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PROBIOTIC POTENTIAL OF MICROORGANISMS OBTAINED FROM THE INTESTINES OF WILD BIRDS

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ABSTRACT

This article presents the study results of the probiotic potential of lactic acid bacteria isolated from the gastrointestinal tract of wild birds. From a series of experiments, results found that lactobacilli (Lactobacillus brevis and Lactobacillus parabuchneri) have high probiotic properties. They do not show an inhibitory effect on the beneficial microbiota of the intestine, as well as each other since when they were jointly cultivated on an agarized nutrient medium in different combinations, growth retardation zones did not appear. When studying the safety of the studied lactobacilli, it revealed that cultures on blood agar do not form lysis zones around themselves, which confirms the negative result of hemolysis, and with the introduction of lactic acid bacteria to laboratory mice, there was no pronounced toxicosis.

Disciplinary: Microbiology, Biotechnology, Animal Science, MicrobioScience.

1. INTRODUCTION

One of the main problems of veterinary medicine is the emergence of microorganisms with pathogenic properties that have become resistant to several antibiotic drugs. The constant use of these drugs in the diet has a negative effect not only on the quality of products but also reduces the immunity of farm animals and poultry. Also, antibiotic substances of drugs inhibit the vital activity of their own microbiocenosis, which is a prerequisite for the development of diseases. In this regard, for the prevention and treatment of diseases caused by microbial imbalance, the use of biological

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products of microbial origin is relevant [4, 9, 13].

Natural normoflora takes an active part in maintaining the colonization stability of the intestinal mucosa and has a large role in preventing diseases. By isolating antibiotic substances into the intestinal environment, they contribute to the inhibition of the growth of pathogenic and conditionally pathogenic microflora, which is the causative agent of various internal non-communicable diseases. The beneficial microflora in the composition of biological products has an anti-infectious, immunomodulatory effect on the body; increases protective functions, and stimulates intestinal motility and excretory functions. Biological products based on live microflora are used both for prophylaxis and therapy. However, each species of animal and bird has its own microflora of the gastrointestinal tract, which has probiotic characteristics. As practice shows, the use of active probiotic microorganisms made based on live strain cultures that were isolated from the gastrointestinal tract from one type of living organism is not always effective when used in another species [7, 10, 11, 12].

It follows that the development of biological products based on their own useful native microflora, which can replace antibiotics, is an urgent area, and the study of the properties of the new isolated useful microflora is both scientific and practical.

The aim of the research work is to study the probiotic potential of lactic acid bacteria isolated from the gastrointestinal tract of wild birds.

2. METHOD

The objects of research were lactic acid bacteria Lactobacillus brevis and Lactobacillus parabuchneri isolated from the gastrointestinal tract of wild quail Coturnixcoturnix.

The antagonistic activity of lactic acid microorganisms with respect to field pathogenic strains was studied by diffusion methods (in-vitro): the method of agar layers [2], holes [1, 8], perpendicular strokes [5, 8] and drops [3] in various modifications. As a test culture used conditionally pathogenic field strains isolated from natural conditions at the poultry farms of the Krasnodar Territory.

We studied the antagonistic properties of lactobacilli with respect to laboratory (reference) test cultures of pathogenic and conditionally pathogenic microflora of various groups (S. aureus ATCC 25923, B. subtilis 534, E. coli ATCC 25922, Sh. Sonnei 941, P. aeruginosa ATCC 27853, Klebsiella pneumonia K1 5054, C. Albicans ATCC 885-653) by the method of delayed antagonism [6].

The ability of the in-vitro probion strains to inhibit the growth of representatives of the intestinal normoflora, as well as each other, was studied according to the recommendations [6]. As test organisms, a pure Lactobacillus acidophilus culture isolated from the Linex medicine (Slovenia) was used; Lactobacillus acidophilus strain n.v. Ep 317/402 (“Narine” preparation, Armenia); Lactobacillus galinarum isolated from the intestines of birds.

A study was made of the release (direct and indirect method) and adhesive properties of strains of lactobacilli in cell models [6]. The study of the release of lactobacilli was carried out by the direct reaction of the release activity of cultures. For confirmation, an indirect method was also used, which is based on the reaction of the anti-adhesive activity of the supernatant of lactobacillus cultures. The adhesive properties of lactobacilli were studied on the intestinal enterocytes of chicken embryos. The

adhesion level of individual bacterial strains was conditionally differentiated into four degrees: non-adhesive (AAI = 0); weakly adhesive (AAI = 1−5); medium adhesive (AAI = 5−10) and highly adhesive (AAI above 10).

As additional methods for studying the safety of used cultures of lactobacilli, the hemolytic activity of invitro and the safety of in-vivo strains were studied. The determination of hemolytic activity was carried out on 5% blood agar. The safety of the studied cultures was in vivo studied in laboratory white mice. Observation of laboratory animals was carried out for five days [6].

To process the results of the experiments, statistical methods were used with finding the arithmetic mean (M), the average error of the arithmetic mean (m) and the probability of error according to Student Tables.

3. RESULT AND DISCUSSION

3.1 ANTAGONISM TO FIELD PATHOGENIC MICROFLORA

The results of a study of the inhibitory activity of lactic acid microorganisms showed that all two strains to varying degrees exhibited an antibacterial effect on the growth of the pathogenic culture. It was found that the zone of delay of pathogenic cultures using various diffusion methods was in the range of 2.0–7.0 mm. Using the modified method of perpendicular strokes showed a growth inhibition zone of 5.0–7.0 mm, a hole method of 2.0–4.0 mm, a drop method of 3.0–6.0 mm, and an agar layer method of 3.0-6.0 mm. Table 1 presents the study results of the antagonistic activity of the tested lactic acid cultures.

Table 1: Zone of growth retardation of field pathogenic cultures from the action of lactobacilli, mm

Pathogenic culture	Lactobacillus studied	
	Lactobacillus brevis	Lactobacillus parabuchneri
Agar layer method		
Escherichia coli	3.0 ± 0.1	3.0± 0.1
Staphylococcus aureus	4.0 ± 0.1	6.0± 0.2
Hole method		
Escherichia coli	3.0 ± 0.1	2.0± 0.1
Staphylococcus aureus	4.0 ± 0.1	4.0± 0.2
Perpendicular stroke method		
Escherichia coli	5.0 ± 0.3	7.0± 0.2
Staphylococcus aureus	7.0 ± 0.3	6.0± 0.3
Drops method		
Escherichia coli	4.0 ± 0.1	3.0± 0.1
Staphylococcus aureus	5.0 ± 0.2	6.0± 0.2

When studying antagonistic activity by the agar layer method, it was found that the growth inhibition zone of Escherichiacoli and Staphylococcus aureus during incubation of Lactobacillus brevis was 3.0 and 4.0 mm, and when growing Lactobacillus parabuchneri, 3.0 and 6.0 mm, respectively.

When studying the antagonism by the hole method, it was found that the growth retardation zone of Escherichia coli and Staphylococcus aureus during the cultivation of Lactobacillus brevis was 3.0 and 4.0 mm, and when growing Lactobacillus parabuchneri, 2.0 and 4.0 mm.

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The method of perpendicular strokes showed that under the influence of Lactobacillus brevis, the growth retardation zone of the test strains was 5.0 and 7.0 mm, respectively. Under the action of inhibitory agents secreted by Lactobacillus parabuchneri, the growth retardation zone of the test cultures was 7.0 mm (Escherichia coli) and 6.0 mm (Staphylococcus aureus).

When testing the antagonistic activity of wild quail lactobacilli isolated from the intestine by the drop method, it was found that all cultures showed an antibacterial effect on the growth of Escherichiacoli. The growth retardation zone of this testing culture was 3.0 and 4.0 mm. The staphylococcus aureus growth inhibition zone when cultured with Lactobacillus brevis and Lactobacillus parabuchneri was 5.0 and 6.0 mm.

Thus, the results of the experiments showed that lactobacilli isolated from the gastrointestinal tract of wild quail exhibit antagonistic properties with respect to field pathogenic microflora, which makes them promising for further experiments.

3.2 ANTAGONISM TO REFERENCE PATHOGENIC MICROFLORA.

Additionally, the antagonistic ability of the studied cultures was studied in relation to the reference test cultures of pathogenic and conditionally pathogenic microflora. The research results are presented in Table 2.

Table 2: Zone of growth retardation of laboratory test cultures from the action of lactobacilli, mm

Test culture	Lactobacillus studied	
	Lactobacillus brevis	Lactobacillus parabuchneri
S. aureus ATCC 25923	21.0± 0.10	21.0± 0.17
B. subtilis 534	14.0± 0.10	11.0± 0.18
E. coli ATCC 25922	21.0± 0.12	25.0± 0.16
Sh. sonnei 941	10.0± 0.11	9.0± 0.14
P. aeruginosa ATCC 27853	22.0± 0.12	22.0± 0.15
Klebsiella pneumonia K_1 5054	23.0± 0.12	24.0± 0.16
C. albicans ATCC 885-653	11.0± 0.10	12.0± 0.14

As a result of the studies, it was found that the antagonistic activity of the studied cultures of lactobacilli in relation to the reference test strains was higher than when influencing field pathogenic test cultures. According to the recommendations, a zone of 20 mm inhibition of the test culture by them is taken as a positive result of the antagonistic properties of microorganisms [6]. In this case, the high antagonistic properties of Lactobacillus brevis are shown for S. aureus ATCC 25923 (21.0 mm), E. coli ATCC 25922 (21.0 mm) and P. aeruginosa ATCC 27853 (22.0 mm), Klebsiella pneumonia K1 5054 (23.0 mm). Lactobacillus parabuchneri inhibits the growth of S. aureus ATCC 25923 (21.0 mm), E. coli ATCC 25922 (25.0 mm), P. aeruginosa ATCC 27853 (22.0 mm) and Klebsiella pneumonia K1 5054 (24.0 mm) well.)

In relation to other reference test strains, the studied lactobacilli also exhibit antagonistic properties, but to a lesser extent, which does not reduce their probiotic potential. So, the delay zone of B. subtilis 534, Sh. sonnei 941, C. Albicans ATCC 885-653 upon the cultivation of Lactobacillus brevis was 14.0; 10.0 and 11.0 mm, respectively. The growth inhibition zone of the corresponding reference test cultures, when exposed to Lactobacillus parabuchneri, was 11.0; 9.0 and 12.0 mm.

Thus, Lactobacillus Brevis and Lactobacillus parabuchneri isolated from the gastrointestinal tract of wild quail exhibit antagonistic properties with respect to laboratory pathogenic test culture.

3.3 ANTAGONISM TO THE NORMOFLORA OF THE INTESTINE

The next stage of the work was to identify the ability of probiotic strains to inhibit representatives of the intestinal normoflora, as well as each other. The determination of the interaction of the studied strains with representatives of normoflora was studied by the method of perpendicular strokes. Sowing the studied culture in the form of a stroke in diameter per cup with nutrient medium MPC. Perpendicular to the first sowing, strokes of other test cultures were made. Cultivation was carried out at 37 ° C for 24–48 hours. The results of the studies were taken into account in the zone of growth inhibition (Table 3)

Table 3: Inhibitory activity of the studied lactobacilli in relation to normoflora, mm

Test culture	Zone of growth inhibition, mm	
	Lactobacillus Brevis	Lactobacillus parabuchneri
Lactobacillus acidophilus (Linex strain)	0	0
Lactobacillus acidophilus strain n.v. Ep 317/402	0	0
Lactobacillus galinarum	0	0
Lactobacillus Brevis	–	0
Lactobacillus parabuchneri	0	–

The research results showed that when co-growing crops in any version, there was no growth retardation of the studied and tested cultures of microorganisms.

Thus, the lactic acid bacteria studied in the research work do not show an inhibitory effect on the beneficial microflora, as well as in relation to each other.

3.4 RELEASE PROPERTIES OF LACTOBACILLI

Anti-adhesive properties of microorganisms are the ability of a culture to prevent or reduce the attachment actions (adhesion) of pathogenic microorganisms to the object of adhesion, in our case, sheep blood red blood cells. As indicated in the "Methods" section, the studied property of lactobacilli was determined by the method of direct and indirect reaction. Anti-adhesive properties of lactobacilli were carried out in relation to test cultures (Escherichia coli and Staphylococcus aureus). The research results are presented in Tables 4 and 5.

Table 4: Results of the release properties of the objects of research to E. coli

Research method	Release agent objects	Release properties to control,%
Direct method	Control	100.0
	Lb. brevis	45.6
	Lb. parabuchneri	43.8
Indirect method	Control	100.0
	Supernatant Lb. brevis	37.4
	Supernatant Lb. parabuchneri	35.6

As a result of the studies, it was established that the studied cultures possessed anti-adhesive properties with respect to test strains, but to a different degree. So, with the influence of lactobacilli on the adhesion of E. Coli to sheep erythrocytes in the direct method, it was revealed that the Lb culture had the highest anti-adhesive properties (45.6%). brevis. Lb. Parabuchneri contributed to the release of E. Coli equal to 43.8%.

When studying the release properties of the supernatant of lactobacilli against E. coli, their

positive effect was also revealed. Lactobacillus supernatants had anti-adhesive properties, which indicates the presence of metabolites that inhibit adhesion, which developed lactic cultures. So, the release properties of the supernatant Lb. brevis in relation to E. Coli amounted to 37.4%, and the release properties of the supernatant of Lb. Parabuchneri 35.6%.

Table 5: The results of the release properties of the objects of research to S. aureus

Research method	Release agent objects	Release properties to control,%
Direct method	Control	100.0
	Lb. brevis	61.2
	Lb. parabuchneri	59.3
Indirect method	Control	100.0
	Supernatant Lb. brevis	39.7
	Supernatant Lb. parabuchneri	37.8

Similar results were obtained in the study of the release properties of lactobacilli against S. aureus. Release properties of Lb. Brevis and Lb. Parabuchneri in the direct method against S. aureus accounted for 61.2 and 59.3%. The supernatants of the studied cultures in relation to S. Aureus also showed anti-adhesive action, but to a lesser extent than native lactobacilli (39.7 and 37.8%).

Thus, the results of the studies showed that live cultures of lactobacilli provide a higher result of the release properties than their metabolic products.

3.5 ADHESIVE PROPERTIES OF LACTOBACILLI

Adhesive activity - the ability of microorganisms to attach to the intestinal epithelial cells and multiply before the enterocytes of the mucous layer are renewed. This ability microflora is provided due to the production of a number of components (drank or fimbriae), which help to gain a foothold on the epithelial cell. These components are called - factors of colonization or adhesion. Microorganisms can express various types of pili, which are encoded by different chromosomal and plasmid genes. This genetic diversity allows the cell culture to adapt to a changing environment and use this opportunity in relation to various surface structures of the host organism [6]. The results of the adhesion of the studied lactobacilli in relation to enterocytes of the intestines of the chicken embryo are presented in Table 6.

Table 6: Average adhesion index (SPA) (when counting on 25 enterocytes) of the studied cultures of lactobacilli

Test lactobacilli	Average adhesion index (AAI)
Lactobacillus Brevis	13.1 ± 0.47
Lactobacillus parabuchneri	13.6 ± 0.50

When counting the number of microbial cells attached to enterocytes, it was found that all the studied cultures of lactobacilli had highly adhesive properties, since the AAI was 13.1 (for Lactobacillus Brevis) and 13.6 (for Lactobacillus parabuchneri).

Thus, the studied lactobacilli exhibit adhesive ability, which also confirms their probiotic potential.

3.6 SAFETY OF THE STUDIED LACTOBACILLI

The safety of lactobacillus cultures used in the study was studied by their manifestation of hemolytic activity on blood agaris when they were orally administered to mice [6].

The production of hemolysins is considered a pathogenicity factor, in this regard, the production of hemolysin in many cases is a marker of virulence. As a result of the experiment, when lactic cultures were grown on 5% blood agar of the lysis zone around them, it was not revealed the result of hemolysis is negative. The studied cultures of lactic acid bacteria do not exhibit hemolytic activity.

The results of studies on the safety of lactobacilli in mice are presented in Table 7.

Table 7: The safety of the studied lactobacilli in mice (n = 5)

Group	Kind of animal	Amount of injected fluid, method of administration	Test result		
			got sick	died	survived
Intact	Mouse	–	0	0	5
Control		0.5 ml, orally (saline)	0	0	5
1st experience		0.5 ml orally (Lb. brevis)	0	0	5
2nd experience		0.5 ml orally Lb. parabuchneri)	0	0	5

As a result of the experiment, within 5 days it was found that in all groups of mice no deaths and diseased animals were recorded. During a daily examination of laboratory animals, it was possible to observe that the mice remained mobile and active, satisfactory food intake was recorded, while maintaining all vital reflexes.

Thus, the lactobacilli cultures used in the research work are safe for use.

4. CONCLUSION

A set of experiments to study the probiotic potential of lactic acid cultures (Lactobacillus brevis and Lactobacillus parabuchneri) showed that microorganisms isolated from the intestines of wild quail have high probiotic properties. They do not show an inhibitory effect on the beneficial microbiota of the intestine, as well as each other since when they were jointly cultivated on an agarized nutrient medium in different combinations, growth retardation zones did not appear. The studied lactobacilli to varying degrees exhibit antagonistic activity against the field and reference conditionally pathogenic and pathogenic test culture, in particular, the growth inhibition zones of field pathogenic microorganisms were in the range 2.0–7.0 mm, while the reference test crops 9.0–25.0 mm. The cultures of the studied microorganisms showed high antiadhesive properties (35.6–61.2%), which were expressed in the fact that the pathogenic strains of Escherichia Coli and Staphylococcus aureus did not attach to sheep erythrocytes. Adhesive properties were revealed in lactobacilli, which were expressed in the ability of cultures to attach to the intestinal enterocytes of the chicken embryo, while the average adhesion rate when counting 25 enterocytes of the studied cultures of lactobacilli was 13.1–13.6 units. When studying the safety of the studied lactobacilli, it was revealed that cultures on blood agar do not form lysis zones around themselves, which confirms the negative result of hemolysis, and with the introduction of lactic acid bacteria to laboratory mice,

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there was no pronounced toxicosis. Thus, these lactobacilli are promising and can be included in the composition of biological products of microbial origin for use in industrial animal husbandry, in particular poultry farming.

5. AVAILABILITY OF DATA AND MATERIAL

Data can be made available by contacting the corresponding authors

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