MICROBIOLOGY AND VIROLOGY

ANTAGONISTIC ACTIVITY OF MONOCULTURES AND CONSORTIA OF LACTOBACILLI AGAINST MULTIDRUG-RESISTANT ISOLATES OF OPPORTUNISTIC BACTERIA AS A SCREENING OF THEIR PROBIOTIC POTENTIAL

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ABSTRACT

Background. *In recent years, special attention has been paid to the studying the consortia of probiotic bacteria. In these associations, the properties of individual microorganisms can be enhanced, in particular, their antagonistic activity which is an effective indicator for screening of probiotic potential. The development of probiotics based on such consortia with antibacterial properties is critical in the light of the growing problem of drug resistance in microorganisms.*

The aim of the work. *To study the antagonistic activity of monocultures and consortia of lactobacilli against multidrug-resistant isolates of opportunistic bacteria.*

Materials and methods. *The antagonistic activity of lactobacilli monocultures and their consortia was assessed simultaneously by two methods: the cross streak method and the well diffusion method.*

Results. *All strains of lactobacilli and their consortia, depending on the research method, had varying degrees of antagonistic activity. Five consortia had stronger antagonism to test cultures as compared to monocultures, while in one consortium, the effect of antagonistic activity was reduced compared to monocultures. The results of studying the antagonistic activity of two consortia (Limosilactobacillus fermentum 44/1 and Lacticaseibacillus rhamnosus 12L, Latilactobacillus curvatus LCR-111-1 and Lactiplantibacillus plantarum 8PAZ) contradict data on the biocompatibility of strains in these consortia. Differences in the degree of antagonistic effects of lactobacilli on gram-positive and gram-negative species of opportunistic bacteria were revealed.*

Conclusion. *The study showed that both the biocompatibility of the probiotic strains and the antagonistic activity of the consortium are the important requirements for creating a probiotic consortium with effective probiotic potential. To study the antagonistic properties of lactobacilli, the number of isolates of target gram-positive and gram-negative bacteria and normobiota should be increased. This will allow us to determine effective strategies for using probiotics in conditions of the spread of drug resistance of microorganisms.*

Key words: *lactobacilli, probiotic consortia, probiotic potential, antagonistic activity, opportunistic bacteria, multidrug-resistant isolates*

Received: 29.03.2024
Accepted: 18.06.2024
Published: 15.07.2024

For citation: Pendyukhova A.S., Belkova N.L., Okhotina Yu.S., Ivanchikov E.A., Shchekotova A.V., Semenova N.V., Rychkova L.V. Antagonistic activity of monocultures and consortia of lactobacilli against multidrug-resistant isolates of opportunistic bacteria as a screening of their probiotic potential. *Acta biomedica scientifica*. 2024; 9(3): 121-129. doi: 10.29413/ABS.2024-9.3.12

АНТАГОНИСТИЧЕСКАЯ АКТИВНОСТЬ МОНОКУЛЬТУР И КОНСОРЦИУМОВ ЛАКТОБАЦИЛЛ В ОТНОШЕНИИ ПОЛИРЕЗИСТЕНТНЫХ ИЗОЛЯТОВ УСЛОВНО-ПАТОГЕННЫХ БАКТЕРИЙ КАК СКРИНИНГ ИХ ПРОБИОТИЧЕСКОГО ПОТЕНЦИАЛА

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РЕЗЮМЕ

Актуальность. *В последние годы особое внимание уделяется изучению консорциумов пробиотических бактерий. В этих ассоциациях свойства отдельных микроорганизмов могут усиливаться, в частности их антагонистическая активность, которая является эффективным показателем для скринига пробиотического потенциала. Разработка пробиотиков на основе таких консорциумов с антибактериальными свойствами имеет решающее значение в свете растущей проблемы лекарственной устойчивости микроорганизмов.*
Цель работы. *Изучение антагонистической активности монокультур и консорциумов лактобацилл в отношении полирезистентных изолятов условно-патогенных бактерий.*
Материалы и методы. *Антагонистическую активность монокультур лактобацилл и их консорциумов оценивали параллельно двумя методами: методом перпендикулярных штрихов и методом лунок.*
Результаты. *Все штаммы лактобацилл и их консорциумы в зависимости от метода исследования обладали разной степенью антагонистической активности. В пяти консорциумах антагонизм к тестовым культурам был сильнее, чем в монокультурах, в то время как в одном консорциуме эффект антагонистической активности снизился по сравнению с монокультурами. Результаты исследования антагонистической активности двух консорциумов (Limosilactobacillus fermentum 44/1 и Lacticaseibacillus rhamnosus 12L, Latilactobacillus curvatus LCR-111-1 и Lactiplantibacillus plantarum 8PA3) противоречат данным о биосовместимости штаммов в этих консорциумах. Выявлены различия в степени антагонистического воздействия лактобацилл на грамположительные и грамотрицательные виды условно-патогенных бактерий.*
Заключение. *Исследование показало, что важными требованиями для создания пробиотического консорциума с эффективным пробиотическим потенциалом являются как биосовместимость пробиотических штаммов, так и антагонистическая активность консорциума. Для изучения антагонистических свойств лактобацилл следует увеличить количество изолятов целевых грамположительных и грамотрицательных бактерий и нормобиоты. Это позволит определить эффективные стратегии применения пробиотиков в условиях распространения лекарственной устойчивости микроорганизмов.*

Ключевые слова: *лактобациллы, пробиотические консорциумы, пробиотический потенциал, антагонистическая активность, условно-патогенные бактерии, полирезистентные изоляты*

Статья поступила: 29.03.2024
Статья принята: 18.06.2024
Статья опубликована: 15.07.2024

Для цитирования: Пеньдюхова А.С., Белькова Н.Л., Охотина Ю.С., Иванчиков Е.А., Щёкотова А.В., Семёнова Н.В., Рычкова Л.В. Антагонистическая активность монокультур и консорциумов лактобацилл в отношении полирезистентных изолятов условно-патогенных бактерий как скрининг их пробиотического потенциала. *Acta biomedica scientifica.* 2024; 9(3): 121-129. doi: 10.29413/ABS.2024-9.3.12

BACKGROUND

Recently, the human body has been considered as a system of symbiotic relationships with the community of microorganisms that inhabit it. This community includes bacteria, archaea, viruses, fungi, and protozoa and is called microbiota [1, 2]. Research shows that an imbalance in the intestinal microbiota not only leads to problems with the digestive system, but also increases the likelihood of cardiovascular and endocrine diseases and causes disturbances in the psychoemotional state [3–5].

Probiotics are an effective means of restoring a healthy balance of intestinal microbiota [2]. Today, special emphasis is placed on the study of associations (consortia) of probiotic microorganisms, in which the diversity of strains and species of bacteria determines the range of positive effects on the body [6]. The scientific literature indicates that one of the important reasons for the inconsistent clinical efficacy of multi-strain probiotic drugs is the lack of consideration of the biocompatibility of microorganisms when creating a consortium. This leads to a decrease in the viability of microorganisms and the loss of significant properties. In addition, it is reported that consortia of microorganisms are often unstable [6]. Therefore, the main goal of developing a complex probiotic is the selection of microorganisms with biocompatibility and similar biological and technological properties and maintaining a constant composition of these strains. The biocompatibility of strains is assessed using the direct co-cultivation method, taking into account the ability of lactobacilli to produce bacteriocins and other biologically active substances that determine the degree of strain antagonism in relation to representatives of its genus and affect the nature of interstrain interactions [7–9].

Modern methods, including next-generation sequencing (genomic, proteomic and metabolomic studies), are used to characterize the fundamental mechanisms of the bacteria probiotic effect on various functions of the macroorganism. New scientific technologies make it possible to assess the role of normal human microbiota and identify the subtle mechanisms of its response to various stressful environmental influences, and determine the factors that maintain the biochemical, metabolic and immunological balance necessary for stable relationships between the macroorganism and symbiotic microorganisms [10, 11].

However, the characterization based on genetic mechanisms and their potential for implementation should be investigated both *in vivo* and *in vitro*. Requirements for probiotic strains include resistance to low pH of gastric juice and bile acids, antagonism to opportunistic and pathogenic microorganisms, stability of composition and viability of bacteria during long-term storage. In addition, the safety of probiotic use should be confirmed in *in vitro* and *in vivo* animal studies and in the first phase of clinical trials [12].

Studying the biocompatibility of strains and the biotechnological potential of the consortium *in vitro* is an initial but very important stage in the development of an effective probiotic drug based on the consortium.

A correctly selected probiotic consortium may produce a synergistic effect, a phenomenon in which the combined action of several factors produces a more significant effect than the action of each of them separately. The synergistic effect allows the consortium to form a single system capable of resisting the effects of other microorganisms. The protective properties of the consortium are due to the antagonistic activity of bacteria and the synthesis of a number of biologically active substances [13].

Antagonistic activity against opportunistic and pathogenic microorganisms is not only one of the classic characteristics of probiotic bacteria, but also an important indicator of the efficacy and safety of a probiotic product, determined *in vitro* [14]. As knowledge of the structure and functions of the intestinal microbiota develops, it is becoming increasingly clear that, in addition to a large number of external factors causing microbial imbalance, antibiotics have a significantly more harmful effect on the microbiota. The resistance of opportunistic microorganisms to antimicrobial drugs, which is growing every year, is one of the global health problems worldwide [15]. In this regard, the development of effective consortia of probiotic strains with antagonism to multiresistant opportunistic microorganisms is extremely necessary.

THE AIM OF THE WORK

To study the antagonistic activity of monocultures and consortia of lactobacilli against multidrug-resistant isolates of opportunistic bacteria.

METHODS

Research objects

Gram-positive facultative anaerobic and/or microaerophilic bacteria of the family Lactobacillaceae

Lactobacillus strains and their consortia were obtained as part of the research work on the topic of "Obtaining microecological agents based on different lactobacillus strains" from the East Siberian State University of Technology and Management (ESSUTM) (agreement No. 6 dated May 10, 2023).

The following strains were obtained: *Lactobacillus curvatus* (*Latilactobacillus curvatus*) LCR-111-1, *Lactobacillus fermentum* (*Limosilactobacillus fermentum*) 44/1, *Lactobacillus acidophilus* 100ASH, *Lactobacillus rhamnosus* (*Lacticaseibacillus rhamnosus*) 12L, *Lactobacillus paracasei* (*Lacticaseibacillus paracasei*) k-406, *Lactobacillus plantarum* (*Lactiplantibacillus plantarum*) 8PAZ *Lactobacillus casei* (*Lacticaseibacillus casei*) MDP-1 [16].

The following consortia were received: *L. fermentum* 44/1 *и L. acidophilus* 100ASH (+); *L. fermentum* 44/1

и *L. rhamnosus* 12L (±); *L. curvatus* LCR111-1 и *L. fermentum* 44/1 (+); *L. curvatus* LCR-111-1 и *L. acidophilus* 100ASH (+); *L. curvatus* LCR-111-1 и *L. plantarum* 8-RA-3 (+); *L. curvatus* LCR-111-1 и *L. casei* MDP-1 (+); *L. acidophilus* 100ASH и *L. rhamnosus* 12L (+); *L. acidophilus* 100ASH и *L. casei* MDP-1 (+) [16].

The biocompatibility of the strains is indicated in brackets: "+" – the strains are compatible; "–" – the strains are incompatible; "±" – moderate antagonism is observed ("exit to the top" of one of the cultures) [16].

Normobiota and polyresistant isolates of opportunistic bacteria

Seven bacterial isolates were used as test cultures for testing antagonistic activity, including one strain belonging to the intestinal normobiota and six isolates of opportunistic bacteria with multiple antibiotic resistance (ABR) included in the "Human Microbiota Collection of the Irkutsk Region" of the Scientific Center for Family Health and Human Reproduction Problems [15]. The species composition of the bacterial test cultures is presented in Table 1.

Research methods

Cultivation of test cultures for in vitro experiments

The test culture strains were plated on the surface of meat-peptone agar (Research Center for Pharmacotherapy LLC, Russia) in a Petri dish and cultured at 37°C until the exponential growth phase was reached. Under sterile conditions, colonies of the exponential culture of the test strain were selected and suspensions were prepared in 10 ml of physiological solution, which were brought to the turbidity of the 0.5 McFarland standard.

Determination of antagonistic activity by the perpendicular streak method

The studied strain/consortium of lactobacilli was streaked on the surface of the Bifidum agar medium (State Research Center for Applied Biotechnology and Microbiology, Russia) in a Petri dish and incubated in an anaerobic jar with gas-generating bags (Anaerogas GasPak, Russia) at 37 °C for 48 h to allow the formation and diffusion of inhibitory compounds into the agar. Then, an exponential culture of the test strain was streaked perpendicularly from the edge of the dish to the streak of the grown culture/consortium of lactobacilli. The dish was incubated again, but under conditions favorable for the growth of the test culture: 37 °C without an anaerobic jar for 24 h. The experiment was repeated 3–4 times.

The presence and width of growth inhibition zones of microorganism test cultures were taken into account. Cultures that formed growth inhibition zones of the indicator strain from 4 to 9 mm were considered weak in antagonistic relation to lactobacilli; cultures that formed a zone from 9 to 14 mm were considered medium in antagonistic relation to microorganisms; cultures that formed a zone from 14 mm and more were considered highly active antagonists [14].

Determination of antagonist activity by the well method

The test cultures were inoculated with three-way streaking movements onto the Müller-Hinton medium (HiMedia Laboratories, India). No later than 15 min later, 10 mm diameter wells were cut in the agar layer containing the test strain using a cork drill and 0.1 ml of the lactobacillus monoculture/consortium suspension (cell count of at least 109 CFU/cm^3) were placed into each well. The cultures were incubated in a thermostat at 37 °C for 48 h. The experiment was repeated 2–3 times. The presence of growth inhibition zones and the diameters of the zones were taken into account with an accuracy of 1 mm, taking into account the diameter of the well itself. Weak antagonists include lactobacilli, the metabolites of which form growth inhibition zones for test cultures from 10 to 15 mm, medium antagonists – from 15 to 20 mm, and strong antagonists – more than 20 mm [14].

Statistical methods

Data on growth inhibition zones are presented as the arithmetic mean of the diameters of growth inhibition zones of test cultures (M) and the standard deviation (m).

Statistical data processing was performed using the PAST v. 4.03 program (Sweden). A nonparametric

TABLE 1

SPECIES COMPOSITION OF BACTERIAL TEST CULTURES

Type of microorganism	Number of isolates, abs.	Labeling of isolates
Human normobiota		
Escherichia coli	1	No. 10
Opportunistic bacteria with multiple resistance to antimicrobial drugs		
Enterobacter hormaechei	1	No. 2
Klebsiella pneumoniae	2	No. 9, No. 12
Pseudomonas aeruginosa	2	No. 34, No. 38
Staphylococcus aureus	1	No. 19

criterion for assessing statistical significance (Mann – Whitney U-test) was calculated for data on the antagonistic activity of consortia and individual lactobacilli strains included in their composition. Differences in statistical indicators were considered significant at $p < 0.05$.

The work was carried out using the equipment of the Collective Usage Center "Center for the Development of Progressive Personalized Health Technologies" and the Scientific Research Institution "Human Microbiota Collection of the Irkutsk Region" of the Scientific Center for Family Health and Human Reproduction Problems (Irkutsk), as well as the equipment of the Collective Usage Center "Progress" and the Biotechnology Center of ESSUTM (Ulan-Ude).

RESULTS AND DISCUSSION

When studying lactobacilli monocultures using the perpendicular streak method, 5 strains showed antagonistic activity against test bacterial cultures: *L. curvatus* LCR-111-1, *L. rhamnosus* 12L, *L. plantarum* 8PAZ, *L. casei* MDP-1 and *L. paracasei* k-406 (Table 2).

According to the experiment results, weak antagonists are strains of *L. plantarum* 8PAZ, *L. rhamnosus* 12L and *L. paracasei* k-406 in relation to the gram-positive isolate of *S. aureus* No. 19. In relation to the gram-negative isolates of *E. hormaechei* No. 2, *K. pneumoniae* No. 9, *K. pneumoniae* No. 12, *P. aeruginosa* No. 34, *P. aeruginosa* No. 38 and *E. coli* No. 10, all 5 strains of lactobacilli showed moderate antagonism.

In a well-based study, two strains showed antagonistic activity: *L. acidophilus* 100ASH and *L. fermentum* 44/1, against all isolates of the test cultures, while the other five lactobacilli strains had no effect. At the same time, the strain *L. acidophilus* 100ASH was a weak antagonist against the gram-positive isolate *S. aureus* No. 19 and a highly active antagonist, along with *L. fermentum* 44/1, against gram-negative isolates No. 2, No. 9, No. 12, No. 34, No. 38, and No. 10.

It should be noted that the effect of antagonistic activity of lactobacilli may be different depending on the type of opportunistic microorganisms with which they interact. This is probably due to the fact that gram-positive and gram-negative bacteria have different cell wall structures and mechanisms of interaction

TABLE 2

ANTAGONISTIC ACTIVITY OF LACTOBACILLI MONOCULTURES AGAINST MULTIDRUG-RESISTANT ISOLATES OF OPPORTUNISTIC BACTERIA AND *E. COLI*

Labeling of test cultures	Growth inhibition zones (M ± m)						
	***L. curvatus* LCR-111-1**	***L. acidophilus* 100ASH**	***L. rhamnosus* 12L**	***L. plantarum* 8PAZ**	***L. casei* MDP-1**	***L. fermentum* 44/1**	***L. paracasei* k-406**
Perpendicular streak method							
No. 2	10.5 ± 2.0	0	10.0 ± 0.2	10.0 ± 1.4	9.5 ± 0.2	0	9.5 ± 2.0
No. 9	12.0 ± 1.4	0	10.0 ± 0.1	12.0 ± 0.4	12.0 ± 0.8	0	11.5 ± 0.7
No. 12	12.5 ± 0.7	0	12.5 ± 0.7	12.0 ± 1.4	11.5 ± 0.7	0	12.0 ± 0.2
No. 19	10.0 ± 0.3	0	8.0 ± 1.4	8.8 ± 0.6	8.6 ± 0.3	0	8.5 ± 1.4
No. 34	10.0 ± 0.1	0	10.0 ± 0.3	10.0 ± 0.3	11.0 ± 1.4	0	10.0 ± 1.4
No. 38	11.5 ± 2.0	0	13.0 ± 0.2	11.0 ± 1.4	16.0 ± 5.6	0	12.5 ± 0.7
No. 10	10.0 ± 4.0	0	12.0 ± 1.4	11.0 ± 1.4	11.5 ± 0.7	0	11.0 ± 1.4
Well method							
No. 2	0	22.0 ± 0.8	0	0	0	23.2 ± 0.6	0
No. 9	0	23.5 ± 0.4	0	0	0	23.3 ± 0.5	0
No. 12	0	22.5 ± 0.7	0	0	0	22.0 ± 0.8	0
No. 19	0	18.5 ± 0.6	0	0	0	23.0 ± 0.8	0
No. 34	0	24.3 ± 1.2	0	0	0	24.5 ± 0.7	0
No. 38	0	24.0 ± 0.8	0	0	0	22.3 ± 1.2	0
No. 10	0	22.7 ± 0.5	0	0	0	22.3 ± 0.9	0

Note. No. 2 – *E. hormaechei;* No. 9 – *K. pneumoniae;* No. 12 – *K. pneumoniae;* No. 19 – *S. aureus;* No. 34 – *P. aeruginosa;* No. 38 – *P. aeruginosa;* No. 10 – *E. coli.*

with other bacteria. In addition, different isolates (No. 34 and No. 38) of the same species – *P. aeruginosa* – showed different results of antagonistic activity (different growth inhibition zones). This may indicate that the more isolates of the same species are tested, the more effectively the antagonistic properties of lactobacilli will be studied.

Among the consortia, antagonistic activity in the study using the perpendicular streak method was noted in two (table 3):

• *L. curvatus* LCR-111-1 and *L. plantarum* 8PAZ, which showed weak antagonism towards isolates No. 2, No. 9, No. 19, No. 34 and moderate antagonism towards isolates No. 12, No. 38 and No. 10. Moreover, the antagonism of the consortium was statistically significantly weaker than the antagonism of individual strains of lactobacilli included in its composition ($p = 0.002$);

• *L. curvatus* LCR-111-1 and *L. casei* MDP-1, which showed weak antagonism towards isolate No. 2 and moderate antagonism towards isolates No. 9, No. 12, No. 19, No. 34, No. 38 and No. 10.

Unlike lactobacilli monocultures, which exhibit different antagonistic activity towards gram-positive and gram-negative isolates of opportunistic microorganisms, such differences are not observed in consortia: according to the degree of antagonistic effect, isolates are combined regardless of the type of cell wall or any specific mechanisms of interaction.

In a well-based study, 6 consortia (highly active antagonists) containing *L. acidophilus* 100ASH and/or *L. fermentum* 44/1 strains showed antagonistic activity, with five of them having statistically significantly more pronounced antagonism than individual strains:

TABLE 3

ANTAGONISTIC ACTIVITY OF LACTOBACILLI CONSORTIUMS AGAINST MULTIDRUG-RESISTANT ISOLATES OF OPPORTUNISTIC BACTERIA AND *E. COLI*

Labeling of test cultures	Growth inhibition zones (M ± m)							
	L. fermentum 44/1 and *L. acidophilus* 100ASH	*L. fermentum* 44/1 and *L. rhamnosus* 12L	*L. curvatus* LCR-111-1 and *L. fermentum* 44/1	*L. curvatus* LCR-111-1 and *L. acidophilus* 100ASH	*L. curvatus* LCR-111-1 and *L. plantarum* 8PAZ	*L. curvatus* LCR-111-1 and *L. casei* MDP-1	*L. acidophilus* 100ASH and *L. rhamnosus* 12L	*L. acidophilus* 100ASH and *L. casei* MDP-1
Perpendicular streak method								
No. 2	0	0	0	0	7.0 ± 0.1*	8.5 ± 0.7	0	0
No. 9	0	0	0	0	8.0 ± 0.3*	9.5 ± 0.7	0	0
No. 12	0	0	0	0	9.0 ± 0.2*	11 ± 0.3	0	0
No. 19	0	0	0	0	6.0 ± 0.3*	9.5 ± 0.7	0	0
No. 34	0	0	0	0	8.0 ± 1.4*	9.0 ± 1.4	0	0
No. 38	0	0	0	0	9.5 ± 2.0*	10 ± 0.2	0	0
No. 10	0	0	0	0	9.5 ± 2.0*	10.5 ± 0.7	0	0
Well method								
No. 2	24.5 ± 0.7*	24.3 ± 0.9*	23.7 ± 1.2*	22.8 ± 1.4*	0	0	23.3 ± 1.2*	19.0 ± 6.3
No. 9	24.3 ± 0.6*	24.3 ± 0.9*	24.3 ± 0.5*	23.3 ± 0.5*	0	0	23 ± 0.8*	23.3 ± 0.5
No. 12	23.5 ± 1.5*	23.3 ± 0.5*	23.3 ± 1.2*	24.7 ± 0.5*	0	0	23.7 ± 0.5*	23.0 ± 0.8
No. 19	23.3 ± 0.9*	23.7 ± 1.2*	24.0 ± 0.3*	24.3 ± 0.6*	0	0	24.7 ± 1.5*	24.2 ± 1.2
No. 34	25.5 ± 1.0*	24.3 ± 0.9*	25.3 ± 1.2*	23.2 ± 1.6*	0	0	23.2 ± 1.4*	22.2 ± 6.7
No. 38	26.5 ± 1.8*	26.3 ± 1.7*	26.5 ± 1.0*	24.7 ± 1.2*	0	0	24.7 ± 1.2*	23.7 ± 0.9
No. 10	23.6 ± 2.0*	23.3 ± 1.2*	23.5 ± 1.9*	24.3 ± 2.0*	0	0	25.7 ± 1.2*	23.3 ± 2.4

Note. No. 2 – *E. hormaechei;* No. 9 – *K. pneumoniae;* No. 12 – *K. pneumoniae;* No. 19 – *S. aureus*; No. 34 – *P. aeruginosa;* No. 38 – *P. aeruginosa;* No. 10 – *E. coli;* * – statistical significance of differences between the consortium and individual strains of lactobacilli included in its composition ($p < 0.05$).

• *L. fermentum* 44/1 and *L. acidophilus* 100ASH ($p = 0.002$);

• *L. fermentum* 44/1 and *L. rhamnosus* 12L ($p = 0.001$);

• *L. curvatus* LCR-111-1 and *L. fermentum* 44/1 ($p = 0.002$);

• *L. curvatus* LCR-111-1 and *L. acidophilus* 100ASH ($p = 0.002$);

• *L. acidophilus* 100ASH and *L. rhamnosus* 12L ($p = 0.002$);

• *L. acidophilus* 100ASH and *L. casei* MDP-1 ($p = 0.198$).

The studied lactobacillus strains in the declared consortia had high biotechnological potential and biocompatibility level, with the exception of the consortium based on *L. fermentum* 44/1 and *L. rhamnosus* 12L strains, which showed moderate antagonism (±) [16]. Comparison of the results of strain biocompatibility and consortia antagonistic activity revealed the following features:

1. In a consortium based on the strains *L. curvatus* LCR111-1 and *L. plantarum* 8PAZ, which was characterized by a high level of biocompatibility (+), a statistically significant decrease in the effect of antagonistic activity was revealed in comparison with the activity of monocultures.

2. In a consortium based on *L. fermentum* 44/1 and *L. rhamnosus* 12L strains, in which moderate antagonism (±) was demonstrated between the strains, the antagonistic activity against opportunistic microorganisms was statistically significantly more pronounced than that of individual strains.

The study showed that a high degree of compatibility of lactobacilli strains does not guarantee a synergistic effect. In the context of lactobacilli, synergy can be expressed as an increase or decrease in antagonistic activity against pathogenic microorganisms, affecting the probiotic potential of the consortium. Therefore, key aspects for the creation of an effective probiotic consortium are both the biocompatibility of probiotic strains and the antagonistic activity of the consortium. A probiotic product developed taking into account these criteria can demonstrate increased efficacy and a wider range of beneficial properties for the body.

The antagonistic activity of probiotic strains is studied using various methods: at the first stage, *in vitro* methods are used (diffusion methods, analysis in liquid nutrient media, etc.), at the second stage, *in vivo* methods (reception of antagonist live culture by a person or experimental animals with subsequent analysis of changes in the intestinal microbiota). All these methods differ in the degree of complexity of implementation, efficiency, the possibility of comparison and the accuracy of the results obtained [17, 18]. For example, the results obtained by two classical methods (the perpendicular streak method and the well method), based on the diffusion of components produced by lactobacilli in the thickness of agar, are difficult to compare. Thus, the perpendicular streak method gives an advantage to individual strains producing inhibitory compounds of small molecular weight. The well method is convenient, in turn, for testing the antagonistic activity of not only monocultures, but also consortia, since a ready-made suspension of bacteria, including their metabolites, is placed in the well. Thus, both of these methods complement each other and should be used in combination, as they provide a more complete picture of the antagonistic activity of lactobacilli and their consortia, which is one of the characteristics of probiotic potential.

CONCLUSION

The results of the study showed that all lactobacilli and their consortia, depending on the study method, had different degrees of antagonistic activity against multiresistant isolates of opportunistic bacteria. In five studied consortia, the antagonistic effect against test cultures was more pronounced than that of individual strains, while one consortium, on the contrary, showed a decrease in the effect of antagonistic activity compared to monocultures. The obtained results of the study of the antagonistic activity of two consortia (*L. fermentum* 44/1 and *L. rhamnosus* 12L, *L. curvatus* LCR111-1 and *L. plantarum* 8PAZ) are not consistent with the data on the biocompatibility of strains in these consortia. Consequently, the compatibility of strains does not always lead to a positive synergistic effect, which can manifest itself in increased antagonistic activity. Thus, the creation of probiotic consortia requires fine-tuning and selection of bacterial strains, taking into account both the biocompatibility of the strains and the antagonistic properties of the consortium to ensure effective and safe action to improve human health.

The detected antagonistic activity against the *E. coli* isolate may be due to the same mechanism of lactobacilli action on both opportunistic bacteria and normobiota. The analysis of the obtained results revealed differences in the degree of lactobacilli antagonistic action on gram-positive and gram-negative bacterial species. Moreover, such differences may also be observed in different isolates of the same species. Given the identified features, a wider range of both gram-positive bacteria and normobiota isolates should be included in the experiment for a more detailed study of the lactobacilli antagonistic properties. In addition, it would be advisable to use not different species, but different isolates of the same species: *E. coli* and target intestinal opportunistic bacteria, for example, *K. pneumonia*, one of the most dangerous types of opportunistic pathogens. This will help to determine more effective strategies for the probiotics use in the context of the mass spread of drug resistance of opportunistic microorganisms.

Funding

The study was carried out within the framework of state topic No. 1023041400005-8-3.4.1.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

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