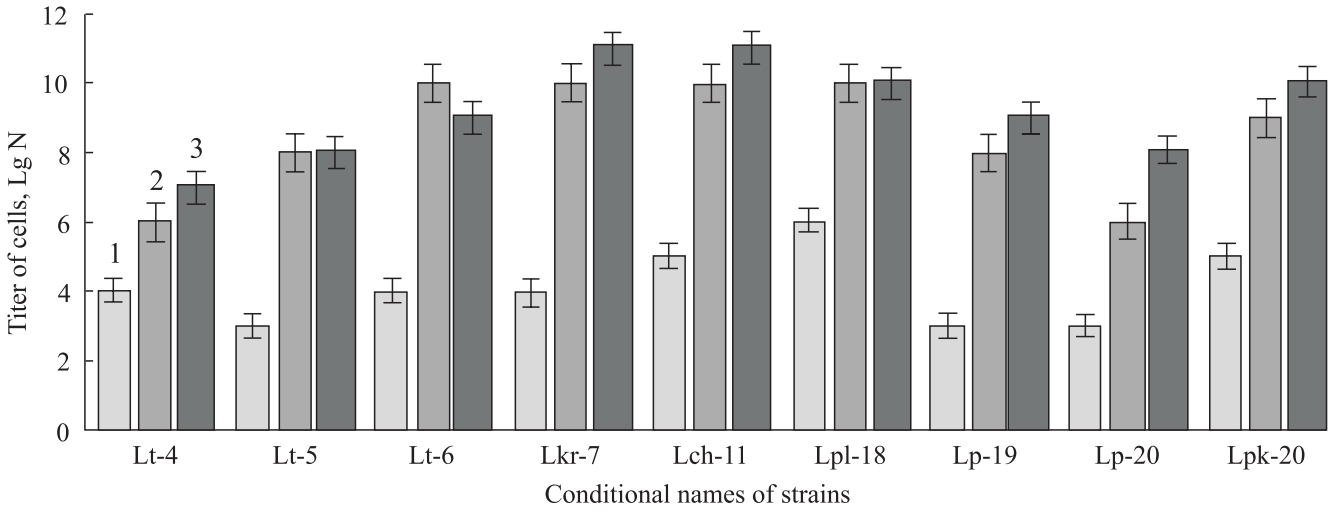

Fig. 1. The influence of aerobic spore-forming bacilli cells on the growth activity of lactic acid bacteria: 1 – control (MRS); 2, 3 – media Nos. 1, 2, respectively

No. 2 − 10^{11} CFU/ml) (Fig. 1). At the same time the growth indices of the multicomponent control medium were not high: even three days later the maximal titers of bacterial cells of lactobacilli were about 10^6 CFU/ml.

The determination of average indices of growth stimulation of LAB demonstrated that the introduction of MCASB to the medium enhances the accumulation of bacterial mass of lactobacilli cells. For instance, the average index of growth stimulation for LAB in medium No. 1 was 2.12, and in the medium No. 2 with the introduction of MCASB − 2.3 (Fig. 2).

Thus, the presence of MCASB in the medium promotes 8 % more intensive growth of LAB compared to the medium without the former.

The results obtained are in good agreement with the literature data on the growth-stimulating properties of biologically active substances, synthesizing aerobic spore-forming bacilli. For instance, it is known that vitamins, enzymes, hormone-like substances and subtilisin *B. subtilis* promote LAB growth [9].

One of relevant properties of LAB is the antagonistic activity regarding pathogenic and conditionally pathogenic microorganisms. The inhibition of the generation of pathogenic and conditionally pathogenic microorganisms of LAB occurs due to the formation of organic acids, antibiotic-like substances, *etc*. The active formation of organic acids – lactic acid, acetic acid – leads to rapid decrease in pH of the medium to the level, when the generation of pathogenic and conditionally pathogenic microorganisms is impossible.

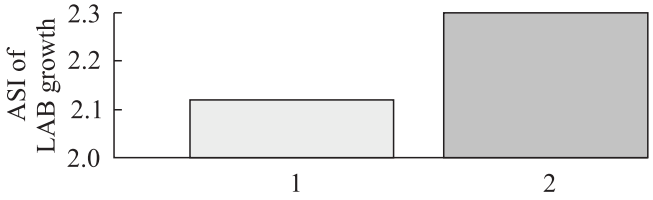

Fig. 2. The average stimulation index (ASI) of the growth of lactic acid bacteria (LAB) at the influence of aerobic spore-forming bacilli cells: 1 – medium No. 1; 2 – medium No. 2

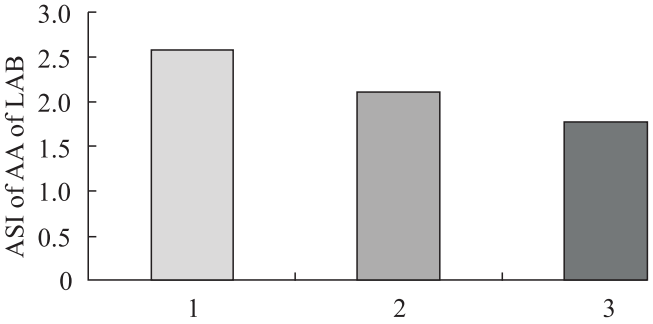

Fig. 3. Average stimulation indices (ASI) of the increase in the antagonistic activity (AA) of lactic acid bacteria (LAB) to the conditionally pathogenic microorganisms at the influence of metabolites and components of aerobic spore-forming bacilli cells: 1 − *E. coli*; 2 − *P. mirabilis*; 3 – *S. aureus*

The stimulation of AA for lactic acid bacilli in the presence of bacteria and, in some cases, their metabolites was first discovered in 1989 [10]. There is a well-known way of enhancing AA of probiotic bacteria by treating them with the bacteria components of indicator culture of *S. aureus* [11].

The influence of medium No. 2 with 10 % content of MCASB on AA of LAB strains have been stu-

The influence of culture medium components on the antimicrobial activity of lactic acid bacteria strains

Conditional name of a strain	Diameter of delay zone of test-culture growth on the culture medium No. 2, mm			Diameter of delay zone of test-culture growth on the control medium MRS, mm		
	Escherichia coli 055k59 3912	*Staphylococcus aureus* 906	*Proteus mirabilis* 3177	*Escherichia coli* 055k59 3912	*Staphylococcus aureus* 906	*Proteus mirabilis* 3177
Lt-4	26.0 ± 0.1	36.0 ± 0.3	23.0 ± 0.2	13.0 ± 0.1	20.0 ± 0.4	12.0 ± 0.1
Lt-5	8.0 ± 0.4	14.0 ± 0.5	12.0 ± 0.4	1.0 ± 0.1	5.0 ± 0.1	8.0 ± 0.4
Lt-6	18.0 ± 0.2	24.0 ± 0.5	15.0 ± 0.5	9.0 ± 0.6	12.0 ± 0.3	10.0 ± 0.6
Lkr-7	44.0 ± 0.3	30.0 ± 0.3	28.0 ± 0.2	21.0 ± 0.4	15.0 ± 0.5	18.0 ± 0.2
Lch-11	32.0 ± 0.7	20.0 ± 0.4	24.0 ± 0.6	18.0 ± 0.5	13.0 ± 0.5	14.0 ± 0.3
Lpl-18	10.0 ± 0.3	15.0 ± 0.3	6.0 ± 0.3	5.0 ± 0.7	15.0 ± 0.1	1.0 ± 0.1
Lp-19	33.0 ± 0.4	28.0 ± 0.1	46.0 ± 0.3	14.0 ± 0.2	12.0 ± 0.3	20.0 ± 0.1
Lp-20	12.0 ± 0.2	15.0 ± 0.2	12.0 ± 0.7	12.0 ± 0.1	13.0 ± 0.4	10.0 ± 0.4
Lpk-21	30.0 ± 0.5	25.0 ± 0.6	20.0 ± 0.4	14.0 ± 0.4	18.0 ± 0.5	11.0 ± 0.6

died. The results obtained testify to the fact that the cultivation of microorganisms on the culture medium with MCASB stimulates AA of LAB strains regarding the test-culture of conditionally pathogenic microorganisms. The increase in AA of the investigated LAB strains regarding *P. mirabilis* 3177 was manifested in the 1.2–2.3-fold increase in the diameters of delay zones of its growth on average compared to the control. The stimulation of antimicrobial activity regarding *S. aureus* 906 was determined in 88 % of the investigated LAB – the diameter of delay zones of test culture growth was increased 1.1–2.8-fold. Over 70 % of LAB strains demonstrated 1.7–2.3-fold increase in the delay zone of the growth of *E. coli* 055k59 3912 (Table) compared to the control.

The results obtained demonstrate that the presence of 10 % MCASB in the medium has the highest stimulating influence on AA of LAB regarding *E. coli*. The average stimulation index (ASI) of the antagonistic activity of LAB to this test-culture increases up to 2.56 (Fig. 3). The lowest values of ASI (1.77) of the antagonistic activity of LAB were reported regarding *S. aureus*.

The analysis of the data obtained allows the assumption that the stimulation of AA of LAB at the influence of MCASB occurs due to either (i) stimulation of lactobacilli growth, or (i) induction of the synthesis of antagonistic activity factors.

CONCLUSIONS

The introduction of metabolites and components of aerobic spore-forming bacilli cells to the culture medium promotes the stimulation of the antagonistic activity of lactic acid bacteria to the pathogenic and conditionally pathogenic microorganisms of intestines – *E. coli*, 055k59 3912, *S. aureus* 906, *P. mirabilis* 3177. The highest average stimulation index (2.56) of the antagonistic activity of lactic acid bacteria was found for *E. coli* 055k59 3912. The results obtained may be used to improve microbiological foundations of elaborating novel probiotic preparations.

Мікробна регуляція антагоністичної активності штамів молочнокислих бактерій за впливу транзиторних бацил роду *Bacillus*

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Мета. Дослідити вплив метаболітів і компонентів мікробних клітин аеробних спороутворюючих бацил роду *Bacillus* (МКАСБ) на антагоністичну активність молочнокислих бактерій. **Методи.** Використано молочнокислі бактерії, виділені з проб ректальних і ротових змивів від телят. Тест-культурами патогенних і умовно патогенних мікроорганізмів слугували *Escherichia coli* (штам 055к59 3912), *Staphylococcus aureus* (штам 906) і *Proteus mirabilis* (штам 3177) з колекції міської санепідемстанції. МКАСБ одержували обробкою автоклавованої мікробної маси виробничого штаму *Bacillus subtilis* 44-р лізоцимом та ультразвуком. Антагоністичну активність молочнокислих бактерій визначали методом

агарових блоків. **Результати.** Показано, що додавання до поживного середовища МКАСБ сприяє стимулюванню антагоністичної активності молочнокислих бактерій до патогенних та умовно патогенних мікроорганізмів кишкової групи. Встановлено найвищий середній індекс стимулювання (2,56) антагоністичної активності молочнокислих бактерій до *E. coli*. **Висновки.** Одержані результати можуть бути використані для удосконалення мікробіологічних основ створення нових про-біотичних препаратів.

Ключові слова: антагонізм, молочнокислі бактерії, патогенні та умовно патогенні бактерії, стимуляція, метаболіти, компоненти мікробних клітин.

Микробная регуляция штаммов молочнокислых бактерий под влиянием транзиторных бацилл рода *Bacillus*

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Цель. Исследовать влияние метаболитов и компонентов микробных клеток аэробных спорообразующих бацилл рода *Bacillus* (МКАСБ) на антагонистическую активность молочнокислых бактерий. **Методы.** Использованы молочнокислые бактерии, выделенные из проб ректальных и ротовых смывов от телят. Тест-культурами патогенных и условно патогенных микроорганизмов служили *Escherichia coli* (штамм 055к59 3912), *Staphylococcus aureus* (штамм 906) и *Proteus mirabilis* (штамм 3177) из коллекции городской санэпидемстанции. МКАСБ получали обработкой автоклавированной микробной массы производственного штамма *Bacillus subtilis* 44-р лизоцимом и ультразвуком. Антагонистическую активность молочнокислых бактерий определяли методом агаровых блоков. **Результаты.** Показано, что добавление к питательной среде МКАСБ способствует стимуляции антагонистической активности молочнокислых бактерий к патогенным и условно патогенным микроорганизмам кишечной группы. Установлен самый высокий индекс стимуляции (2,56) антагонистической активности молочнокислых бактерий к *E. coli*. **Выводы.** Полученные результаты могут быть использованы для усовершенствования микробиологических основ создания новых пробиотических препаратов.

Ключевые слова: антагонизм, молочнокислые бактерии, патогенные и условно патогенные бактерии, стимуляция, метаболиты, компоненты микробных клеток.

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